HiTS

Harvard Program
in Therapeutic Science

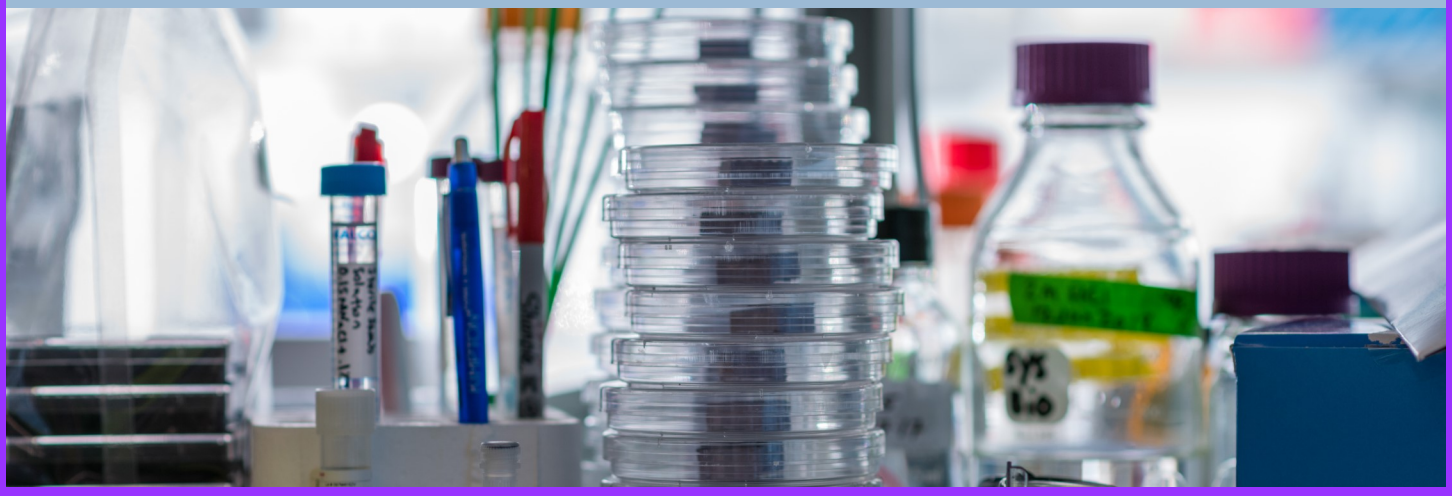
HiTS Symposium

December 5, 2018

Harvard Business School, Spangler Auditorium

Soldiers Field Road

Boston, MA



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4:45 – 5:15pm	**Session 4, Part 2: Detection Methods for Biological Systems** **Chair: Alyce Chen**, Scientific Program Manager, Harvard Program in Therapeutic Science **Michael Baym**, Harvard Medical School, Department of Biomedical Informatics *Antibiotic Resistance Evolution*
5:15 – 6:45pm	**Poster Session, Poster Prizes and Refreshments – Williams Room**

Speaker Bios and Abstracts

Welcome and Special Session: Oncology as a Test Bed for Precision Medicine

Chair: Cheryl Hutt

Peter Sorger, Harvard Medical School

Peter Sorger is the Otto Krayer Professor of Systems Pharmacology at Harvard Medical School. He received his AB from Harvard College and PhD from Trinity College, Cambridge University U.K., working under the supervision of Hugh Pelham. He trained as a postdoctoral fellow at the University of California, San Francisco with Harold Varmus and Andrew Murray. Prior to coming to HMS Peter served as a Professor of Biology and Biological Engineering at MIT. Sorger was cofounder of Merrimack Pharmaceuticals and Glencoe Software and is an advisor to multiple public and private companies and research institutes in the US, Europe and Japan.

Peter's research focuses on the signal transduction networks controlling cell proliferation and death, dysregulation of these networks in cancer and inflammatory diseases and mechanisms of action of therapeutic drugs targeting signaling proteins. His group uses mathematical and experimental approaches to construct and test computational models of signaling in human and murine cells as a means to understand and predict responses to drugs applied individually and in combination. The Sorger group also develops open-source software for analyzing biological networks and drug mechanism of action and it participates in multiple collaborative programs working to improve data access and reproducibility. Recent research extends a systems pharmacology approach to analysis of clinical samples and interpretation of clinical trials.

A Systematic Approach to Pharmacology

Precision approaches to cancer therapy are frequently equated with the use of gene sequencing to identify sequence variants that are predictive of drug response. This approach has proven particularly effective when mutations lie in diving oncogenes that are themselves targets of inhibitory biologic drugs and small molecules. This approach has yielded multiple "silver bullet" drugs with ability to dramatically improve the health of patients with hitherto untreatable diseases. Silver bullets include Trastuzumab for HER2amp breast cancer, imatinib for of BCR-Abl positive CML and vemurafenib for BRAFV600E melanoma. However, it is increasingly clear that similarly simple predictors of drug response are unlikely to be identified for many other types of cancer. For example, development of drugs that inhibit PI3K/Akt kinases was motivated by the high prevalence of mutations in breast and other cancers.

However, such drugs are struggling in the clinic, in part because the presence of nutations in PI3K/Akt pathway proteins is poorly predictive of response.

In this talk I will describe approaches to quantifying and studying drug response at two points in the drug development pipeline: early pre-clinical studies and post approval development of combination therapies. Drug sensitivity and resistance in cell lines are conventionally quantified by IC50 or Emax values, but these metrics suffer from a fundamental flaw when applied to growing cells: they are highly sensitive to the number of divisions that take place over the course of a response assay. Division rate varies with cell line and experimental conditions. Seeding density is a particularly strong and unpredictable confounder. The dependency of IC50 and Emax on division rate creates artefactual correlations between genotype and drug sensitivity while obscuring important biological insights and interfering with biomarker discovery. I will describe alternative metrics that are insensitive to division number. These are based on estimating growth rate inhibition (GR) in the presence of a drug using endpoint or time-course assays. The latter provides a direct measure of phenomena such as adaptive drug resistance. Theory and experiments show that GR50 and GRmax are superior to IC50 and Emax for assessing the effects of drugs in dividing cells. GR metrics promise to improve our ability to identify drugs effective on specific patient-derived tumor cells.

Combination therapy is widely regarded as the key to treating cancers with widely varying oncogenic drivers. Combinations commonly improve tumor control compared to monotherapy and their development is currently motivated in most cases by pre-clinical data on synergism in cell lines. However, because drug response is commonly variable from one patient to the next, combinations may be beneficial not only through additive effects in individuals, but also by combining agents that produce favorable responses in different individuals. I will describe a mathematical model of between-patient variability in drug response and its use to re-analyze multiple human clinical trials as well as a remarkable dataset of drug responses in patient derived tumor xenograft (PDX) mice published by Gao H, Korn JM, Ferretti S, et al. (Nature medicine 2015;21:1318-25). I will argue that, with some exceptions, the observed benefits of combination therapies are consistent with a simple principle: combinations improve the likelihood that each tumor is treated with at least one therapy to which it is sensitive. Using this principle I will describe how analysis PDX trials of monotherapies to nominate combinations that may improve response rates through optimal management of response variability. Thus, even in the absence of additive or synergistic tumor inhibition, combinations are superior to monotherapies due to imperfect knowledge of which patient should receive which monotherapy, providing a scientific rationale for the use of combination therapy whenever toxicity is acceptable.

H. Kim Lyerly, Duke University

Dr. Lyerly the Executive Director of the Accelerating Anticancer Agent Development and Validation (AAADV) Workshop, which has been held annually for the past 16 years near the FDA headquarters in Bethesda, MD. The three-day, on-site interactive AAADV Workshop offers a mix of small group lectures, moderated discussions, plenary lectures and facilitated case studies of successful drug applications. AAADV is the only workshop held in collaboration with the U.S. Food and Drug Administration designed specifically to help participants understand and negotiate the drug development approval process so that effective cancer treatments can reach patients more quickly.

Dr. Lyerly is the former director of the Duke Comprehensive Cancer Center, and currently George Barth Geller Professor of Cancer Research, Professor of Surgery, Professor of Pathology and Professor of Immunology at Duke University in North Carolina. He was appointed in 2008 by President George Bush to serve on the National Cancer Advisory Board, which oversees the National Cancer Institute. He served on the Global Health Sub-Committee of the National Cancer Advisory Board, the National Institute of Health Council of Councils, and the Board of the National Institute of Health Office of AIDS Research.

Accelerating Drug Development for Molecular Subsets, Rare, and Pediatric Cancers

Advances in genetic sequencing and other diagnostic technologies have enabled the use of precision medicine in clinical cancer care, as well as the development of novel therapies that are targeted to specific molecular drivers of cancer. Developing these new agents and making them accessible to patients requires global clinical studies and regulatory review and approval by different national regulatory agencies. Whereas these global trials present challenges for drug developers who conduct them and regulatory agencies who oversee them, they also raise practical issues about patients with low-frequency cancers who need these therapies. A lack of uniform standards in both regulatory approval for marketing and reimbursement for approved agents across countries may make the newly developed agent either unavailable or inaccessible to patients in certain countries or regions, even if patients from those countries or regions participated in the clinical research that established the safety and efficacy of the agent. As a matter of justice, agents being developed for rare cancers, pediatric cancers, or uncommon molecular subsets of common cancers need a pragmatic, science-based regulatory policy framework to clearly specify the type and quantity of evidence needed to demonstrate efficacy from these trials and evidence to support accessibility.

Russell Jenkins, MGH Cancer Center

Russ Jenkins completed his clinical oncology training in the Dana-Farber/Harvard Cancer Center and his post-doctoral research training in David Barbie's lab at Dana-Farber. He is now a faculty member of the MGH Cancer Center in the Center for Melanoma and is the inaugural Termeer Early Career Investigator in Systems Pharmacology and a member of the Laboratory for Systems Pharmacology at HMS. The Jenkins laboratory uses sophisticated tools and techniques to study and investigate the complex and dynamic interactions between cancer cells and the immune system.

Ex Vivo Profiling of PD-1 Blockade Using Organotypic Tumor Spheroids

Ex vivo systems that incorporate features of the tumor microenvironment and model the dynamic response to PD-1 blockade may facilitate efforts in precision immuno-oncology. We have developed a system for for ex vivo profiling of PD-1 blockade using 3D microfluidic culture of murine- and patient-derived organotypic tumor spheroids (MDOTS/PDOTS) using established murine models as well as clinically relevant patient specimens.

Speaker Bios and Abstracts

Session 1: Informatics and Data Analysis

Chair: Artem Sokolov

Anupam Jena, Harvard Medical School

Anupam Jena is the Ruth L Newhouse Associate Professor is Health Care Policy and Medicine at Harvard Medical School and a physician at Massachusetts General Hospital.

Natural Experiments in Health Care

Clarence Yapp, Harvard Medical School

Clarence obtained his MSc and DPhil from the University of Oxford, England in the labs of Professor Udo Oppermann and Professor Alison Noble. He studied Biomedical Engineering and prior to that, Mechatronics Engineering. Now, Clarence is the Platform Manager for Microscopy and Image Analysis at the Laboratory of Systems Pharmacology (LSP) under Professor Peter Sorger, as well as a research associate at the Image and Data Analysis Core (IDAC) under Dr. Hunter Elliott, and regularly works with the Nikon Imaging Center (NIC) under Dr. Jennifer Waters at Harvard Medical School (HMS). At the LSP and IDAC, Clarence aims to apply his interests in new microscopy techniques and unsupervised machine learning image analysis algorithms to solve problems.

Image Processing and Segmentation for CyCIF Data

In this talk, we will take a look at methods used to process and segment individual cells in plate-based multidimensional data (such as that found in CyCIF data), and preparing the data for future visualization and clustering. No prior imaging experience required!

Denis Schapiro, Harvard Medical School/Broad Institute

Denis Schapiro studied Technical Biology at the University of Stuttgart (Germany) with two lab rotations (in Cell and Systems Biology) at the Harvard Medical School and an internship at Bayer AG (Leverkusen, Germany) in PBPK modeling. Afterward, he joined the laboratory of Bernd Bodenmiller at the University of Zurich (Switzerland). Currently, Denis Schapiro is an Independent Fellow at the Harvard Medical School and the Broad Institute.

histoCAT: Analysis of Cell Phenotypes and Interactions in Multiplex Image Cytometry Data

We have developed a computational histology topography cytometry analysis toolbox (histoCAT) to enable the interactive, quantitative, and comprehensive exploration of phenotypes of individual cells, cell-to-cell interactions, microenvironment, and morphological structures within intact tissues.

