HiTS

Harvard Program
in Therapeutic Science

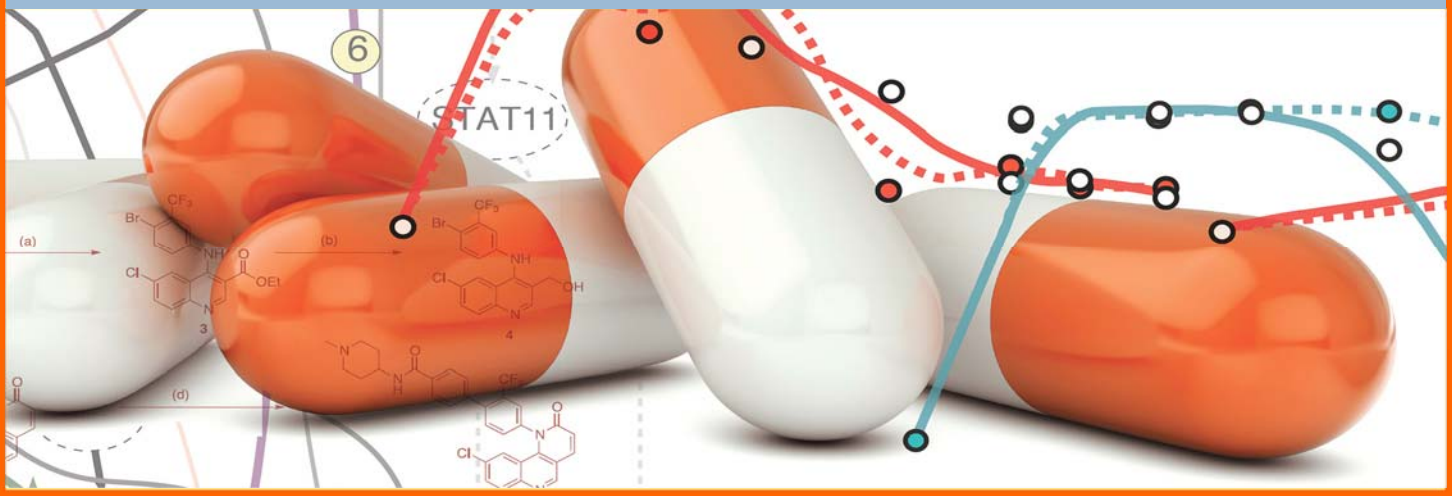
HiTS Symposium

December 8, 2017

Novartis Institutes of BioMedical Research

250 Massachusetts Avenue

Cambridge, MA



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Schedule

8:30 – 9:00am	Registration and Light Breakfast
9:00 – 9:30am	**Welcome Remarks: Peter Sorger**, Head of Harvard Program in Therapeutic Science, Harvard Medical School
9:30 – 10:10am	**Keynote Talk**: **Mark Borowsky**, Executive Director of Scientific Data Analysis, Novartis Institutes for BioMedical Research *Machine Learning and Drug Discovery*
10:10 – 10:20am	Coffee Break
10:20 – 12:15pm	**Session 1: Biology and Pharmacology at the Single Cell Level** **Chair: Michael Baym** **Bree Aldridge,** Assistant Professor, Tufts University *Mycobacterial cell size control and antibiotic susceptibility* **Ben Izar**, Instructor, Harvard Medical School *Cancer cell-autonomous mechanisms of resistance to immune checkpoint inhibitors revealed by single-cell RNA-sequencing* **Adam Palmer**, Postdoctoral Fellow, Harvard Medical School *Identifying the origins of benefit in combination cancer therapy* **Bill Forrester**, Senior Research Investigator, Novartis Institutes for BioMedical Research *A regulated ubiquitin catastrophe* **Sandro Santagata**, Assistant Professor, Dept. of Pathology, Brigham and Women's Hospital *High dimensional pathology for clinical research*
12:15 – 1:20pm	Lunch
1:20 – 2:30pm	**Session 2: Computation and Data Science in Biomedicine** **Chair: Bree Aldridge** **Debbie Marks,** Assistant Professor of Systems Biology, Harvard Medical School *Methods to identify combinations of alleles that are associated with antimicrobial resistance* **Toufiq Parag**, Research Scientist, Harvard School of Engineering and Applied Sciences *EM Connectomics : Discovering the biological neural network of animal brain*

1:20 – 2:30pm	**Session 2: Computation and Data Science in Biomedicine (cont.)**
	Artem Sokolov, Director of Informatics and Modeling, Harvard Medical School *Towards integration of single-cell data*
2:30 – 2:40pm	Coffee Break
2:40 – 4:20pm	**Session 3: Measuring and Modeling Therapeutic Responses**
	Chair: Sandro Santagata
	Leonid Peshkin, Lecturer on Systems Biology, Harvard Medical School *Bayesian Confidence Intervals for Multiplexed Proteomics*
	Kris Sarosiek, Assistant Professor of Radiation Biology, Harvard T.H. Chan School of Public Health *Dynamic regulation of apoptosis in healthy tissues as a driver of therapy-associated toxicities*
	Michael Baym, Assistant Professor of Biomedical Informatics, Harvard Medical School *Evolutionary approaches to antibiotic resistance*
	Steve Rodriguez, Instructor in Neurology, Massachusetts General Hospital *Neurodegeneration mediated by a neuronal antiviral innate immune signaling pathway*
4:20 – 4:30pm	Coffee Break
4:30 – 5:15pm	**Session 4: Cheminformatics and Computational Chemistry (Discussion)**
	Moderator: Peter Sorger
	Jeremy Jenkins, Novartis Institutes for BioMedical Research *Cheminformatics in phenotypic drug discovery*
	Nienke Moret, Graduate Student, Harvard Medical School **Jeremy Muhlich**, Director of Software Engineering, Harvard Medical School *Building cheminformatics tools and resources for LSP*
5:15 – 6:45pm	**Poster Session, Poster Prizes and Refreshments**

Speaker Bios

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 (systems biology) approaches to construct and test computational models of signaling in human and murine cells as a means to understand and predict the responses of cells and tumors 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.