Speaker Bios and Abstracts

Session 2: Regulatory Science: Challenges and Opportunities in the 21st Century

Chair: Ariel Stern

Ariel Dora Stern is an Assistant Professor of Business Administration and Hellman Faculty Fellow at Harvard Business School. Her research focuses on the management of innovation in health care, with a focus on the medical device and pharmaceutical industries. Ariel is particularly interested in the intersection of the regulation, firm strategy, and economics of health care. Her research has been cited by Bloomberg, The New York Times, and National Public Radio. Ariel received her Ph.D. from Harvard, where she was a National Bureau of Economic Research Predoctoral Fellow in the Economics of Health and Aging, and an undergraduate degree from Dartmouth College, where she was a Presidential Scholar.

Sebastian Schneeweiss, Brigham and Women's Hospital/Harvard Medical School

Sebastian Schneeweiss, MD, ScD, is a Professor of Medicine and Epidemiology at Harvard Medical School and Chief of the Division of Pharmacoepidemiology, Department of Medicine, Brigham and Women's Hospital.

His research focuses on the comparative effectiveness and safety of biopharmaceuticals. He has developed analytic methods to improve the scientific validity of epidemiologic analyses using complex longitudinal healthcare databases for newly marketed medical products. The overarching theme of his research is applying advanced real-world data analytics for regulatory decision making transparently and in rapid cycles. His work is published in >400 articles. His work is funded by NIH, PCORI, Arnold Foundation, IMI, and FDA where he is also a voting consultant.

Dr. Schneeweiss is Director of the Harvard-Brigham Drug Safety Research Center funded by FDA/CDER and Methods Lead of the FDA Sentinel program. He is Past President of the International Society for Pharmacoepidemiology and is Fellow of the American College of Epidemiology, the American College of Clinical Pharmacology, and the International Society for Pharmacoepidemiology.

Real World Evidence and Regulatory Decision Making

By 2021 the US Food and Drug Administration is to develop a guidance on when and how FDA may rely on real-world evidence to regulate drugs, including drug approvals.

I provide examples of the various opportunities for pharmacoepidemiologic analyses of real-world data (RWD) to inform on the causal relationship between drugs and outcomes. Key

questions when considering whether RWD analyses can augment RCTs for regulatory decision making are: When can we study drug effects without randomization and how should we implement such RWD analyses? The when is primarily driven by externalities not controlled by investigators, whereas the how is focused on avoiding known mistakes in RWD analyses.

I describe a regulatory process that includes analytic platforms that will enable transparency of the study implementation process, sharing of analyses without sharing individual-level data, and remote sensitivity analyses by regulators.

Tony Letai, Dana-Farber Cancer Institute

Dr. Letai is an independent investigator at Harvard Medical School and Dana-Farber Cancer Institute where he is Professor of Medicine. His laboratory studies how apoptosis can be activated, particularly in cancer cells. Key to these studies is a novel assay - BH3 profiling. BH3 profiling can detect what blocks cancer cells use to evade apoptosis and profiling detect cells that are dependent on BCL-2. Using BH3 profiling to identify cancers vulnerable to such drugs, he has led efforts to translate BCL-2, BCL-XL, and MCL-1 inhibitors into the clinic. These include venetoclax, a BCL-2 inhibitor made by AbbVie approved by the FDA for CLL and AML and now being tested across nearly all blood cancers. The laboratory will be testing whether BH3 profiling can be used as a predictive biomarker in clinical cancer therapy.

I Don't Know What I Don't Know About Regulatory Barriers for Laboratory Derived Tests

The work of over a decade in my laboratory by has produced a predictive biomarker platform for oncology based on BH3 profiling. This platform works well at predicting what drugs will work for individual cancer patients and so has obvious utility. At this point, the major barriers to clinical testing and clinical application are regulatory, not scientific. As a laboratory scientist, I have been confronted by a Kafka-esque landscape of overlapping regulatory bodies and processes that apparently stand between us and clinical use. I am not sure what questions I should be asking, whom to ask, or what marks success. I know that there are IRBs, CLIA/CAP, the FDA, HMS, DFCI, COI, pathology departments, patient tissue custody, HIPAA issues all to be considered. Believe me, this talk is not going to present any solutions, just a description of where we are, what sense I have made out of the system, and some vague suggestions about how we could improve both locally and nationally at getting innovative diagnostic ideas to the clinic. My overall sense is that while much of this regulation may be well-meaning, it would be hard to discern the benefit patients obtain from it. Also, that if we locally want to facilitate innovation from the lab to the clinic in the diagnostic space, we need someone whose job it is to pilot through the regulatory environment.

Speaker Bios and Abstracts

Session 3: Harvard Business School: Value and Incentives in Precision Medicine Development (Panel Discussion)

Moderator: Josh Krieger, Harvard Business School

Josh Krieger is an assistant professor of business administration in the Entrepreneurial Management Unit, teaching The Entrepreneurial Manager to first-year MBA students.

Josh's research focuses on R&D strategy and the economics of innovation. His work examines project selection decisions, R&D competition, and how firms can benefit by learning from their competitors' failures. His current work explores how firms and research organizations adjust their R&D efforts in response to new information and resources.

Josh has a BA in economics and government from Cornell University. He received his PhD at the MIT Sloan School of Management.

Richard Hamermesh, Harvard Business School

Richard Hamermesh is a Senior Fellow at the Harvard Business School, where he was formerly the MBA Class of 1961 Professor of Management Practice. Currently, Richard is the Faculty Co-Chair of the Kraft Precision Medicine Accelerator. Richard created and teaches the second-year MBA elective, Building Life Science Businesses. Previously, he was the course head for the required first-year course, The Entrepreneurial Manager.

From 1987 to 2001, Richard was a Co-Founder and a Managing Partner of the Center for Executive Development, an executive education and development consulting firm. Prior to this, from 1976 to 1987, he was a member of the faculty of the Harvard Business School.

Richard is also an active investor and entrepreneur, having participated as a principal, director and investor in the founding and early stages of more than 20 organizations. He was the founding president of the Newton Schools Foundation and served on the editorial board of the Harvard Business Review.

Richard is the author or co-author of five books, including "New Business Ventures" and "The Entrepreneur". His best-known book, "Fad-Free Management", was published in 1996. He has published more than 100 case studies and numerous articles, including his recent publications "What Precision Medicine Can Learn from the NFL" and "One Obstacle to Curing Cancer: Patient Data Isn't Shared."

Rodrigo Martinez, VERITAS

Rodrigo lives at the crossroads of Biology + Technology + Design. As Chief Marketing & Design Officer at Veritas Genetics, he creates experiences to engage people in the genomic revolution. For the last 20 years, Rodrigo has lead some of the most exciting projects in the life sciences revolution. Before joining Veritas, Rodrigo was Life Sciences Chief Strategist at IDEO, where he led & worked on 20+ projects from creating health services & products to tech for the year 2079.

His work has won awards including Fast Company Most Innovative Cos 2018, MIT Tech Review's 50 Smartest Cos, MDEA Gold Award, Core 77 Design Strategy, Webby; appeared in publications including LA Times, WIRED, HBR Brasil, EDGE-Serpentine Gallery, and several books. Rodrigo has been a keynote speaker & presenter at: TED Unplugged, DENT, World's Top 50 Innovators, Summit at Sea, ARC Fusion, TEDx events, among other.

Before IDEO he was a principal with The Boston Consulting Group. With Juan Enriquez, Rodrigo co-founded Harvard Business School's Life Sciences Project and coined the term 'bioeconomy' in a series of papers and articles starting in 1997.

Rodrigo is a regular guest lecturer at Harvard and MIT. He was a Bailleres Scholar at ITAM in Mexico City and a World Bank Scholar at Harvard. He lives with his wife and daughter in Massachusetts. He is a freediver and loves spicy food.

Ellie McGuire, Polaris Partners

Ellie is a partner of the LS Polaris Innovation Fund and works out of the Boston office. She has held leadership and consulting positions in business development, operations, and strategy in multiple life science companies including Arsia Therapeutics, SQZ Biotech, XTuit Pharmaceuticals, and Lyndra.

Prior to co-foundingLS Polaris Innovation Fund, McGuire was the head of business development at Lyndra, where she was also a member of the founding team. She was recently awarded the Up and Coming Woman of the Future Award by WEST. McGuire studied neuropsychology prior to attending Tuck School of Management Business Bridge Program. McGuire graduated Colby College magna cum laude and Phi Beta Kappa.

Speaker Bios and Abstracts

Session 4: Detection Methods for Biological Systems

Chair: Alyce Chen

Michael Baym, Harvard Medical School

Assistant Professor of Biomedical Informatics, LSP core member.

Antibiotic Resistance Evolution

Mike Springer, Harvard Medical School

Mike Springer is an associate professor of Systems Biology at Harvard Medical School. Mike was a double major in Chemistry and Biology at Stanford. He did his graduate work at UCSF with Erin O'Shea, pioneering single-cell quantitative experimental approaches together with mathematic modeling to uncover signaling principles in the phosphate starvation response in yeast. He did his post-doctoral work with Marc Kirschner at Harvard Medical School exploring cellular feedback in response to changes in protein level along with methods development. He started his own independent lab in the Systems Biology department at HMS in 2009.

Microbial Movement and Biosecurity

Jeffrey Moffitt, Boston Children's Hospital

Jeffrey Moffitt received his PhD from the University of California at Berkeley where he studied molecular motors using optical tweezers with Dr. Carlos Bustamante. He then received postdoctoral training from Dr. Philippe Cluzel, and then Dr. Xiaowei Zhuang. In Dr. Zhuang's laboratory, he developed methods to study the expression level and distribution of the transcriptome via direct single molecule imaging within single cells.

Mapping the Brain With In Situ Single-cell Transcriptomics

Image-based approaches to single-cell transcriptomics, in which the identity of individual RNAs are determined in situ via imaging, are emerging as powerful and exciting complements to single-cell techniques based on cell dissociation and RNA sequencing, in part, because these techniques naturally preserve the native spatial context of both RNAs within cells and cells within tissues. Here I describe the development of multiplexed error robust single-molecule fluorescence in situ hybridization (MERFISH)—a technique capable of identifying and quantifying hundreds to a thousand different RNAs simultaneously in single fixed cells. This

approach achieves this level of multiplexing by using combinatorial RNA labeling to assign unique barcodes to individual RNAs and sequential rounds of single-molecule FISH (smFISH) imaging to readout these barcodes. Remarkably, this degree of multiplexing comes with little cost in performance: MERFISH maintains the high dynamic range and sensitivity typical of single-molecule FISH, the gold-standard for RNA quantification in single-cells. Recent improvements to this protocol now allow tens of thousands of human cells to be characterized in a single-day-long measurement, and with the use of a clearing approach designed for smFISH, MERFISH can be used to quantify RNA expression levels in slices of adult mouse brain.

Using this approach in combination with single-cell RNA sequencing, we have identified a striking diversity of transcriptionally distinct neuronal types in a portion of the mouse hypothalamus, mapped out their intricate spatial organization, and associated specific neuronal types with the hypothalamic control of instinctive social behaviors such as aggression, parenting, and mating. By measuring the transcriptional profile of millions of single cells within in tact tissue slices, MERFISH promises the exciting ability to create molecularly defined, spatially resolved, atlases of a wide range of tissues.

Speaker Bios and Abstracts

Session 5: Student Nano Talks

Chair: Catherine Dubreuil

Carmen Sivakumaren, Harvard Medical School

Carmen Sivakumaren is a 6th-year graduate student in the Biological and Biomedical Sciences (BBS) program at Harvard University. Hailing from Malaysia, she majored in Chemistry and Psychology at the Johns Hopkins University where she developed a keen interest for the chemistry-biology interface and pharmacology of cancer therapeutics. In the Gray Lab, she is currently delving into lipid kinase signaling inhibition, focusing on the PI5P4K family and PIKfyve in cancer and Ebola. Carmen is in the Therapeutics graduate certificate program and is interested in the science-business intersection of drug discovery.

Targeting the PI5P4K Lipid Kinase Family in Cancer Using Novel Covalent Inhibitors

The phosphatidylinositol 5-phosphate 4-kinases, PI5P4Kα, β and γ been shown to be crucial in driving a number of cancers as well as involved in other disease pathologies such as proliferative vitreoretinopathy, diabetes, and neurodegenerative disorders. However, the therapeutic potential of targeting these lipid kinases is understudied due to a lack of potent and specific small molecules available. Here we present the discovery and characterization of a novel pan-PI5P4K inhibitor, THZ-P1-2, that covalently targets cysteine residues on a disordered loop region in PI5P4K// proximal to the ATP-binding site. Cancer cell line profiling demonstrates AML and ALL cell lines to be sensitive to THZ-P1-2 covalent targeting, consistent with PI5P4K's reported role in leukemogenesis. THZ-P1-2 causes lysosomal disruption, and defects in the clearance of autophagosomes, halting autophagy in a manner dependent on cysteine-targeting and phenocopying the effects of genetic deletion of PIP4K2A/B. THZ-P1-2 treatment also causes an increase in TFEB nuclear localization, upregulation of TFEB target genes and subsequent cancer cell growth inhibition. These studies provide evidence that covalent inhibition of PI5P4K by THZ-P1-2 compromises autophagy, an essential alternative energy source during periods of metabolic stress which cancer cells depend on to maintain cellular homeostasis and prolonged cell viability, further suggesting PI5P4K as a novel therapeutic target in cancer metabolism. Taken together, our studies demonstrate that the PI5P4K enzymes are tractable targets, with THZ-P1-2 serving as a useful tool to further interrogate the therapeutic potential of PI5P4K inhibition and inform drug discovery campaigns for these lipid kinases in the context of cancer and potentially other autophagy-dependent diseases.

Elaine Garcia, Harvard Medical School

Elaine is pursuing her doctorate degree at Harvard Medical School, with a focus on cancer therapeutics. Specifically she studies the cellular and molecular roles of enzymes in aggressive and treatment-resistant T-cell Acute Lymphoblastic Leukemia. Elaine has a passion for science communication, policy, and outreach. Since joining Harvard, she has participated in and organized many groups including the Minority Biomedical Scientists of Harvard (MBSH), Science in the News (SITN), and Harvard Graduate Student Leadership Institute (HGSLI).

Upon graduation, Elaine hopes to pursue a career in portfolio management or regulatory science to understand and influence novel and innovative biological and pharmaceutical treatments in America. During Elaine's spare time, she leads and coaches the Harvard Dragon Boat Team, enjoys organizing wine and hors d'oeuvres events, bakes intricate pastries, and avidly reads science-fiction novels.

PRL3: A Novel Oncogenic Phosphatase in T-cell Acute Lymphoblastic Leukemia

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive and unpredictable malignancy of thymocytes and a major clinical challenge with <30% of children and <10% of adults able to survive relapsed disease. The current first-line cytotoxic treatments are inadequate for relapsed disease treatment. Therefore, new therapeutics that specifically target relapse pathways in malignant T-cells are needed. In normal hematopoietic progenitor cells, phosphatase of regenerating liver (PRL3) is not expressed, while in T-ALL, PRL3 is co-amplified with MYC in 12% of patients by partial or complete trisomy 8 events. Interestingly, PRL3 is highly expressed in metastatic lesions of colon and breast tumors, while non-metastatic and primary tumors do not over-express PRL3, suggesting that PRL3 has a role in tumor progression and maintenance. Analysis of RNA gene expression from primary patient T-ALL samples identified that PRL3 was expressed in 32% of samples and was most highly expressed in the common TLX subtype and ETP leukemia, an aggressive form of ALL. In a zebrafish model of T-ALL, PRL3 collaborates with MYC to increase T-ALL onset and aggression. In human cultures of T-ALL, genetic and enzymatic inhibition of PRL3 activity kills human T-ALL. Through time-course tandem mass spectrometry phosphoproteomics and genetic studies on putative PRL3-interacting proteins, PRL3 seems to contribute to tumor progression in aggressive and hard-to-treat T-ALLs by manipulating T-cell signaling pathways and apoptotic pathways. By studying the mechanisms by which PRL3 promotes tumor survival and evades death, a novel therapeutic approach to block relapse in T-ALL may be generated.

Sanchez Jarrett, Harvard Medical School

Molecular Basis for Feedback Inhibition of Notch Signaling by the Notch Regulated Ankyrin Repeat Protein, NRARP

The Notch regulated ankyrin repeat protein (NRARP) acts as a negative feedback regulator of Notch signaling in higher organisms, but the molecular basis for NRARP function has remained elusive. Using an unbiased proteomics approach, we show that NRARP interacts with the core Notch transcription activation complex (NTC), containing the RBPJ transcription factor, the Notch intracellular domain (NICD), and Mastermind-like co-activators. Binding experiments using recombinant molecules establish that binding of NRARP into the complex requires both RBPJ and NICD, and that NRARP binds to Notch-RBPJ complexes independently of Mastermind-like proteins or DNA. The X-ray structure of an NRARP/RBPJ/Notch1/DNA complex, determined to 3.75 Å resolution, reveals a non-canonical binding mode for engagement of one ankyrin protein, NRARP, with the ankyrin domain of Notch1. Mutations disrupting this interface result in loss of feedback inhibition in cell-based Notch signaling assays. Collectively these studies establish the structural basis for NTC engagement by NRARP and provides insights into the feedback inhibition mechanism of NRARP as a modulator of Notch signaling.

Zebulon Levine, Harvard Medical School

Zeb is a G6 in the chemical biology program in Suzanne Walker's lab.

Which Catalytic Activity of O-GlcNAc Transferase is Essential?

O-GlcNAc Transferase (OGT) is an essential enzyme that is solely responsible for the stress-protective and nutrient sensitive post translational modification O-GlcNAc on thousands of proteins. It also matures the essential transcriptional co-regulator host cell factor 1 (HCF-1) by catalyzing its proteolysis. We have developed a strategy to replace OGT with separation of function mutants, allowing us to determine which activity, proteolysis or glycosylation is required for cell survival.

Attendee List

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Poster Abstracts

1. DNA Origami Nanocask for Controllable Drug Delivery

Frances Anastassacos

DNA origami allows for nanometer precision when positioning and designing structures at the nanoscale. The Shih lab is interested in designing drug delivery vehicles for therapeutic applications. Here, we are designing and constructing MDa scale a smart DNA origami nanocarrier from 7 individual components that come together through self-assembly.

2. Drug-Induced Receptor Tyrosine Kinase Overexpression Drives Resistance to Vemurafenib in Melanoma

Mariya Atanasova

Approximately 50-60% of melanoma patients carrying a BRAFV600E mutation show initial response to the BRAF kinase inhibitors vemurafenib or dabrafenib. However, the response is fractional and resistance invariably develops, making long-term progression-free survival the outstanding clinical challenge of targeted therapies in melanoma. We have shown that over the course of four weeks of vemurafenib treatment, cell lines exhibit pronounced changes in the abundance of several receptor tyrosine kinases (RTKs), such as EGFR, ERBB2, MET, and AXL. Inhibition of these upregulated receptors results in significantly increased cytotoxicity, suggesting that signaling through these RTKs provides survival advantage to cells in the presence of vemurafenib. Interestingly, the magnitude of the effect of RTK inhibitors varies depending on the time of addition after treatment with vemurafenib. Our results point to a selective temporal sensitivity to RTK inhibitors, which develops as a result of vemurafenib treatment and may prove to be clinically relevant.

3. A Computational Tool for Discovery-based Immunophenotyping Identifies a Novel Cytotoxic T cell State in Glioblastoma

Greg Baker

Glioblastoma (GBM) is a rapidly progressing and invariably lethal brain tumor with few treatment options. Although the tumor is known to profoundly dampen effector function of tumor-infiltrating immune cells, relatively little is understood about its impact on the number, function, and network-level architecture of cells constituting the peripheral immune system. We developed a set of complementary experimental and computational workflows to screen for tumor-induced changes in the frequency and intercellular correlation of 2,048 distinct cell states across 5 lymphoid organs of C57BL/6 mice bearing syngeneic GL261 GBM. The innovation, referred to as Systemic Lymphoid Architecture Response Assessment (SYLARAS), is of general applicability to the field of immunology, as it allows for the supervised exploration and tidy visual display of the time and tissue-dependent changes occurring in systemic cellular immune composition in response to experimental perturbation. Among the many insights gained through our analysis, our study reveals that expression of the CD45R/B220 isoform of the Ptprc gene product CD45 defines a subset of CD8+ T cells that respond differentially to GBM compared to other CD8+ T cells. CD45R/B220+ CD8+ T cells (simply referred to a B220 T cells) are absent from the thymus and exhibit brighter CD8α signal intensity relative to conventional cytotoxic T cells. Cyclic Immunofluorescence (CyCIF) performed on GBM- bearing brain tissue sections reveals the presence of B220 T cells within the GBM tumor microenvironment that are morphologically and topographically-distinct from other tumor-infiltrating CD8+ T cells. Next-generation RNA-sequencing also shows that B220 T cells are transcriptionally-distinct from their B220- counterparts. The work of others has described an extant population of CD45R+ CD8+ T cells in humans that function to suppress immune activation in response to autologous antigens (PMIDs: 29805553, 2567246, 17163450). Further experimental data demonstrating that mouse B220 T cells are functionally orthologous to human CD45R+ CD8+ T cells would have important implications for brain cancer immunotherapeutic strategies that enhance immune responses to tumor-specific (TS) and tumor- associated (TA) autoantigens through the selective ablation of B220 T cells.

4. Proteomics Platform in the Laboratory of Systems Pharmacology

Matt Berberich

The Proteomics Platform of the Laboratory of Systems Pharmacology (LSP) is part of an inter-institutional effort to improve the science underlying drug development, drug evaluation in clinical trials and use of new medicines in patients. Our role is to provide collaborative research projects with custom tailored analysis pipelines of highly advanced mass spectrometry (MS) based proteomics. This begins with scientific advice on experimental design and continues with sample preparation of MS samples by our platform including support with processing and analysis of collected data.

The cornerstone of the Platform is a state of the art Orbitrap Lumos Tribrid MS instrument which provides superior resolution and quantification accuracy. By combination with isobaric labeling of up to 11 multiplexed experimental conditions with Tandem Mass Tag (TMT) reagents we achieve highly precise relative quantification of thousands of proteins in parallel. Fractionation by alkaline reversed-phase chromatography allows deep analysis of very complex peptide mixtures such as whole proteome digests. Addition of internal standards can allow for absolute quantification.

Studying the effects of kinase inhibitors or other biological perturbations of cells requires precise quantification of changes in the phospho-proteome. Our platform has extensive expertise in quantifying phospho-peptides by isobaric tagging (phospho multiplex TMT). We analyze even subtle changes of this important post-translational modification by applying phospho-peptide enrichment techniques such as iron metal affinity chromatography (IMAC) and antibody-based enrichment of phosphorylated tyrosines (pY). Profiling of kinase inhibitors can be assessed through Kinobeads technology. In addition to these systems-wide approaches, our Platform applies targeted proteomics using synthesized trigger-peptides or -phospho-peptides. Triggered by Offset, Multiplexed, Accurate mass, High-resolution, Absolute Quantification (TOMAHAQ) allows for monitoring changes of peptide-targeted proteins or phosphorylation sites.

The Proteomics Platform of LSP has successfully applied these technologies to cell culture, tissue and clinical samples. Statistical analysis (volcano plots, data clustering, principal component analysis [PCA]) and database searches (Enrichr, Kinase Set Enrichment Analysis) facilitate biological interpretation of extensive multiplex datasets.

5. Hybridization-enhanced Identification and Enrichment of Engineered Sequences

Sarah Boswell

Synthetic biology has advanced to the point that researchers can engineer organisms to produce useful compounds and monitor and record signals in the world around them.

While the vast majority of synthetic biology activities are well-intentioned, engineered organisms could be used by our adversaries to create pandemics, disrupt food supplies and generally inflict economic damage. Consequently, the Intelligence Community (IC) has a growing need for novel tools for rapid identification and attribution of engineered organisms in complex environmental samples.