As founding head of the Harvard Program in Therapeutic Science (HiTS) and its Laboratory Systems Pharmacology (LSP) Peter leads a multi-institutional effort to advance the basic and translational science used to develop new medicines, create novel drug combinations and identify responsive patients. The LSP also applies systems approaches to understanding and mitigating adverse drug effects and to designing new clinical trials. The recently established Harvard-MIT Center of Regulatory Sciences focuses on improving how drugs are evaluated, brought to market and used in patients. HiTS includes faculty from seven institutions.

Mark Borowsky, Novartis Institutes of BioMedical Research

Mark Borowsky is Global Head of Scientific Data Analysis at the Novartis Institutes for BioMedical Research. His team creates and applies data analysis approaches to answer biological questions that advance drug discovery efforts. Mark received a bachelor's degree in Molecular Biology and Biochemistry from Harvard, a PhD in Biology from MIT, and he performed post-doctoral work at UC Berkeley. Since then Mark has moved between industry and academia, pursuing interesting opportunities to work at the interface of biology and computational sciences. He has held positions at Incyte Genomics, the Broad Institute, MGH, and Novartis.

Machine Learning and Drug Discovery

A brief overview of machine learning and examples of how we're testing its utility in drug discovery.

Bree Aldridge, Tufts University

Bree Aldridge is an Assistant Professor in the Department of Molecular Biology and Microbiology and Department of Biomedical Engineering. Her lab investigates tuberculosis infection and drug response at single-cell resolution. She specializes in combining live-cell microscopy and mathematical modeling to create intuitive descriptions of complex cell biology. She earned double B.S. degrees in computer engineering and molecular and cellular biology and the University of Arizona as a Flinn Scholar. She completed her PhD in Biological Engineering at MIT with Douglas Lauffenburger and Peter Sorger with the support of a DOE Computational Science Graduate Fellowship. She brought her interdisciplinary perspective to tuberculosis and infectious diseases during her postdoctoral training at the Harvard School of Public Health with Sarah Fortune. She is an Alfred P. Sloan Research Fellow and is the recipient of an NIH Director's New Innovator Award.

Mycobacterial cell size control and antibiotic susceptibility

Mycobacterium tuberculosis infects billions of people worldwide and kills more than 1.5 million per year. TB remains extremely difficult to treat with antibiotics, requiring months to years of therapy for cure. The variable course of disease and treatment response suggests that functionally heterogeneous populations of mycobacteria respond differently to stress. Using a quantitative single-cell approach, we show that mycobacteria deterministically generate diversity in their growth characteristics through asymmetric growth and maintain a controlled but asymmetric chromosomal organization pattern. Coupled with a unique mechanism of cell size regulation utilizing parallel adders from initiation, this asymmetry creates subpopulations of cells with distinct growth rates and cell sizes that are differentially susceptible to clinically relevant classes of antibiotics. We find that combinations of inherent and temporal properties

of individual cells describe subpopulations susceptible to different antibiotics. Thus, the polar growth pattern intrinsic to mycobacteria deterministically creates a diverse population structure that may underlie phenotypes previously thought to be controlled by external stressors.

Ben Izar, Broad Institute/Harvard Medical School

Post-doc: Rudolf Grosschedl, “Functional elements in the immunoglobulin heavy chain enhancer region”

Cancer cell-autonomous mechanisms of resistance to immune checkpoint inhibitors revealed by single-cell RNA-sequencing.

Immune checkpoint inhibitors (ICI) have revolutionized the therapeutic landscape of several cancers, including metastatic melanoma. While some patients experience durable responses, most patients are either have either intrinsic or acquired resistance to ICI. The mechanisms of ICI resistance (ICR) are poorly understood and current profiling technologies are do not recapitulate the inherent heterogeneity of the tumor ecosystem. We applied single-cell RNA-seq (scRNA-seq)to >10,000 cells isolated

from 31 patients with metastatic melanoma, including 15 patients with ICR and 15 with treatment naive (TN) disease. We identify several previously unknown cancer cell-autonomous mechanisms of ICR and mechanisms of T cell exclusion. Signatures generated by scRNA-seq are prognostic and predictive in public data sets. Furthermore, we assembled tan independent cohort of 112 melanoma patients who undergo pre-treatment bulk tissue RNA-seq, followed by treatment with anti-PD1 therapy. Signatures generated by scRNA-seq are predictive of clinical benefit (CB), intrinsic and acquired resistance, and progression-free survival (PFS) in this cohort. Together, these data indicate that a scRNA-seq derived predictive signature may be useful in stratifying patients based on a pre-treatment biopsy. Ongoing work is focussed on validating the spatial context of identified ICR mechanisms using highly multiplexed single-cell protein assessment of corresponding FFPE specimens.

Adam Palmer, Harvard Medical School

Adam is a postdoctoral fellow in Peter Sorger's group, where he applies experiments and computation to study combination cancer therapies.