In collaboration with teams from Draper Labs and Boston University, we are part of an effort known as Hybridization-enhanced Identification and Enrichment of Engineered Sequences (HIdE-En-Seq), with the aim to identify whether an organism has been engineered. The HIdE-En-Seq team's approach will use microorganisms as a model system with a focus on developing tools for microbial forensics; however, the proposed tools are organism agnostic and are broadly extensible to higher-order organisms.

The approach the Springer Lab is working is is targeted engineering marker sequencing (TEM-Seq), where we will identify whether an organism, even unknown, has been engineered using a comprehensive set of known markers of engineering, e.g. synthetic promoters, plasmid elements, and toxins. The innovative workflow we propose uses DNA probes to selectively capture and enrich both the engineered sequences and the surrounding DNA of the host organism. Critically, sequencing the junctions of inserted DNA will discriminate whether the marker of engineering is from an environmental sample or an engineered organism.

6. Sequencing Technology Platform

Sarah Boswell

The LSP Platforms are not cores in the conventional sense, but are specialized resources for the development and implementation of approaches relevant to the overall mission of the LSP.

The Sequencing Technology Platform has worked with LSP researchers in a variety of capacities such as:

- Selecting the best sequencing method for the experiment
- Optimization of sample extraction
- Testing/teaching new extraction methods
- Perform/teach high-throughput library preparation
- Walk through the sequencing process
- Assist in alignment/early stage data processing
- Research & adopt new sequencing methods

7. Lineage Calling Can Identify Antibiotic Resistant Clones Within Minutes

Karel Břinda

Surveillance of circulating drug resistant bacteria is essential for healthcare providers to deliver effective empiric antibiotic therapy. However, the results of surveillance may not be available on a timescale that is optimal for guiding patient treatment. Here we present a method for inferring characteristics of an unknown bacterial sample by identifying the presence of sequence variation across the genome that is linked to a phenotype of interest, in this case drug resistance. We demonstrate an implementation of this principle using sequence k-mer content, matched to a database of known genomes. We show this technique can be applied to data from an Oxford Nanopore device in real time and is capable of identifying the presence of a known resistant strain in 5 minutes, even from a complex metagenomic sample. This flexible approach has wide application to pathogen surveillance and may be used to greatly accelerate diagnoses of resistant infections.

8. Kinase Inhibition Shows Minimal Effects on Microtubule Dynamics in Retinal Pigmented Epithelial (RPE1) Cells at Baseline

Sergine Brutus

Dynamic instability is a key intrinsic property of microtubules that facilitates many integral microtubule-dependent functions such as intracellular trafficking, migration, and mitosis. This process is regulated by a myriad of microtubule-binding proteins that determine the parameters of dynamic instability - growth and shrinkage rates, as well as rescue and catastrophe events. One notable family of microtubule-binding proteins are the end-binding proteins (EBs) that track along growing microtubule ends, which includes end-binding protein 3 (EB3). The ability of microtubule-binding proteins to bind, and thus regulate, microtubule dynamics is heavily dependent on phosphorylation. However, the kinases involved in the regulation of microtubule dynamics have not been extensively explored. To address this knowledge gap, we set out to use an unbiased method to systematically identify kinase regulators of microtubule dynamics by screening a library of 40 target-profiled kinase inhibitors. This library was designed to cover ~250 kinases via polypharmacology with a small number of compounds and consists of preclinical, clinical, and approved compounds. High throughput EB3-GFP comet counting was used to score for response of microtubule dynamics to kinase inhibition. Human retinal pigmented epithelial (RPE1) cells expressing an EB3-GFP reporter are seeded on 384-well plates. Comets were imaged prior to and following a four-hour drug treatment (3nM - 10uM). Automated image processing and analysis were conducted in Python to generate a comet count that is background subtracted and normalized for cell area with respect to t=0, with fold-change calculated with respect to the DMSO control. Given the known importance of phospho-regulation in microtubule dynamics, we expected majority of the compounds to affect EB3 comet count. Surprisingly, we found minimal effects on EB3 comet count due to kinase inhibition, as our screen produced no true hits. Half of the compounds had negligible effects on EB3 comet count. The remaining compounds demonstrated intermediate to strong effects at high doses, indicating those effects are not target specific. These compounds have been advanced to a secondary screen to determine if they demonstrate direct activity on microtubules by affecting in vitro microtubule polymerization, which could explain the high-dose effects. In conclusion, our results suggest that phospho-regulation is not integral in maintaining the number of growing microtubules in RPE1 cells at baseline.

9. Proteomics-based Substrate ID in the Ubiquitin Proteasome System

Jonathan Bushman

We are developing robust and scalable methods for interrogating substrates of E3 ubiquitin ligases and DUBs across the proteome. With an increasing number of selective chemical probes targeting components of the ubiquitin proteasome system, shotgun proteomics serves as a valuable tool to discover substrates and explore the poly-pharmacology of these novel molecules. We are leveraging selective probes for E3s and DUBs alongside orthogonal UPS perturbations to discover enzyme-substrate pairs in their native cellular context.

10. Under the Hood: Improved Processing of Multiplexed Tissue Images to Investigate Tumor Architecture

Yu-An Chen

Multiplexed optical imaging of fixed tissue sections provides deep, single cell phenotypes at tissue-scale to guide discovery, diagnosis and therapeutic decisions. However, the physical limitations of optical microscopy and the natural complexity of tissues impact the types and quality of the resulting data sets. Here, we describe a suite of image pre-processing and image segmentation solutions to be incorporated in our current image workflow to improve cell phenotyping and quantitative whole slide imaging analysis from data sets obtained by cyclic immunofluorescence (CyCIF) imaging of FFPE tissues sections. These include correcting illumination to improve quantitative phenotyping, stitching and templated image registration of large tissue section images, sample extraction from tissue microarrays and benchmarking cell segmentation methods in biopsies and large data sets. With continuing improvements in image processing and visualization at LSP, we aim to deliver on the potential of CyCIF imaging for discovery, biomedical education, and clinical impact.

11. Investigating the Therapeutic Potential of Targeting ACVR1 in Diffuse Intrinsic Pontine Glioma

Amanda R Clark

Diffuse intrinsic pontine glioma (DIPG) is the most common pediatric brainstem tumor and represents a serious unmet clinical need. DIPG has been sub-classified based on transcriptomic differences into three molecular subtypes: histone 3 lysine-27-methionine (H3-K27M), silent, and MYCN. Additionally, mutations in the activin receptor gene, ACVR1, were identified in approximately 20% of DIPG tumors. Due to its sensitive location in the brainstem, surgical resection is not a therapeutic option. Rather, standard of care treatment for newly diagnosed DIPG is focal radiation therapy and corticosteroids for the control of peritumoral edema. Recently, a first-in-human use of TP-0184, a nanomolar inhibitor of mutant ACVR1, in a single DIPG patient was performed at Boston Children's Hospital/Dana-Farber Cancer Institute and elicited several questions regarding its therapeutic relevance for the treatment of DIPG. To determine if drug resistance mechanisms arise when targeting ACVR1 in DIPG by TP-0184, we studied the effect of drug treatment on the cell signaling network of primary DIPG neurosphere cell lines by phosphoproteomics. Our results suggested phosphorylation of platelet-derived growth factor receptor alpha (PDGFR-a) increased in two of the three DIPG cell lines in response to drug exposure, while the third cell line had increased basal levels of phosphorylated PDGFR-a relative to the other two cell lines. This prompted us to study the effects of dual inhibition against ACVR1 and PDGFR-a, using TP-0184 and crenolanib, respectively, on the transcriptome of primary DIPG neurosphere cell lines. Gene enrichment analysis comparing combination treatment against single treatment (PDGFR-a) suggested that inhibition of PDGFR-a in ACVR1 R206H (BT869) cells affected cell cycle regulation, but ACVR1 wild-type (DIPG-XIII) and ACVR1 G328V (SU-DIPG-IV) both showed different responses. More work is underway to better understand the potential clinical value of targeting ACVR1 and/ or PDGFR-a in DIPG.

12. A Novel Antiparasitic Compound with Unique Mechanism of Action Targets Multiple Pathways in Plasmodium Falciparum and is Prone to Non-canonical Drug Resistance

Rebecca Clements

Malaria is a deadly disease caused by Plasmodium parasites. Rapidly emerging resistance to first-line antimalarials imposes an urgent need for novel therapeutics. Our group developed a nano-luciferase reporter in Plasmodium falciparum that allows us to monitor parasite viability via luminescence intensity. Using this reporter system, we identified a compound, BCH070, that inhibits growth of P. falciparum asexual stage parasites and Trypanosoma cruzi amastigotes with an EC50 of ~100-200 nM. BCH070 also inhibited asexual growth of a panel of antimalarial-resistant P. falciparum strains, suggesting that BCH070 may act via a novel mechanism of action. BCH070 preferentially kills ring-stage P. falciparum. To identify the target(s) of BCH070, we selected for BCH070-resistant parasites after chemical mutagenesis with N-ethyl-N-nitrosourea (ENU). These strains demonstrate a 3-10-fold increase in EC50 compared to the parental strain. We performed whole genome sequencing on clonal parasites to identify putative targets of BCH070, but the sequencing results suggest non-canonical drug resistance mechanisms. Metabolomic analysis of BCH070 demonstrates that this compound likely targets multiple pathways in the parasite, including the mitochondrial electron transport chain and hemoglobin catabolism pathways. The long-term goal of this work is to identify and characterize a novel class of antimalarials and to demonstrate the potential of chemical mutagenesis to aid in target identification in Plasmodium falciparum parasites.

13. A Combinatorial-logic Underlies Multivalent Motif-mediated Interactions.

Joe Cunningham

The interaction of motifs with peptide-binding domains (PBD) are a critical set of interactions thought to underlie a significant fraction of protein-protein interactions. The apparent modularity of PBDs has given rise to an interpretation of PBDs as core cellular components tying together partitioned components of the proteome. Here, we interrogate the composition and resulting function of PBDs and motifs as distributed in the human proteome. Our results identify a set of multivalent, functional modules, repeated across multiple proteins, that define distinct logical units. Taken together, these modules imply a combinatorial logic defined over the human proteome comprised of PBDs and motifs. Extension of these mechanisms in dysregulated contexts provides insight into the different architecture associated with disease pathogenesis.

14. Identification of New Metabolic Vulnerabilities of Triple Negative Breast Cancer by High Throughput BH3 Profiling

Veerle Daniels

Reprogrammed cancer energy metabolism might be the ideal target for chemotherapy, as cancer cells are often more dependent on a certain metabolic pathway and therefore more sensitive to inhibition of that specific pathway than normal cells. Despite great efforts in this research area, few metabolic compounds have made it to clinical trials so far. One possible reason why researchers might fail to identify metabolic compounds to use as single agents or in combination therapies to target cancer, might be because they are only considering cancer cells as either alive or dead and they are not taking into account a third cellular state i.e. “primed for death”.

In this study, we set out to identify previously unrecognized metabolic vulnerabilities of triple negative breast cancer (TNBC), looking for metabolic perturbations that shift mitochondrial apoptotic priming rather than inducing cell death as a single agent. To do so, we developed a High Throughput Dynamic BH3 Profiling (HT-DBP) technique and used it to screen 192 chemical compounds with known metabolic targets on the SUM149 TNBC cell line. We identified 33 metabolic compounds that increase mitochondrial apoptotic priming without inducing cell death. Twenty six of these compounds were validated in three additional TNBC cell lines and are being counter screened in MCF10A and HMEC.

Together, these data show that cellular metabolism is closely intertwined with mitochondrial apoptotic signaling and HT-DBP as an excellent screening tool for the identification of valuable metabolic perturbations that enhance apoptotic priming even if these perturbations alone do not rapidly cause complete cell death as a single agent.

Acknowledgements:

This work is supported by the Ludwig center at Harvard Award (03/01/15-09/30/19). I thank the Laboratory of System Pharmacology and ICCB Longwood Screening Facility for the use of the high throughput screening equipment.