Previously Adam received his Ph.D in Systems Biology from Harvard University, where he worked with Roy Kishony on mechanisms of drug action and the evolution of drug resistance.

Identifying the origins of benefit in combination cancer therapy

Combination cancer therapies are widely thought to be the key to improving rates of response and reducing the likelihood of acquired resistance, and indeed multi-drug combinations can cure some cancers. Today, combination therapies are commonly developed based on pre-clinical evidence of drug synergy. I will present studies of clinically successful combination therapies that identify the control of within-tumour and between-tumour heterogeneity, irrespective of drug synergy, as critical contributors to efficacious and even curative combination therapy.

Bill Forrester, Novartis Institutes for BioMedical Research

PHD: Mark Groudine, “Identification of the beta-globin locus control region”
Post-doc: Rudolf Grosschedl, “Functional elements in the immunoglobulin heavy chain enhancer region”
Asst Prof: Dept Pathology, Harvard Med School
Senior Research Investigator: Novartis

A regulated ubiquitin catastrope

We have identified a subset of ovarian cancer patients that are deficient for the expression of a gene critical for the establishment of cellular ubiquitin levels. The polyubiquitin gene UBB is transcriptionally repressed in approximately 30% of high grade serous ovarian cancers (HGSOC) leading to a dependence on the polyubiquitin gene, UBC. Inhibition of UBC is lethal in UBB-repressed cells. This synthetic lethal relationship was validated in vivo, finding robust regressions of established orthotopic ovarian tumors following inducible expression of shRNA targeting UBC. Transcriptional repression of UBB is a cancer subtype-specific alteration that occurs in a significant population of patients with cancers of the female reproductive tract, including uterine carcinosarcoma, endometrial carcinoma, as well as HGSOC, where it is associated with poor survival outcomes. The prognostic value and demonstrated efficacy establishes these observations as clinically relevant and defines UBC as an important target for precision medicine among ovarian cancer patients. This work describes for the first time a recurrent cancer-specific lesion at the level of ubiquitin production.

Sandro Santagata, Brigham and Women's Hospital

Sandro is an assistant professor in the pathology department at Brigham and Women's Hospital where he practices neuropathology. His group investigates stress responses in cancer and the molecular mechanisms of brain tumors. Along with the Lab for Systems Pharmacology he is implementing multiplexed methods for characterizing tissue responses at a single cell level.

High dimensional pathology for clinical research

Debora Marks, Harvard Medical School

Debora Marks received her BSc in Mathematics from the University of Manchester. After a career in the pharmaceutical industry, she returned to research and obtained her PhD in Mathematical Biology from Humbolt University where she was the first to define and quantify the potential targeting scope of microRNAs and their combinatorial regulation of protein expression. Marks conducted her postdoctoral work at Harvard Medical School where she developed an evolutionary approach to accurately predict large pharmaceutically relevant membrane proteins (600 amino acids), and the 3D structures of multi-protein complexes. While performing this work, she initiated an extensive network of collaborations to investigate the structures of proteins of specific biological and medical importance.

Marks joined the Systems Biology Department as an Assistant Professor in 2014. She is the Inaugural Director of the Raymond and Beverly Sackler Laboratory at Harvard Medical School and in 2016, she was awarded the Overton Prize by the International Society for Computational Biology.

Methods to identify combinations of alleles that are associated with antimicrobial resistance.

The acquisition of multidrug resistance does not always decrease biological fitness and can therefore result in the persistence of resistant strains, even in the absence of antimicrobial selection pressure. Genome sequencing of pathogens from infected patients tested against a range of antimicrobials provides a unique opportunity to investigate genomic background of multidrug-resistance in some of the World Health Organization "highest priority" pathogens. There is a great deal of knowledge about specific alleles and their contribution to resistance to a wide range of antimicrobials. However, little is known about whether there are combinations of alleles that contribute and the identification of any combinations has been recalcitrant to statistical techniques due mostly to the combinatorial explosion. Debbie will present a new statistical approach that identifies background alleles that may act in combination with known alleles to contribute to the persistence of resistance.

Toufiq Parag, Harvard School of Engineering and Applied Sciences

Dr Toufiq Parag is a research scientist in Professor Hanspeter Pfister's lab at SEAS, Harvard University. He received in PhD in computer vision and machine learning from Rutgers University. After receiving his PhD, Dr Parag worked at HHMI Janelia Research Campus on EM connectomics for multiple years. At Janelia, he developed machine vision algorithms for automated neural reconstruction that have been widely used in connectomics community and enabled Janelia researchers to reconstruct multiple areas of fruit fly brain. Novel biological findings from these studies have been published in PNAS, eLife journals; in addition, Dr Parag has many publications in both machine vision and medical vision venues and has been serving on the program committees of these meetings for several years. At Harvard, he is involved in a large scale effort for mouse brain EM reconstruction funded by IARPA.

EM Connectomics : Discovering the biological neural network of animal brain

Exhaustive knowledge of biological neural networks possesses immense value to the field of neuroscience. Recent advances in Electron Microscopy (EM) imaging have enabled neurobiologists to reveal the finest detail of brain wiring diagram. However, recording neurons at nanometer scale yields vast amount of data even from a few hundreds of cubic microns of brain tissue. In addition, the extremely intricate organization of neural cells makes it infeasible to determine the wiring diagram from an enormous dataset in a reasonable amount of time through human tracing. One of the goals of EM connectomics is to reconstruct the biological neural network using intelligent computational platforms. I will present one such intelligent computing framework for neural reconstruction, show some existing results and briefly sketch future research directions towards fully automated reconstruction.