15. The Use of In Vivo Models in the LSP

Stephanie Davis

It is common practice in translational research to use in vivo models to validate promising results found using in vitro systems. This poster will outline ongoing animal work in the Laboratory of Systems Pharmacology (LSP). Syngeneic models, standard xenografts established from conventional cell lines, as well as patient derived xenograft (PDX) models are currently in use for breast cancer and melanoma research in LSP. Standard xenografts established in nude mice are grown to ~200 mm3 and then used for acute dose response studies or long term tumor response experiments. PDX models are established in NSG mice, propagated from mouse to mouse, and used for similar studies. Syngeneic models are established in C57BL/6 mice and are used to explore tumor immunology and tumor biology, particularly to study resistance or sensitivity to immune checkpoint blockade (ICB) as well as metastasis. Upon study completion, tumors are resected and used for a variety of downstream analyses including RNA sequencing, proteomics, and cyclic immunofluorescence. We have introduced fluorescent and/or bioluminescent reporters into cells (PDX tumors can be dissociated ex vivo and cultured) prior to engraftment to facilitate separating tumor cells from stroma, and to enable imaging for more accurate measurement of tumor burden, respectively.

16. Hierarchical Modularization of Rule Base Modeling Enables Construction of Complex Mechanistic Models

Fabian Froehlich

Mechanistic models are mathematical formulations of biological hypothesis, which can be quantitatively tested on experimental data. Accordingly, our increasingly detailed understanding of biological systems naturally leads to larger and more complex models. Often, the increase in understanding is incremental, which could be captured as refinement of an existing model. Yet, models are typically formulated in an integrated fashion, where reuse or refinement of individual parts of a model is difficult. Consequently, most models are reformulated from scratch, which becomes unmanageable with increasing model complexity.

Here, we introduce a computational framework that allows formulation of rule-based models as hierarchical composite of individual modules. The modularized composition of smaller, testable submodels ensures manageability of large models. To this end, we provide a set of visualization routines for temporal dynamics, input-output relationships and model structure at varying level of detail. Complimentarily, the hierarchical composition enables simple model refinement through coarse- and fine-graining of individual modules.

We apply the framework to a model of adaptive resistance in melanoma where modularization was employed for incremental model formulation and refinement. In the future, we anticipate

that models are created from libraries of modules in a plug and play fashion, which would ensure better reusability of models and substantially simplify model formulation.

17. PRL3: A Novel Oncogenic Phosphatase in T-cell Acute Lymphoblastic Leukemia

Elaine Garcia

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive and unpredictable malignancy of thymocytes and a major clinical challenge with <30% of children and <10% of adults able to survive relapsed disease. The current first-line cytotoxic treatments are inadequate for relapsed disease treatment. Therefore, new therapeutics that specifically target relapse pathways in malignant T-cells are needed. In normal hematopoietic progenitor cells, phosphatase of regenerating liver (PRL3) is not expressed, while in T-ALL, PRL3 is co-amplified with MYC in 12% of patients by partial or complete trisomy 8 events. Interestingly, PRL3 is highly expressed in metastatic lesions of colon and breast tumors, while non-metastatic and primary tumors do not over-express PRL3, suggesting that PRL3 has a role in tumor progression and maintenance. Analysis of RNA gene expression from primary patient T-ALL samples identified that PRL3 was expressed in 32% of samples and was most highly expressed in the common TLX subtype and ETP leukemia, an aggressive form of ALL. In a zebrafish model of T-ALL, PRL3 collaborates with MYC to increase T-ALL onset and aggression. In human cultures of T-ALL, genetic and enzymatic inhibition of PRL3 activity kills human T-ALL. Through time-course tandem mass spectrometry phosphoproteomics and genetic studies on putative PRL3-interacting proteins, PRL3 seems to contribute to tumor progression in aggressive and hard-to-treat T-ALLs by manipulating T-cell signaling pathways and apoptotic pathways. By studying the mechanisms by which PRL3 promotes tumor survival and evades death, a novel therapeutic approach to block relapse in T-ALL may be generated.

18. Baseline Omics and Drug Response Profiling of Breast Cancer Cell Lines and Models

Benjamin Gaudio

Baseline omics and drug response profiling of breast cancer cell lines and models.

19. MicroRNA-132 Mediates Tubular Cell Sensitivity and Increases Kidney Injury in Mice

Cory Gerlach

Background:

MicroRNAs regulate critical signaling pathways that affect the initiation and progression of kidney disease. We previously reported that miR-132 was upregulated in injured kidney tubular epithelial cells (TECs) of mice and humans, yet the role of miR-132 in these cells was unknown.

Methods:

MiR-132 global knockout (miR-132-/-) mice and wild-type littermate controls were subjected to folic acid nephropathy. Tissue-based cyclic immunofluorescence (CyCIF) was conducted on mouse samples, and isobaric tag-based proteomics was performed on primary human TECs transfected with miR-132 mimics.

Results:

MiR-132-/- and WT mice incurred similar acute kidney damage, but miR-132-/- mice had improved recovery, less inflammation, and developed less fibrosis. CyCIF determined that TECs from miR-132-/- mice sustained less injury as opposed to more proliferative repair. Proteomics identified that miR-132 suppressed mediators of cytoresistance, including RASA1 and SOD2, and cell cycle regulators, such as p21. In vitro, miR-132 overexpression caused TECs to bypass critical cell cycle checkpoints. MiR-132 overexpression also resulted in TECs being more sensitive to nephrotoxic injury. In vivo, CyCIF identified that injured TECs from miR-132-/- mice had more p21 expression relative to WT mice. Injured kidneys also had more RASA1 expression in the absence of miR-132.

Conclusions:

These results suggest that miR-132 contributes in the progression of acute-to-chronic kidney disease by suppressing cytoprotective mechanisms such as cell cycle checkpoints. Therefore, inhibition of miR-132 may offer therapeutic benefits for patients with acute kidney injury and limit the extent of kidney fibrosis.

20. Pulsatile ERK Reactivation in BRAFV600E Melanoma Drives Adaptive Resistance to RAF Inhibitors

Luca Gerosa

Cancer cells treated with targeted kinase inhibitors can escape treatment through adaptations of their signaling networks, a phenomenon termed ‘adaptive resistance’. Our limited ability to predict the response of signaling pathways to drug perturbations is a key obstacle to design drug strategies that can prevent adaptive resistance. Here, we use a system-level approach based on experimental and modelling cycles to build a predictive model of drug adaptation in colorectal, thyroid and skin cancers bearing BRAFV600E, a mutation that is present in 10% to 50% of these cancers and is responsible for hyper-activation of the pro-growth RAF/MEK/ERK signaling pathway. By incorporating the biochemistry of the ERK signaling pathway and the mechanisms of action of targeted kinase inhibitors into an Ordinary Differential Equation model, we reproduced the adaptive response of these cancers to targeted inhibitors. Model analysis led us to hypothesize that adaptive resistance in these cancers is governed by lineage-specific receptor dynamics and feedback regulation strengths. We validated the approach by proving that low receptor abundances in BRAFV600E melanoma allow adaptation to RAF inhibitors trough sporadic and brief pulses of ERK reactivation in single cells. To the best of our knowledge, this is the first example of pulsatile reactivation of an oncopathway during targeted therapy, which might provide novel insights into the precise role that single-cell signaling dynamics exert on cancer growth.

21. Antibody Resource Knowledge-base to Enable Deep Phenotyping of FFPE Tissues with Cyclic Immunofluorescence (t-CycIF)

Connor A Jacobson

Multiplexed, single-cell analysis of tissues can provide a deeper understanding of disease processes in situ. Cyclic immunofluorescence (CycIF) was developed as an open-source method for high dimensional imaging of protein and phosphoprotein epitopes in tissues by rounds of staining with fluorescent-antibody conjugates, imaging and chemical fluorochrome inactivation (or antibody elution). This method can be performed using conventional fluorescent microscopes with commercial antibodies and reagents. However, extensive antibody qualification for a given species and antigen recovery protocol is required to realize its potential for plug-and-play assembly of robust biomarker panels for deep phenotyping of tissues sections. To integrate with clinical workflows and access archival specimens, this poster describes our current workflow for qualification of more than 500 antibodies in human tissues and tumor samples, with approximately 100 antibodies also tested in mouse FFPE tissue or PDX models. The resulting high-dimensional images and segmented image data is available for review online by pathologists and subject matter experts in the community.

22. Molecular Basis for Feedback Inhibition of Notch Signaling by the Notch Regulated Ankyrin Repeat Protein, NRARP

Sanchez Jarrett

The Notch regulated ankyrin repeat protein (NRARP) acts as a negative feedback regulator of Notch signaling in higher organisms, but the molecular basis for NRARP function has remained elusive. Using an unbiased proteomics approach, we show that NRARP interacts with the core Notch transcription activation complex (NTC), containing the RBPJ transcription factor, the Notch intracellular domain (NICD), and Mastermind-like co-activators. Binding experiments using recombinant molecules establish that binding of NRARP into the complex requires both RBPJ and NICD, and that NRARP binds to Notch-RBPJ complexes independently of Mastermind-like proteins or DNA. The X-ray structure of an NRARP/RBPJ/Notch1/DNA complex, determined to 3.75 Å resolution, reveals a non-canonical binding mode for engagement of one ankyrin protein, NRARP, with the ankyrin domain of Notch1. Mutations disrupting this interface result in loss of feedback inhibition in cell-based Notch signaling assays. Collectively these studies establish the structural basis for NTC engagement by NRARP and provides insights into the feedback inhibition mechanism of NRARP as a modulator of Notch signaling.

23. Rab18 is Not Necessary for Lipid Droplet Biogenesis or Turnover in Human Mammary Carcinoma Cells

Christina BK Jayson

Rab GTPases recruit peripheral membrane proteins and can define organelle identity. Rab18 localizes to the ER but also to lipid droplets (LDs), where it has been implicated in effector protein recruitment and in defining LD identity. Here, we studied Rab18 localization and function in a human mammary carcinoma cell line. Rab18 localized to the ER and to LD membranes upon LD induction, with the latter depending on the Rab18 activation state. In cells lacking Rab18, LDs were modestly reduced in size and numbers, but we found little evidence for Rab18 function in LD formation, LD turnover upon cell starvation, or the targeting of several proteins to LDs. We conclude that Rab18 is not a general, necessary component of the protein machinery involved in LD biogenesis or turnover.

24. Spatial Identification of Apoptosis-resistant and Apoptosis-sensitive Cells in Tissues Using in Situ BH3 Profiling

Gaurav Joshi

Inadequate cell death is causally linked to disorders such as cancer or autoimmune disease, suggesting that modulation of cell death may be beneficial therapeutically. Recently, the apoptotic potential of various tissues has been measured, showing that some tissues such as the hematopoietic system, are “primed” for apoptosis. Other tissues, such as the lung and gastrointestinal system are considered “unprimed” while largely post-mitotic tissues such as the brain and heart are “apoptosis refractory.” However, since tissues consist of heterogeneous cell types with highly specialized roles and functions, it is hypothesized that cells within these tissues may regulate apoptosis differently, which may impact cellular and tissue response to injury or damage. We have therefore developed tissue-based (in situ) BH3 profiling to spatially differentiate the apoptotic sensitivity of various cells directly in intact tissues.

The in situ BH3 profiling assay utilizes 20 amino acid length peptides that resemble pro-apoptotic activator proteins BIM and BID. In a primed cell, these peptides efficiently activate BAX and BAK on mitochondria resulting in mitochondrial outer membrane permeabilization (MOMP) that can be measured using cytochrome c release from mitochondria. In an unprimed cell, higher concentrations of these peptides are required to induce MOMP as compared to a primed cell. An apoptosis refractory cell doesn’t undergo MOMP in response to pro-apoptotic peptides, typically due to insufficient expression of BAX and BAK.

Using in situ BH3 profiling on lung slices from healthy mice, we found differential priming of various specialized cell types. Based on the location of the cells within the tissue airway epithelial cells, alveolar type 1 epithelial cells, and macrophages are primed to undergo apoptosis. In contrast, type 2 epithelial cells are not primed. This has important implications for tumor development since the transformation of type 2 epithelial cells into lung cancers would potentially result in tumors that are less sensitive to apoptosis and thus resistant to anti-cancer treatments. Cells in ileum of small intestine were found to highly primed. In this ongoing work, we will further characterize the effect of oncogenic activation on cellular priming and role of apoptotic preset of parental cells in determining treatment outcomes.

25. Nucleocytoplasmic Glycosylation is Required for Cell Proliferation and Directed by the O-GlcNAc Transferase TPR Domain

Zebulon G. Levine

O-GlcNAc Transferase (OGT) is the sole enzyme responsible for nuclear and cytoplasmic O-GlcNAc, the reversible addition of a monosaccharide to serine or threonine residues of thousands of proteins. This modification, which is nutrient-responsive and increases in due to cellular stress, has been correlated with pathogenesis of neurodegeneration, cancer, and diabetes. OGT also has a second catalytic activity: it acts as a protease to mature the essential cell cycle associated transcriptional coregulator host cell factor 1 (HCF-1). It is not clear how OGT selects its substrates or why it is essential for mammalian cell proliferation. We have developed tools to address these questions. Using biochemical methods, we demonstrate that OGT uses its TPR domain to select substrates, and identified key residues driving substrate selection. We've further developed genetic methods to replace OGT with mutant copies, and demonstrated that Ser/Thr O-GlcNAc is required for mammalian cell proliferation, but HCF-1 proliferation is dispensable for survival.

26. Identifying Potential Targets for Slowing Antibiotic Resistance Evolution by Measuring Robustness of Sensitive and Resistant E. coli

Anurag Limdi

Robustness is the ability of biological systems to tolerate random genetic perturbations. It is typically measured by creating a library of mutations and measuring fitness effects of mutations in a specific environment. Such experiments can identify genes important for biological processes, like antibiotic resistance or virulence in pathogens. However, how the fitness effects of mutations changes over evolutionary trajectories remains unexplored. Here, we propose to use random transposon insertion mutagenesis (TnSeq) to measure fitness effects of insertion mutations in ciprofloxacin sensitive and resistant E. coli. We will investigate whether resistant bacteria are more or less robust to mutations (on average). We will also identify potential targets for slowing resistance evolution by pinpointing genes which when disrupted in sensitive E. coli are neutral (or even beneficial), but strongly deleterious in ciprofloxacin resistant E. coli.

27. Highly Multiplexed Immunofluorescence Imaging with t-CyCIF for Probing Human Diseases

Jerry (Jia-Ren) Lin

We recently developed cyclic immunofluorescence (t-CyCIF), a method that (unlike current alternative methods) acquires highly multiplexed fluorescence images from formalin-fixed, paraffin-embedded (FFPE) tissue sections using standard instrumentation and reagents. Here, we report use of t-CyCIF to profile immune cell populations within the tissue microenvironment. We developed a 16-antibody panel that identifies various adaptive and innate immune cells and robust protocols for antibody validation, image acquisition, data processing, analysis and visualization. In lung cancer, we illustrate how t-CyCIF enumerates and localizes immune cells, including rare immune subtypes, within routinely collected and processed surgical specimens. In the current workflow, one week is required for users to perform spatially resolved immune profiling of more than 30 samples, each up to 4cm2 in size. Because t-CyCIF antibody panels are readily customized and can be used to analyze samples in clinical FFPE archives, t-CyCIF enables the detailed immunophenotypic characterization of a broad spectrum of human diseases.

28. Investigating New Therapeutic Vulnerabilities in NRAS-mutant Melanomas

Haley Manchester

Cutaneous melanoma is a metastatic and treatment refractory skin cancer, and the discovery of critical oncogenic mutations in the RAS/RAF/MEK/ERK pathway led to the development of highly selective kinase inhibitors. In BRAF-mutant melanomas, the combination of MEK inhibitors (MEKi) and BRAF inhibitors (BRAFi) stimulates a response in 76-90% of patients. However, for NRAS-mutant melanomas, a small percentage of patients (~20%) partially respond to allosteric inhibitors of MEK, with an increase in progression free survival of only 3.7 months. Because of this, mutations in NRAS are generally linked to poor overall survival. In this study, we performed an unbiased genome-scale negative selection CRISPR screen to identify targets that, when suppressed, cooperate with MEKi to produce a cytotoxic response in NRAS-mutant melanomas. We identified and further characterized one druggable target of interest, the de-ubiquitinase USP7.

29. Cyclic Immunofluorescence (CycIF) Multiplexed Imaging for High Resolution and High Dimension View of TB Infection

Amy Moody

Tuberculosis (TB), caused by infection with Mycobacterium tuberculosis (Mtb), kills 1.4 million and infects 10.4 million people per year. Strikingly, the immune response is often sufficient to combat Mtb infection, but the local immune response is also highly variable, with different outcomes between different areas of the lung. Identifying the root causes of restrictive vs permissive macrophage response to Mtb infection will improve our fundamental understanding of TB virulence.

Macrophages are the first line of defense against Mtb and drive granuloma formation and later immune signaling. Here, we have adapted the highly multiplexed imaging technique, cyclic immunofluorescence (CycIF), to early macrophage TB infection to identify aspects of immune signaling that are most beneficial for bacterial killing. We used CycIF to assess bacteria burden, bacteria pH stress, and a panel of 19 key innate immune signaling and cell surface markers during the first 72h of TB infection. Strikingly, many infected cells did not activate to detectable levels most of the innate immune signaling pathways that we assayed. However, we identified an important role for early p38 activation with p38 inhibition leading to decreased macrophage cell death and increased bacteria burden.

We have also applied CycIF to TB infected lung tissue samples from Mtb-infected patients to get a more complete view of granuloma morphology and to investigate interactions between immune cells. By combining a set of immune cell identification markers with the innate immune signaling patterns used above, we can investigate specific signaling patterns within certain cell types and correlate the patterns with local microenvironment features. By applying this method to patient samples and in vitro infections, we aim to develop a quantitative, dynamic, and single-cell resolution understanding of TB infection to identify deterministic or predictive factors of infection outcome.

30. ASHLAR: An Accurate and Efficient Algorithm for Stitching, Registration and Mosaicing of Multiplexed Microscopy Images of Large Samples

Jeremy Muhlich

To obtain a single seamless image of a tissue sample much larger than a microscope's field of view, a microscopist typically captures a regular grid of images across the sample and then "tiles" them to form a single large mosaic image. However it is nearly impossible to both construct and calibrate a microscope precisely enough to produce a perfectly aligned grid of images directly. Instead one can introduce a small amount of overlap in neighboring tile images, then align common features in the overlapping regions to adjust the tile positions. This alignment and adjustment process is known as stitching. When combining tiled acquisition of large samples with multiplex imaging techniques (e.g. CyCIF or CODEX), in which a sample is imaged in multiple rounds each with a different set of fluorescent markers, one must furthermore apply a similar alignment and adjustment process to the images acquired across the several rounds of imaging. This process is known as registration. To perform simultaneous stitching and registration of tiled, multiplexed image data, we wrote a tool called ASHLAR (Alignment by Simultaneous Harmonization of Layer/Adjacency Registration). We use phase correlation to align images pairwise followed by a statistical model of microscope stage behavior to detect and correct outliers. Once corrected stage positions have been computed for all image tiles across all imaging cycles, a single seamless many-channel mosaic image is produced. The mosaic images can be uploaded directly to the OMERO microscopy data management server for visualization, or processed further via single-cell segmentation and quantification. ASHLAR is provided as a command-line tool for batch processing as well as a Python package for advanced users to write custom scripts.

31. The Impact of Latent Viruses on Bacterial Sensitivity to Antibiotics

Siân Owen

A chemical stress that is commonly faced by clinically-relevant bacteria is exposure to antibiotics which kill or inhibit cell growth. Like antibiotics, bacterial viruses (bacteriophages) are a pervasive selective force acting on the evolution of bacterial genomes. Temperate bacteriophages can integrate into bacterial genomes (where they are termed 'prophages'), and are ubiquitous in the genomes of bacterial pathogens.

A number of antibiotics have been shown to induce the SOS response, a bacterial DNA-damage response pathway which also frequently activates the lytic replication (and phage-mediated cell death) of many prophages. However, the interaction between prophages and antibiotic exposure at an evolutionary level has not been studied.

Here I present data suggesting that the removal of prophage elements from the genomes of Salmonella strains increases the minimum inhibitory concentration of Ciprofloxacin necessary to inhibit growth. The data are consistent with a model where the presence of inducible viral elements in the bacterial genome increase sensitivity to antibiotics.

This poster explores the physiological and evolutionary mechanisms by which prophages and antibiotics may interact.

32. Drugs in the Curative RCHOP Combination Exhibit Low Cross-resistance but not Pharmacological Synergy

Adam Palmer

Almost all existing curative cancer therapies involve multi-drug combinations developed by experimentation in humans, and the mechanistic basis for their success has not been investigated in detail. Here we use isobologram analysis to score pharmacological interaction and clone-tracing and CRISPR screening to measure cross-resistance among the five drugs comprising R CHOP, a combination therapy that frequently cures Diffuse Large B-Cell Lymphomas. We find that drugs in R CHOP exhibit very low cross-resistance but no synergy; their combined activity is close to dose-additive (Loewe model) and effect-independent (Bliss model). These data provide direct evidence for the 50-year old hypothesis that a curative cancer therapy can be constructed on the basis of non-overlapping resistance among individually effective drugs, rather than synergistic pharmacological interaction.

33. Cofactors and Mediators that Control Dose-dependent Response in Gene Expression

Jeehae Park

34. Targeting the Prolyl Isomerase Pin1 with Covalent Inhibitors

Benika Pinch

Pin1 regulates the function and stability of specific phosphoproteins by catalyzing the cis/trans isomerization of peptidyl-prolyl bonds that follow phosphorylated serine or threonine residues. While Pin1 is dispensable for viability in mice, it cooperates with activated Ras to induce tumorigenesis, suggesting a role for Pin1 inhibitors in Ras-driven tumors, such as pancreatic ductal adenocarcinoma (PDAC). Through iterative rounds of synthesis and characterization, we rationally designed inhibitors that form a covalent adduct with a critical cysteine residue, Cys113, in the Pin1 active site. The lead compounds were optimized to give two structurally distinct, highly potent, cell permeable, and Pin1-selective covalent inhibitors that serve as versatile tool compounds with which to probe Pin1 biology. We show that Pin1 inhibition diminishes viability of human PDAC cell lines, which can be fully rescued in corresponding Pin1 knockout cells generated using CRISPR/Cas9, showing that this phenotype is on-target. In parallel to inhibitor development, we used CRISPR/Cas9 GFP-dropout screens to further validate the dependence of these cell lines on Pin1. Genetic disruption of Pin1 led to antiproliferative effects, confirming the results of inhibitor treatment. We also employed the degradation tag (dTAG) approach to assess the effects of rapid and selective Pin1 degradation through generation of FKBP12F36V-Pin1, Pin1-/- human PDAC cell lines. Treatment with a small molecule FKBP12F36V-degrader led to rapid ubiquitination and degradation of FKBP12F36V-Pin1, enabling comparisons of targeted inhibition and Pin1 degradation. Through the development of selective Pin1 inhibitors coupled with genetic approaches and the chemical-genetic dTAG strategy, we demonstrate that Pin1 inhibition represents a tractable strategy in PDAC.

35. Iterative Screen-seq: A Means to Reprogram a Cell Into any Somatic Cell Type

Feodor Price

The principle of cellular reprogramming has profoundly altered the landscape of regenerative medicine. In order for reprogrammed cells to be a feasible option for cell therapy, fundamental advancements in the time, understanding of the mechanisms involved, and resources required for generating a specific cell type are required. While conventional platforms used for

therapeutic screening are appropriate for phenotypic screens where the target is unknown or in the case of genomic screens (shRNA, CRISPRi or CRISPa) where mechanistic data is generated, rarely does a screening platform provide mechanistic data on a genome wide scale and identify therapeutically relevant compounds simultaneously. To address these issues we have established a novel RNA-seq based screening platform (Screen-seq) and are in the process of applying this platform to study cellular reprogramming in a manner that is applicable to all somatic cell types. Screen-seq leverages large quantities of gene expression data to answer the fundamental questions that surround cellular reprogramming. To date, we have confirmed that Screen-seq can detect the subtle transcriptional changes indicative of cellular reprogramming, and have identified early modulators of the fibroblast state using combinatorial screening. Using InDrop single cell RNA-seq at specific time points in the our Screen-seq pipeline we have increased the resolution of effects caused by a given perturbation.