Artem Sokolov, Harvard Medical School

Artem completed his PhD in Computer Science and Bioinformatics at Colorado State University under the supervision of Asa Ben-Hur, with whom he worked on developing novel state-of- the-art methods for protein function prediction. His postdoctoral work at the University of California Santa Cruz focused on building robust, interpretable in silico models of human cancers and correlating these model with biological and clinical outcomes as part of his involvement in The Cancer Genome Atlas (TCGA) and the West Coast Dream Team (WCDT) consortia.

As Director of Informatics and Modeling at the LSP, Artem leads a group of computational biologists and software engineers who model pre-clinical, translational and clinical data using a wide range of machine learning and artificial intelligence approaches. The LSP is unusual in that nearly half of the total academic effort is dedicated to computation and modeling. Artem plays a leading role in training and mentoring a diverse group of students and postdocs in managing the lab’s collaborations with academic and industrial groups

Towards integration of single-cell data

As single-cell technologies take center stage in data collection, the need arises for effective data integration methods. A specific question is how to utilize single-cell data to glean additional information from bulk RNAseq, which is still the most readily available and prevalent form of sequencing today. In this talk, I explore a simple sample deconvolution task, where the goal is to infer tumor purity from matching CyCIF and bulk RNAseq data. The talk highlights a number of challenges presented by each platform and discusses how prior biological knowledge can be used to overcome these.

Leonid Peshkin, Harvard Medical School

Leon Peshkin is a member of the Research Faculty at the Department of Systems Biology, Harvard Medical School. Leon's graduate studies were done in the field of Applied Mathematics at the Weizmann Institute of Science and Machine Learning at the Brown University and MIT. His current interests span systems embryology, quantitative proteomics and reverse engineering of developmental pathways.

Bayesian Confidence Intervals for Multiplexed Proteomics

Multiplexed proteomics has emerged as a powerful tool to measure protein expression levels across multiple conditions. The relative protein abundances are inferred by comparing the signal generated by isobaric tags, which encode the samples' origins. Intuitively, the trust associated with a protein measurement depends on the similarity of ratios from different peptides and the signal level of these measurements. Up to this point in the field, peptide-level information has not typically been integrated into confidence, and only the most likely results for relative protein abundances are reported. If confidence is reported, it is based on protein-level measurement agreement between replicates. Here we present a mathematically rigorous approach that integrates peptide intensities and peptide-measurement agreement into confidence intervals for protein ratios (BACIQ). The main advantages of BACIQ are: 1) it removes the need to threshold reported peptide signal based on an arbitrary cutoff , thereby reporting more measurements from a given experiment; 2) confidence can be assigned without replicates; 3) for repeated experiments BACIQ provides confidence intervals for the union, not the intersection, of quantified proteins; 4) for repeated experiments, BACIQ confidence intervals are more predictive than confidence intervals based on protein measurement agreement. Therefore, our method drastically increases the value obtained from quantitative proteomics experiments and will help researchers to interpret their data and prioritize resources.

Kris Sarosiek, Harvard School of Public Health

Kristopher received his Ph.D. in Molecular and Cellular Pharmacology from the University of Miami School of Medicine under the mentorship of Izidore Lossos, MD. After his graduate studies, Kris joined the laboratory of Anthony Letai, MD, PhD, at the Dana-Farber Cancer Institute/ Harvard Medical School for his postdoctoral fellowship. Working with colleagues, Kris found that some cancer cells are more primed to undergo apoptosis (programmed cell death) than others, as measured by a novel assay called BH3 Profiling (Ni Chonghaile & Sarosiek, et al., Science, 2011). Cancer cells and patient tumors that are more primed to undergo apoptosis are consequently more sensitive to chemotherapy treatment. This finding potentially explains why some patients respond favorably to chemotherapy while others do not. Kris has also utilized BH3 profiling to identify novel interaction preferences among the BCL-2 family of proteins, finding that BID preferentially activates BAK while BIM preferentially activates BAX to trigger apoptosis (Sarosiek, et al., Molecular Cell, 2013). More recently, Kris has characterized how apoptosis is regulated in healthy tissues and how this impacts cell fate decisions in response to damage and stress (Sarosiek, et al., Cancer Cell, 2017). In 2016, Kris started his laboratory at the Harvard School of Public Health. The long-term goal of his laboratory is to develop a better understanding of how cell death is regulated in healthy and diseased cells in order to expose novel opportunities for therapeutic intervention.

Dynamic regulation of apoptosis in healthy tissues as a driver of therapy-associated toxicities

Pediatric cancer patients treated with radiation therapy or chemotherapeutic agents are particularly at risk of developing devastating toxicities including neurocognitive decline and chronic heart failure, limiting the use of potentially curative therapies. These treatments, which rely on the preferential induction of an apoptotic cell death in cancer cells over healthy tissues, are comparatively well tolerated in adults, yet the molecular basis for this contrast in sensitivity is unknown. By using BH3 Profiling to test the functional state of the mitochondrial apoptotic pathway, we make the surprising discovery that many adult somatic tissues including the brain, heart, kidneys and skeletal muscle are apoptosis refractory, or lack the ability to undergo apoptosis. In stark contrast, we find that these same tissues in young mice and humans are extremely primed for apoptosis, resulting in hypersensitivity to ionizing radiation and cytotoxic chemotherapies. The high level of priming in young tissues is actively driven by c-Myc, which promotes the expression of many critical pro-apoptotic genes including BAX and BAK, which are then strongly downregulated postnatally during the transition to apoptotic resistance in adult tissues. Importantly, we use mouse models to show that these pathways may be modulated to potentially prevent treatment-associated toxicities. Our findings identify the molecular mechanisms that contribute to the devastating vital organ sensitivity to genotoxic damage in children and strategies for its prevention.

Michael Baym, Harvard Medical School

Michael Baym received his PhD in Mathematics from MIT and was a postdoctoral fellow at Harvard Medical School in Systems Biology. Baym's research is centered around the problem of antibiotic resistance, at the intersection of experimental, theoretical and computational techniques. His work ranges from understanding the basic mechanisms of evolution to the development of algorithms for computation on massive biological datasets.

Evolutionary approaches to antibiotic resistance

Steve Rodriguez, Massachusetts General Hospital

Steven got his Ph.D. studying the development and connectivity of neurons in the mouse olfactory neural circuit in the lab of David Lin at Cornell University. During his work he found a novel non-cell-autonomous role for Notch2, a gene known for its role in development, in maintaining neuronal viability in adult mice. This work got him interested in studying mechanisms of neurodegeneration, so he joined Mark Albers' lab at the MassGeneral Institute for Neurodegenerative disease at MGH. In Mark's lab, he has been using the main olfactory system to study mechanism of neurodegeneration where he found a novel mechanism for DNA damage induced activation of antiviral innate immune pathways. Steven joined the Lab of Systems Pharmacology in 2015 where he continues to work on antiviral signaling and identifying therapeutic approaches to treat neurodegenerative disease.

Neurodegeneration mediated by a neuronal antiviral innate immune signaling pathway

Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) are two related neurodegenerative diseases that afflict tens of thousands of people. There are currently no therapies to treat these diseases necessitating a better understanding of the mechanisms of neurodegeneration. We find evidence for elevated antiviral innate immune signaling and elevated dsRNA, an established antiviral innate immune pathway stimulant, in brains with FTD and ALS. We show that dsRNA can trigger neuronal activation of antiviral signaling and is sufficient to cause neurodegeneration in vivo and in vitro. Multiple studies performed with multiple non-CNS cell types show that TBK1 is required to activate antiviral innate immune signaling. Paradoxically, autosomal dominant null mutations in TBK1 (TANK-binding Kinase 1) have been reproducibly linked to both FTD and ALS. Using small molecule inhibitors and genetics we find that TBK1 is not required for dsRNA mediated antiviral signaling in a human neuronal stem cell culture model. Interestingly, a proteomic analysis of TBK1 deficient neuronal cultures shows elevated expression of antiviral innate immune signaling pathways, which normally require TBK1 for activation. These data highlight how signaling pathways that have been defined in the periphery can be unique in the nervous system. The development of better therapeutic strategies will require a better understanding of intracellular signaling networks, including innate immune signaling, in the nervous system.

Jeremy Jenkins, Novartis Institutes for BioMedical Research

Jeremy L. Jenkins is Executive Director, Head of Chemical Biology & Therapeutics (CBT) Informatics, and Novartis Leading Scholar at the Novartis Institutes for BioMedical Research in Cambridge, MA. He is responsible for data science teams involved in computational profiling, screening, and engineering. Jeremy's research has focused on integrating the fields of cheminformatics and chemical biology, with an emphasis on structuring knowledge. Jeremy was the 2011 Corwin Hansch Award winner for contributions to the field of QSAR. He was a Novartis Presidential Postdoc, following postdoctoral research in the lab of Dr. Robert Shapiro at Harvard Medical School and receiving a PhD in Molecular Genetics from The Ohio State University in 2000.

Cheminformatics in phenotypic drug discovery

As chemical biology increasingly shifted drug discovery towards more phenotypic approaches, a biology-infused form of cheminformatics evolved as the discipline in silico chemical biology. Impactful methodologies developed in NIBR will be briefly described.

Nienke Moret, Harvard Medical School

Nienke's work revolves around understanding functional polypharmacology in small molecule probes and drugs. Where it was once thought that the most selective molecules would make the most efficacious and safe therapeutic compounds, we now come to understand that – especially for cells that are genomically stable– the genomic redundancy of a cell requires drugs to inhibit more than one enzyme to be effective. However, discovering which enzymes should be targeted concomitantly remains a daunting task.

After studying the cello, envisioning a career music, Nienke started her scientific studies at the university of Amsterdam studying chemistry and pharmaceutical sciences. Currently, Nienke is a PhD candidate in Harvard's chemical biology program. Nienke is a graduate student in the Laboratory in Systems Pharmacology (LSP) and a part-time intern at the Novartis Institute for Biomedical Research (NIBR) where she works with Jeremy Jenkins. At NIBR, Nienke works at understanding genomic redundancy in more detail, while she applies that knowledge to develop small molecule based immune-therapies in the LSP.

Jeremy Muhlich, Harvard Medical School

As the Director of Software Engineering for the LSP, Jeremy Muhlich helps bring computational tools to bear in the areas of mathematical modeling, data management and reproducible research. He and the other Informatics team members are also working to teach Python programming to all LSP scientists to empower them in their own computational analyses.

Building cheminformatics tools and resources for LSP

High throughput screening (HTS), classically developed as a method for screening millions of small molecule compounds at a time for activity against a single gene product or other simple phenotype, has become a standard tool in translational research and provides a key ingredient for large-scale data warehouses in industry. However, even the more modestly-scaled experiments routinely performed by LSP scientists can yield broadly useful information about compound activity given the right experimental designs and analytical framework.