36. Anti-CRISPR Dynamics in Plasmids

Natalia Quinones

37. A Multi-targeted Probe-based Strategy to Scan the Kinome for Targetable Cysteines

Suman Rao

Recently approved covalent kinase inhibitors that target specific cysteine residues in the ATP-binding site of the kinase, represent an important class of clinically relevant compounds. Within the human kinome, there are approximately 215 kinases with potentially targetable cysteines distributed across 18 spatially distinct locations in and around the ATP-binding pocket. Despite nearly half the kinome having targetable cysteines, only 40 kinases have been covalently targeted. We hope to address this gap using our strategy that combines the use of a multi-targeted acrylamide-modified inhibitor, SM1-71, with a suite of complimentary chemical proteomic approaches to identify additional targetable cysteines. We successfully identified 41 cysteines across 38 kinases that are amenable to covalent inhibition including SRC, YES1, MEK1/2, ERK1/2, RSK1/2/4, LIMK1, PLK1/4, TNK1 and MARK1-4. Among these were 25 kinases previously not known to be covalently targeted. Our findings more than doubles the number of covalently targetable kinases and highlights new opportunities for covalent kinase inhibitor development.

38. Tissue Specific Response and Resistance to Immune Checkpoint Inhibitors in a Pre-clinical Melanoma Model

Meri Rogava

Immune checkpoint inhibitors (ICI) produce durable responses in a portion of melanoma patients. However, most patients experience intrinsic or acquired resistance, and the underlying mechanisms may be determined by the tissue site of metastasis. Understanding the site-specific resistance mechanisms may inform rationale development of novel therapies, however, preclinical immunocompetent models poorly reflect metastatic patterns observed in patients and their variable responses to anti-CTLA-4 or anti-PD-1 therapy. In this work, we characterize a syngeneic melanoma mouse model harboring a CDK4 mutation which renders it insensitive to p16-mediated inhibition of CDK4 and CDK6, thereby promoting a RB1-hyperphosphorylating phenotype that is resistant to CDK4/6 inhibition to palbociclib and abemaciclib in vitro. Upon titration of tumor load injected either via tail vein or subcutaneous injection, we find that this model develops metastases to lung, brain, liver and lymph nodes with remarkable resemblance to the metastatic pattern in melanoma patients. The model is resistant to anti-CTLA-4 and shows partial response to anti-PD-1 therapy, while combination produces long-term disease control. Overall, this model lends itself to study fundamental questions, such as site-specific tropism, and large-scale perturbations thereof in the context of immunotherapy. To begin understanding how tissue-specific immunity may determine responses to immune-based therapies, we examined the combination of TLR-agnosits and anti-PD-1 therapy. Upon bilateral flank injection, ipsilateral intra-lesional TLR-stimulation produces complete responses in some animals, and partial responses in the contralateral site when co-administered with PD-1 therapy. Interestingly, while anti-PD-1 therapy alone had little effect on the metastatic burden to the lung, combined therapy abrogated metastasis to the lungs, indicating cross-talk of tissue specific immune response enhanced by local stimulation of innate immunity. Together, these studies indicate that the model recapitulates disease behavior as seen in patients and exhibits similar responses to immunotherapies providing a useful platform for further studies of metastasis, drug resistance and development of new combinatorial treatment strategies.

39. Seizure Diary Simulator

Juan Romero

Objective: To create a simulation of seizure diaries from patients with epilepsy where the population of simulated patients mimics statistical properties found in actual patient populations for the purpose of investigating RCTs (Randomized Clinical Trials)

Methods: Daily seizure counts for each individual patient were generated via a statistical model which takes in a set of population-wide parameters. This set of parameters was fitted to closely match two statistical properties found in patient populations: the slope of the line of best fit for the log-log plot of the mean vs the standard deviation of the two-week seizure frequency (previously found to be 0.7), and the median of the monthly seizure count (previously found to be 2.7). The mean and standard deviation of the RR50 and MPC for the placebo arm of an RCT was calculated for both 5000 simulated RCTs and 23 historical RCTs reviewed from the medical literature.

Results: The monthly median seizure frequency was fitted to be 2.562, the slope of the line of the log-log plot was fitted to be 0.682, and the averages of the RR50 and MPC were calculated to be 21.1 ± 3.4 % and 21.4 ± 4.4 % for the simulated RCTs, and 21.7 ± 9.9 % and 16.7 ± 10.3 % for the actual RCTs.

Conclusion: This simulator implements a statistical model of daily seizure diaries which reflects important statistical properties found in the overall patient population, and will aid in the investigation of statistical properties of RCTs.

40. Selective USP7 Inhibitors for Probing p53-USP7 Interactions in Cancer Cells

Nathan Schauer

Deubiquitinating enzymes (DUBs) counteract E3 ubiquitin ligases by removing ubiquitin tags from protein substrates. K48- and K11-polyubiquitin chains target proteins for proteasomal degradation, and alteration of the ubiquitin-proteasome system has proven clinically useful, either through direct inhibition of the proteasome (bortezomib) or by targeted recruitment of E3 ligases to specific substrates (lenalidomide). In the same way that lenalidomide recruits new substrates to the proteasome, DUB inhibition should increase the proteasomal trafficking of native DUB substrates by preventing their de-ubiquitination. DUBs are nominally “druggable” enzymes that contain a solvent-accessible binding pocket, but this binding pocket is both very large (accommodating ubiquitin, an 8.6-kDa protein) and highly conserved. It is thus still an open question whether potent and selective substrate-competitive DUB inhibitors can be achieved. Previously, we identified a compound that binds 12 angstroms from the active site cysteine of USP7 in a binding site that, surprisingly, is important for ubiquitin binding but not conserved in any other DUBs. Here, we show that extending the inhibitor scaffold from this binding site to the active site cysteine of USP7 results in a dramatic increase in enzymatic and cellular potency while still retaining a high degree of USP7 selectivity across the entire human proteome. USP7 has been hypothesized as a therapeutic target in p53-WT tumors, but previous small molecule studies have shown conflicting effects of USP7 inhibition on p53. Here, we show that our covalent USP7 inhibitors rapidly induce MDM2 degradation and exhibit cell killing in a p53-dependent manner across multiple cell lines. In a panel of > 500 cancer cell lines, response to USP7 loss, MDM2 loss, and TP53 loss all predict response to our inhibitors, implying that the MDM2 / p53 axis is a key therapeutic target for USP7 inhibitors across all cancer types.

41. Optimization of Medulloblastoma Therapy by Modulating Apoptosis in Tumor Cells and Healthy Brain Tissue

Rumani Singh

Medulloblastoma (MDB) is the most common childhood malignant brain tumor, which comprises 40% of all pediatric tumors in children. In addition to surgical resection, postoperative radiotherapy and chemotherapy are the mainstays of treatment. The standard radiation regimen consists a dose of 36 Gy to the entire craniospinal axis followed by a boost to the whole posterior fossa, for a total dose of 55 Gy. This treatment strategy effectively controls tumor growth and is curative in up to 80% of patients, but also causes severe neurologic, cognitive and endocrinologic sequalae. A variety of different chemotherapeutic agents are being utilized before or after radiotherapy to reduce irradiation dose and minimize toxicity in brain. Besides encouraging results of combination therapy, children who received a reduced dose of radiation showed inferior event-free survival. Radiation therapy or chemotherapy alone or in combination has shown to cause significant decline in cognitive functions, drop in IQ levels, ototoxicity and other associated dysfunctions.

The goals of the current study were to 1) understand how apoptotic pathways are regulated in pediatric brain tumors and utilize apoptotic vulnerabilities to improve treatment outcomes; and 2) to establish animal models of radiation- or chemotherapy-induced neurotoxicity and understand what molecular or cellular mechanisms are involved in therapy-induced toxicity.

The mitochondrial apoptosis pathway, which is controlled by the BCL-2 family of proteins, is the most dominant form of physiological cell death and plays a pivotal role in radiation- and chemotherapy-induced tumor cell death. We have previously found that cancers that are “primed” for apoptosis respond more favorably to chemotherapy than those cancers that are “unprimed” for apoptosis. We measured apoptotic priming in medulloblastomas using BH3 profiling and tested their response to chemotherapies and ionizing radiation using chemosensitivity assays. We found that medulloblastomas are primed cancer cell lines, which contributes to their sensitivity to therapy. In addition, medulloblastoma cell lines exhibited dependence on pro-survival protein BCL-xL and underwent apoptosis in response to a BCL-xL inhibitor (WEHI-539) alone and in combination treatments. To build on these findings, we are currently exploring the potential for combining BH3 mimetics with standard therapies in order to improve outcomes for patients. Furthermore, treatment-induced apoptotic cell death in heathy tissues drastically limits the use of radiation and chemotherapy in the clinic. This is especially true for pediatric cancer patients, who frequently experience higher cure rates than adults but also higher rates of treatment-associated toxicities and adverse long-term health effects. We aim to establish radiation- and chemotherapy-induced toxicity models and elucidate their molecular and cellular mechanism that regulate radiation- and chemotherapy-induced neurotoxicity.

42. Targeting the PI5P4K Lipid Kinase Family in Cancer Using Novel Covalent Inhibitors

Carmen Sivakumaren

The phosphatidylinositol 5-phosphate 4-kinases, PI5P4Kα, β and γ been shown to be crucial in driving a number of cancers as well as involved in other disease pathologies such as proliferative vitreoretinopathy, diabetes, and neurodegenerative disorders. However, the therapeutic potential of targeting these lipid kinases is understudied due to a lack of potent and specific small molecules available. Here we present the discovery and characterization of a novel pan-PI5P4K inhibitor, THZ-P1-2, that covalently targets cysteine residues on a disordered loop region in PI5P4K// proximal to the ATP-binding site. Cancer cell line profiling demonstrates AML and ALL cell lines to be sensitive to THZ-P1-2 covalent targeting, consistent with PI5P4K's reported role in leukemogenesis. THZ-P1-2 causes lysosomal disruption, and defects in the clearance of autophagosomes, halting autophagy in a manner dependent on cysteine-targeting and phenocopying the effects of genetic deletion of PIP4K2A/B. THZ-P1-2 treatment also causes an increase in TFEB nuclear localization, upregulation of TFEB target genes and subsequent cancer cell growth inhibition. These studies provide evidence that covalent inhibition of PI5P4K by THZ-P1-2 compromises autophagy, an essential alternative energy source during periods of metabolic stress which cancer cells depend on to maintain cellular homeostasis and prolonged cell viability, further suggesting PI5P4K as a novel therapeutic target in cancer metabolism. Taken together, our studies demonstrate that the PI5P4K enzymes are tractable targets, with THZ-P1-2 serving as a useful tool to further interrogate the therapeutic potential of PI5P4K inhibition and inform drug discovery campaigns for these lipid kinases in the context of cancer and potentially other autophagy-dependent diseases.

43. Drug Mechanisms of Action Predict Neurodegeneration in Alzheimer's Patients

Artem Sokolov

Alzheimer's Disease (AD) is a growing epidemic as longer life expectancy fuels its principal risk factor - aging. As understanding of AD grows in the setting of many failed clinical trials, the concept of AD as a single disease is giving way to the hypothesis that it is a syndrome with multiple disease pathways progressing towards a common end-stage clinical presentation. Here, we aim to identify FDA-approved drugs that target these pathways and thus are candidates for repurposing in AD.

Given an FDA-approved drug, we asked if its mechanism of action is related to AD biology by training a predictor of disease stage. The predictor was limited to using expression of genes known to be associated with the drug, and its performance was compared to predictors

constructed on randomly-selected gene sets of equal size. Thirty top-performing drugs were subsequently profiled on human neuroprogenitor cell lines that differentiate into a mixed culture of neurons, glia and oligodendroctyes to further refine their mechanisms of action in relevant cell types. Jak inhibitors Tofacitinib and Ruxolitinib were among the top performers, and additional in vitro experiments demonstrated that the two drugs can rescue inflammatory-induced neuronal death, suggesting their potential as repurposing candidates for AD.