During this session, we will discuss our ongoing efforts to improve the cheminformatics tools and resources in the LSP. We will begin by demonstrating tools that assist individual researchers with small molecule selection and mechanism of action deconvolution, and conclude by outlining vision for an LSP database of small molecule profiles built from both public and internal datasets.

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Poster Abstracts

1. Programmable Nanocapsules for Drug Delivery Using DNA Origami

Frances Anastassacos

This poster presents the development pipeline of therapeutically applied DNA origami nanocapsules and the preliminary results of their development. Our final objective is the fabrication of a pH-responsive, modular nanocapsule able to transport versatile DNA-conjugated payloads. We further envision these nanocapsules to act as versatile nanoscale pegboards for 3D arrangement of decorative surface ligands. By investigating the effects size, shape, surface decoration and cargo release mechanisms, we present a highly versatile and programmable drug delivery vehicle that can be optimized for a wide range of diverse drug targets.

2. Systemic Lymphoid Architecture Response Assessment (SYLARAS): an approach to discovery-based, system-wide immunophenotyping

Greg Baker

Interactive flow cytometry analysis software allows users to make binary decisions on immunomarker status of single-cells through arbitrary placement of one- and two- dimensional gates. However, manual gating through a point-and-click graphical user interface (GUI) is impractical for large datasets. Here we describe SYLARAS, a suite of experimental and computational tools for the systematic and chronological evaluation of lymphoid tissue architecture. SYLARAS takes multidimensional, quantitative single-cell data as input, bins cells in n-dimensional orthant space, and returns schematized summary statistics for immunophenotypes of interest. We programmatically curated data on 33 immune cell types from a dataset consisting of 96.2 million peripheral leukocytes of immunocompetent mice bearing syngeneic, orthotopic glioblastoma (GBM). SYLARAS permitted the discovery of multi-dimensional immune signatures whose divergence from mock engrafted, age-matched controls was quantified and statistically analyzed. By correlating peripheral immune composition with the levels of 111 blood cytokines from the same mice, we uncovered novel cytokines, cell types, and correlation networks altered by this malignant brain cancer.

3. Proteomics Platform in the Laboratory of Systems Pharmacology

Matt Berberich

Matthew J. Berberich, Marian Kalocsay, Suman Rao, Kartik Subramanian, Alison Erickson, Robert A. Everley, Peter K. Sorger

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 also 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.

4. 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

5. Prediction of penicillin resistance in pneumococcus within minutes

Karel Břinda

Antibiotic resistance has become a major threat in public health. For instance, multidrug resistant gram-negative organisms have been associated with mortality rates ranging from 30% to 70%, largely due to an inappropriate use of antibiotics. Traditional methods for detecting resistance, based on cultivation, typically require several days. However, modern sequencing technologies enabled to detect resistance from sequencing reads obtained directly from a patient metagenomic sample. For instance, assembly- and alignment-based approaches have recently been shown to detect resistance of M.tuberculosis within the same day.

In this poster, we present a method for detecting antibiotic resistance in the order of minutes using mobile nanopore sequencers. Our approach combines rapid alignment-free techniques with the knowledge of the underlying population structure of the pathogen, and assessing the resistance based on the known resistance profiles.

6. Phospho-regulation of Microtubule-Binding Proteins in Response to Microtubule Destabilization

Sergine Brutus

Microtubules are constantly remodeled via plus-end dynamics in order to facilitate integral cellular functions, such as intracellular transport, migration, and mitosis. We hypothesize that cells monitor microtubule dynamics, with kinase-phosphatase signaling in place to respond to perturbations to restore healthy dynamics. This hypothesis is informed by preliminary data showing the rapid dephosphorylation of dozens of microtubule-associated proteins (MAPs) in response to microtubule destabilization, including several that are known to stabilize microtubules. The response seen with microtubule-stabilizing proteins suggest the existence of a homeostatic regulatory circuit that responds to microtubule perturbations. We have identified two such microtubule-stabilizing MAPs as potential biomarkers of microtubule destabilization that will be used to investigate this signaling circuit. We propose to identify the kinase-phosphatase regulators of this circuit, investigate the effect of MAP phosphorylation on MT binding affinity and dynamics, and determine if and how this signaling affects mitosis. In addition to uncovering fundamental aspects of microtubule biology, this work has the potential impact of providing insight into how cells respond to microtubule destabilizers, which are an important class of chemotherapeutic drugs used to treat patients in the clinic.

7. Identification of mechanisms of chemotherapy resistance using CRISPRi/a screens

Christopher Chidley

Cancer cells can evade chemotherapy through inherent or acquired mechanisms of drug resistance, which ultimately prevent a therapeutic response or lead to cancer relapse. In order to provide patients with effective treatment, it is essential to characterize the genes and pathways that enable these resistance mechanisms. Screening to determine the effect of perturbations to gene expression on the chemotherapy resistance phenotype is a powerful tool for identification of such candidate genes. Here, we developed genetic tools to reduce or increase gene expression using the CRISPRi/a platform in melanoma and lymphoma cell lines with demonstrated chemotherapy resistance. We confirmed that these tools allow specific and robust perturbation of expression of any gene. In a first application, we are performing genome-wide screens to identify genes involved in resistance to a 5-drug chemotherapy program which is clinically used for the treatment of non-Hodgkin lymphoma. In a second application, we are performing more targeted screens to find the genes in the ERK pathway which modulate the development of adaptive resistance to B-Raf inhibitor drugs used to treat melanoma. The resulting map of the effect of gene level perturbations on ERK pathway activity will also provide a quantitative framework for understanding interactions within the ERK

pathway. In sum, we demonstrate the power of systematic gene perturbation to identify mechanisms of chemotherapy resistance and explore biological networks.

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

Mirra Chung

Mirra Chung, Catlin E. Mills, Kartik Subramanian, Marc Hafner, Artem Sokolov, Chris Chen, Sarah A. Boswell, Matthew Berberich, Robert A. Everley, Peter K. Sorger

Several publications have addressed concerns surrounding drug response screens by pointing out sources of variability and by presenting recommendations for better experimental methods and more robust analytical approaches. In the first phase of the HMS LINCS breast cancer profiling effort, we selected 32 breast cancer cell lines with a strong bias towards triple negative (TNBC) lines as well as 4 cell lines established from relevant patient-derived xenografts. We evaluated a panel of 34 clinically relevant agents, biased towards kinase inhibitors and the PI3K pathway, using the microscopy-based dye-drop dose response assay to measure drug potency, and to quantify drug efficacy in terms of growth inhibition (GR metrics) and cell death. The use of GR metrics to quantify drug sensitivity enabled us to identify and study differences between cytostatic and cytotoxic responses. This systematic dose response dataset is complemented by measurements of baseline transcript expression levels by mRNAseq, quantification of absolute abundance of ~12,000 proteins, and relative phosphoprotein levels by shotgun mass spectrometry across all cell lines. Additionally, the baseline activity of transcription factors and kinases were inferred from the mRNA (using VIPER) and phosphoprotein (using kinase enrichment analysis) data, respectively. The second phase of the HMS LINCS breast cancer profiling effort is underway covering ~30 other cell lines with an emphasis on hormone receptor positive, Her2 amplified, and patient-derived models. Richer phenotypic dose response data for panel of 34 additional agents, biased towards CDK inhibitors, will be collected using the deep-dye-drop assay, and baseline omics profiles of all new cell lines will also be acquired. Overall these datasets will be a valuable resource for understanding drug response in breast cancer models, and the molecular mechanisms that influence it.

9. Distinct lipidomic signatures in high-grade pediatric brain tumors using MALDI mass spectrometry imaging

Amanda Clark

Medulloblastoma is the most common primary pediatric malignant brain tumor. Genomic and transcriptomic analyses have altered the classification of medulloblastomas by delineating subgroups. The mRNA expression patterns in these subgroups have distinctive driver mutations and genetic alterations. These genetic subtypes of medulloblastoma have distinct clinical characteristics with different ages of onset, clinical aggressiveness, and response to standard-of-care therapies. To expand our understanding of the molecular underpinnings of these tumors, we have employed matrix assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) to investigate the lipid signatures within tissue sections of medulloblastoma subtypes and have compared these signatures with those of pineoblastoma, another histologically related high-grade pediatric tumor. Our results show that medulloblastoma and pineoblastoma can be differentiated based on the lipid signature analysis. More work is warranted in this area to explore the distinct lipid signature profiles of medulloblastoma and pineoblastoma and whether such profiles can provide the framework for establishing lipid based diagnostic, prognostic, and predictive signatures for these very aggressive malignancies.

10. Characterization and target identification of novel anti-malarial candidates

Rebecca Clements

Malaria, which is caused by the Plasmodium parasite, results in more than 200 million infections and 400,000 deaths annually. Rapidly emerging resistance to first-line anti-malarials imposes an urgent need for novel therapeutics that are safe and effective. Our group has developed a nano-luciferase reporter in Plasmodium falciparum, the parasite responsible for the deadliest cases of malaria, that allows us to monitor parasite viability in order to screen for novel anti-malarial compounds. Using this assay, we identified a compound, BCH070, which has an EC50 of ~400 nM and is most effective against ring stage parasites. This compound remains active against other strains that are resistant to currently used anti-malarials, suggesting a novel mechanism of action. To identify the target(s) of BCH070, we performed chemical mutagenesis with N-ethyl-N-nitrosourea (ENU) to generate resistant parasites and are in the process of raising and cloning these lines. The long-term goal of this work is to identify and characterize a novel class of anti-malarials and to demonstrate the potential of chemical mutagenesis to aid in target identification in Plasmodium falciparum parasites.

11. The use of in vivo models to support in vitro findings 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 murine work in the Laboratory of Systems Pharmacology (LSP). 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 Crl:Nu-Foxn1nu (Nude) mice are grown to ~200 mm3 and then used for acute dose response studies or longterm tumor response experiments. PDX models are established in NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice, propagated from mouse to mouse, and used for similar studies. 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.