44. Using Knockout Cell Lines to Probe the Cytosolic DNA Innate Immune Response and its Relationship to Micronucleating Chemotherapeutics

Emma Spady

Human cells interpret DNA in the cytoplasm as a signal of viral infection or cellular damage. The cast of molecules underlying these signaling pathways have recently been identified; the most important are thought to be cGAS, a cytosolic DNA sensor synthesizing the dinucleotide cGAMP, and STING, the signal mediator activated by cGAMP. Nevertheless, the way these components interact with each other to mediate the overall response remains poorly understood. For example, micronucleation is a well-documented result of genotoxic chemotherapeutics, but has only recently been shown to trigger these DNA-sensing innate immune pathways and induce an inflammatory response. Furthermore, the proteomic responses downstream of the activation of various DNA-sensing pathways remain unexamined. We are exploring the nature of the cytosolic viral-DNA and micronucleation responses, and the relationship between them, using CRISPR knockout cell lines. We have recently made clonal knockouts of the DNA sensors cGAS, DDX41, ZBP1, and the IFI16/AIM2 cluster in an immunocompetent fibroblast cell line, along with a STING knockout. Preliminary data indicates cGAS involvement in the micronucleation response after taxane treatment, as detected by a co-seed luciferase reporter assay. The transcriptional response to both DNA transfection and micronucleation in the different knockout lines will be determined via RT-PCR of NFKBIA, TNF-α, CXCL10, IFN-α2, IFIT1, IRF-1, and MX1. The downstream proteomic response to DNA transfection will be measured with label-free protein mass spectrometry at the Pacific Northwest National Laboratory. In parallel with the transcriptional and proteomic work, the co-seed luciferase assay will determine if all micronucleating chemotherapeutics induce comparable inflammatory signals through similar innate immune pathways.

45. Single-cell Analysis of Apoptotic Regulation in Healthy Tissues

Johan Spetz

Cancers are typically treated with agents that induce cell death, not only in diseased cells but also in the healthy tissues of the body, resulting in toxic side effects which limit the use of therapeutics that could potentially cure the patient. These side effects may be more or less toxic to different lineages of cells within healthy tissues. Additionally, previous studies have revealed that the mitochondria of cells within certain somatic tissues (e.g. brain, heart, and kidney) become refractory to pro-apoptotic signaling as the organism reaches adulthood, suggesting that adult toxicity in these tissues is mediated by other means. The blood vasculature supports the metabolic needs of tissues by supplying oxygen and nutrients and removing waste products to facilitate tissue growth and maintenance of homeostasis. In fact, cardiovascular dysfunction is a major potential complication following cytotoxic cancer treatments. We therefore hypothesized that vascular endothelial cells may remain highly sensitive to pro-apoptotic stimuli, and thereby contribute to the formation of toxicity or secondary disease in otherwise apoptosis-resistant tissues. Commitment to cell death via the mitochondrial apoptotic pathway requires activation of the mitochondrial pore-forming proteins BAX or BAK by activator proteins BIM or BID. Using the flow cytometry-based BH3 Profiling assay, it is possible to measure the proximity of cells to the threshold of apoptosis, a property termed apoptotic priming. It has been previously shown that the level of apoptotic priming within healthy tissues governs their sensitivity to sources of damage or stress such as ionizing radiation or chemotherapy. The aims of this project are to 1; measure apoptotic priming at a single-cell level in mammalian tissues during normal development and aging to identify cells that are most at risk of undergoing apoptotic cell death in response to damage or stress, and 2; measure the sensitivity of vascular endothelial cells to cytotoxic stress such as radiation or chemotherapy. BH3 profiling of tissue-specific cells in postnatal mouse frontal cortex, liver, lungs, kidneys, and heart revealed a decrease in apoptotic priming with age, in coherence with previous results obtained for whole-tissue analysis. Of the analyzed cell types, CD31+ blood vessel endothelial cells as well as CD45+ peripheral blood mononuclear cells showed the highest apoptotic priming in the tissues analyzed, a result that was consistent with age (from postnatal day 0 to 90). Furthermore, Immunofluorescence analyses of cleaved caspase-3 in mouse tissues revealed apoptotic activation in endothelial cells following radiotherapy. In conclusion, our results indicate that apoptotic sensitivity of healthy cells is regulated at the single-cell level by lineage programs and developmental age. Importantly, vascular endothelial cells are highly sensitive to apoptosis-inducing stress independent of age, which may be a major driver of tissue toxicities in response to anti-cancer treatments.

46. Structure and Function of the CD19-CD81 B Cell Co-Receptor Complex

Katherine Susa

47. ATM-independent Activation of p53 Following Failure of the DNA Damage Checkpoint

Michael Tsabar

Following ionizing irradiation, the levels of p53 protein exhibit an oscillatory dynamics that are thought to persist as long as the DNA damage is not resolved. By monitoring MCF7 cells following irradiation using live cell imaging we find that a subset of the population switches from oscillatory to sustained p53 dynamics. We show that this switch is a result of failure to maintain cell cycle arrest following irradiation, and that the switch in p53 dynamics is associated with reduced cell proliferation following irradiation. Further, we demonstrate that this switch occurs independently of ATM signaling, but that increasing p53-induced death domain 1 (PIDD1) protein levels following irradiation leads to a dynamic switch. Taken together, our results propose a way to optimize irradiation regimens and expose a new regulator of the p53 response.

48. Evaluating Perturbagen-driven Phenotypic Changes on a Single-cell Level

Yunguan Wang

Background

The NIH Microenvironment Perturbagen (MEP) LINCS Center studies how microenvironment signals affect cell intrinsic intracellular transcriptional- and protein-defined molecular networks to generate experimentally observable cellular phenotypes. In a project across LINCS centers, cellular phenotypes were assessed using various approaches including live cell imaging, immunofluorescence, transcriptomics and proteomics. As part of this effort, the HMS LINCS center used cyclic immunofluorescence (CycIF) to study cellular phenotypic changes on both the population and single-cell levels.

Methods

EGF-deprived MCF10A cells were treated with ligands, including epidermal growth factor (EGF), bone morphogenetic protein 2 (BMP2) + EGF, hepatocyte growth factor (HGF), interferon gamma (IFNg) + EGF, oncostatin M (OSM), transforming growth factor beta 1 (TGFB) + EGF for one to 48 hours. The treated cells were stained with 24 antibodies over nine rounds of CycIF. Single cell data were extracted from CycIF images using ASHLAR and in-house random forest based segmentation scripts. Population level phenotypic changes were assessed using student's T test and Kolmogorov–Smirnov (KS) tests. Heterogeneity and subpopulations within treatments were assessed using a pipeline of unsupervised analysis including dimension reduction and clustering.

Results

Population level analysis showed that all treatments introduced significant deviation from PBS controls by the 48 hour time point. When the EGF group was used as the control, the treatments generally showed less deviation with the BMP2 + EGF group showing the least. Unsupervised analysis showed similar trends with the EGF, HGF, and BMP2 + EGF groups showing similar phenotypes and the IFN-g group showing the strongest phenotype. Differences in treatment groups were primarily driven by differential expression of S6, STAT1, NDRG1 and PD-L1. Subgroups of cells with consistent phenotypes were also identified which may suggest unresponsiveness to the selected ligands. In summary, the generalizable analysis pipeline we designed was able to extract not only population level statistics but also extract patterns between different treatment groups and identify subpopulations within each.

49. Myc Modulation in the Treatment of Medulloblastomas

Stacey Yu

Medulloblastomas are the most common malignant brain tumor of childhood, accounting for about 20% of all childhood brain tumors. Current treatments include a combination of surgery, radiation, and/or chemotherapy. While patients respond well to radiation treatment, there are long term neurotoxicities associated with radiation exposure. In protecting healthy neurons from undergoing radiation induced cell death, we target the BAX protein of the intrinsic apoptotic pathway via myc inhibition. Using BH3 profiling and known myc inhibitors, we study the effects of myc inhibition on medulloblastoma cell lines in order to develop a model for studying the potential protective effects in primary mouse neurons.

50. Elucidating the Role of Fibroblasts in HER2-targeted Therapy Resistance in Breast Cancer

Ioannis Zervantonakis

Introduction: HER2 overexpressing (HER2+) breast cancer accounts for 15-20% of all breast cancer cases, and although HER2-targeted therapies offer improved patient outcomes, advanced disease is rarely cured. Although several studies have demonstrated the role of tumor cell growth factor pathways in HER2-therapy resistance, the roles of extrinsic signals stemming from the tumor microenvironment remain poorly understood. Here, we investigate the role of fibroblasts in HER2-therapy resistance by utilizing high-throughput screening, cyclic immunofluorescence, proteomic measurements and in vivo xenograft models.

Materials and Methods: We measured the dose-response of seven HER2+ breast cancer cell lines to the FDA-approved HER2-targeted agent lapatinib under monoculture and direct co-culture with breast fibroblasts (Fig 1A). We also performed viability assays using fibroblast conditioned medium and paracrine assays. To identify signaling pathways that are induced by fibroblasts in tumor cells we perform reverse phase protein arrays (Figure 1B) and in situ cyclic immunofluorescence under direct co-culture conditions. A high-throughput direct co-culture screen was also performed using a library of 144 selective inhibitors to identify novel combination therapies that restore drug sensitivity. The in vivo relevance of the fibroblast-induced drug resistance was investigated using tumor xenografts formed using mammary fat pad and intraductal injections to control for the extend of stroma infiltration.

Results and Discussion: In vitro drug sensitivity analysis to lapatinib treatment showed that direct co-culture with fibroblasts induced lapatinib resistance (~10fold increase in IC50) in 4/7 HER2+ breast cancer cell lines. Exposure to fibroblast conditioned medium in a subset of the HER2+ breast cancer cell lines could phenocopy the effects of direct fibroblast co-culture suggesting that paracrine factors are sufficient to induce HER2-therapy resistance.

To gain insight into the underlying response mechanisms we examined fibroblast-induced alterations in protein expression in the cell line (EFM192) that exhibited the strongest fibroblast-mediated protection from lapatinib. Factors secreted by fibroblasts upregulated pro-survival signaling in EFM192 cells noted by high RAS/MAPK activity, reduction in pro-apoptotic proteins (BIM and BAX) and increased anti-apoptotic protein activity (MCL-1). In situ immunofluorescence analysis confirmed that pro-survival signaling was also upregulated in direct co-culture with fibroblasts. Using a drug combination screening approach we identified that SRC inhibition reduced tumor cell viability under coculture with fibroblast and did not affect fibroblast viability or tumor cell viability under monoculture. Finally, our in vivo results of an enriched fraction of Ki67+ tumor cells in lapatinib-treated tumors at the tumor-fibroblast in vivo interface compared of untreated tumors suggest that stroma-rich areas mediate drug resistance in vivo.

Conclusion: Our studies highlight the critical role of fibroblasts in mediating drug resistance in a subset of HER2+ breast cancers that is mediated by activation of anti-apoptotic pathways. Furthermore, we identified a new combination therapy using SRC inhibitors to restore HER2-therapy sensitivity in breast cancer models that exhibit fibroblast-mediated resistance.

51. DNA Nanoswitches: Programmable Sensors of Biomolecular Interactions with Application to Drug Discovery

Mark Lipstein

Our understanding of cellular signaling has grown to encompass pathways and their constituent components acting as complex microprocessors encoding, integrating, and relaying information by dynamically altering conformation and/or subunit composition. When deregulated, these processes are central to the etiology of diseases like neurodegeneration and malignant neoplasms. Their underlying signaling biology remains poorly characterized with a dearth of approved therapies. Capabilities allowing for measurement of these phenomena represent opportunities for mechanistic and therapeutic insight. Tools developed in our lab, DNA Nanoswitches, engender quantitative measurement of multi-bodied biological interactions1. Using the principles of nucleic-acid self-assembly, putatively interacting components can be hybridized to a linear, single-stranded backbone. An interaction bridges DNA bending, which can be measured by gel electrophoresis. Utilizing In-vitro library technologies such as mRNA display in-tandem with nanoswitches may engender high content, one-pot discovery of binders with DNA-looping as the selection readout. The goal of this combined assay is to demonstrate, in a reconstituted, biologically relevant multi-component system, the ability to discover chemical and biologic tools with novel mechanisms of action.

52. Chemically Induced Degradation of Anaplastic Lymphoma Kinase (ALK)

Chelsea Powell

We present the development of the first small molecule degraders that can induce anaplastic lymphoma kinase (ALK) degradation, including in non-small-cell lung cancer (NSCLC), anaplastic large-cell lymphoma (ALCL), and neuroblastoma (NB) cell lines. These degraders were developed through conjugation of known pyrimidine-based ALK inhibitors, TAE684 or LDK378, and the cereblon ligand pomalidomide. We demonstrate that in some cell types degrader potency is compromised by expression of drug transporter ABCB1. In addition, proteomic profiling demonstrated that these compounds also promote the degradation of additional kinases including PTK2 (FAK), Aurora A, FER, and RPS6KA1 (RSK1).

53. Development and Characterization of Prolyl tRNA Synthetase Inhibitors for Malaria

Mark Tye