12. Degrading the breast cancer oncoprotein Pin1 through deubiquitinating enzyme inhibition

Laura Doherty

Deubiquitinating enzymes (DUBs) cleave ubiquitin from specific protein substrates, protecting them from proteasomal degradation. The inhibition of a specific DUB is an attractive strategy to degrade oncoproteins that are challenging to target directly. The work presented here aims to identify DUBs that stabilize oncoproteins in breast cancer in order to identify potential novel therapeutic targets. Pin1, an oncoprotein implicated in triple negative breast cancer, isomerizes proline residues of specific substrates causing conformational changes and ultimately leading to the activation/stabilization of more than forty oncoproteins and the inactivation/destabilization of more than twenty tumor suppressors. The inhibition of a single oncogenic pathway is often insufficient to prevent cancer progression; however, targeting Pin1 presents the opportunity to block multiple oncogenic pathways simultaneously, thus having the potential to be more effective. Furthermore, Pin1 is suggested to have scaffolding functions that are dependent on its binding to substrates but are independent of its catalytic activity. Therefore, degrading Pin1 by the inhibition of its DUB is attractive because it would enable the suppression of catalytic dependent and independent function of Pin1. To identify the DUB stabilizing Pin1, both small molecule and genetic approaches will be used. A unique library of pre-clinical small molecule DUB inhibitors was screened to identify degraders of Pin1 using immunofluorescence to quantify change in endogenous Pin1 abundance in response to treatment. The next steps of this work will be to use CRISPR Cas9 to systematically knockout each of the approximately one

hundred DUBs individually and assay Pin1 abundance, as well as a set of other oncoproteins, signaling pathways, and cellular processes. This broadened approach will identify the DUB stabilizing Pin1, as well as identify DUBs modulating other targets and pathways of interest using highly multiplexed cyclic immunofluorescence imaging.

13. Interferon Effectors as a Platform for Broad Acting Antiviral Design

Dahlene Fusco

definitions: interferon effector gene (IEG): gene whose product is required for interferon antiviral effects. interferon stimulated gene (ISG): gene upregulated at mRNA level by interferon

PROBLEM: The Zika virus (ZIKV) epidemic has coincided with a rise in incidence of microcephaly and congenital malformation in North and South America. The relative contributions of viral invasion and host antiviral response to ZIKV teratogenicity remain incompletely understood. No specific treatments or vaccine for ZIKV exist. Type I interferons (IFN) suppress ZIKV and other flaviviridae in vitro, but clinical utility is limited due to viral inhibition and multiple side effects. Like other flaviviridae, ZIKV subverts the IFN response by inhibiting STAT1/2. Flaviviral pathology studies and therapeutic development for have been hampered by lack of in vivo models that reproduce both infection and the innate antiviral immune response. Current mouse models for flaviviral infection include IFNAR1/2 and STAT2 knockout mice.

BACKGROUND DATA: We performed a whole genome RNAi screen to identify host factors required for IFN-mediated suppression of fully infectious hepatitis C virus (HCV), using high throughput microscopy readout, and identified 120 IFN effector genes. The majority of these genes had not previously been linked to the host IFN response. We subsequently performed RNAi screening of these 120 HCV IFN effectors and found that 56 of the 120 gene candidates were also required for IFN-mediated suppression of fully infectious DENV NGC2. The HCV-DENV IEGs are notably enriched for genes encoding nuclear receptor interacting factors. One of these genes, HELZ2, is both an IFN effector gene (gene product required for IFN antiviral effects) and an IFN stimulated gene (upregulated at the mRNA level by IFN). As an ISG product, HELZ2 lies downstream of the STAT1/2 viral inhibition point. Our preliminary data reveals that HELZ2 is required for IFN-mediated suppression of fully infectious ZIKV in cell lines. These data have led to the following

HYPOTHESES:

1. HELZ2 knockout will enhance ZIKV infection in vivo.

2. HELZ2 knockout mice will exhibit enhanced teratogenicity phenotype, compared to ABR mice, due to presence of partial IFN signal transduction. PRELIMINARY RESULTS: We are now performing in vivo comparisons of ZIKV teratogenicity in HELZ2-/- vs WT mice using ZIKV

Uganda and Puerto Rico strains delivered through intravaginal infection at E5. In our pilot studies, we have established timed breedings of HELZ2-/- and WT control mice, and have detected and quantified ZIKV RNA in liver, spleen, and blood of dams, and fetal tissue, in HELZ2 knockout mice, revealing ZIKV susceptibility of this mouse model. Comparison of number of fetuses between infected and uninfected HELZ2-/- and WT mice, fetal weights and morphology, and head circumference / cranial bone and retinal pathology are in progress. No behavioral differences have been noted between infected and uninfected dams to date. Skeletal CT scan studies are pending at this time.

SUMMARY: Whole genome RNAi screening, followed by a second targeted RNAi screen, identified 56 IFN effectors, required for IFN-mediated suppression of both HCV and DENV. Because HELZ2 lies distal to the STAT1/2 common viral subversion point, HELZ2 may be a useful target for broad-acting host directed antiviral design. Prior to pursuing such studies, validation of HELZ2 as an IFN effector in vivo is necessary. The first step in this process is determining whether HELZ2 knockout leads to enhanced flaviviral permissivity, or IFN resistance, compared to WT controls. An advantage of HELZ2 knockout mice, compared to the current standard of ABR mice, is maintenance of the first several steps of IFN signal transduction, allowing improved resolution of host innate inflammatory contribution to flaviviral pathology. Ultimately these mice, and other IEG knockout mice, may serve as invaluable tools for the preclinical testing of much needed therapeutics for acute flaviviral infections.

Authors: Kamela Bellovoda, Marco Menara, Scarlett Se Yun Cheon, Magali St. Geniez, Maria Troulis, Dahlene Fusco.

14. mIFN anti DENV : a Targeted Therapy for DENV Infection

Dahlene Fusco

There are limited treatments for acute viral infections. Acute infection with dengue virus (DENV) is a global problem and can be complicated by hemorrhagic fever and death. No effective therapies exist for DENV infection, and limited data is available to accurately predict which patients will succumb to severe DENV, either during primary or secondary infection.

Type I interferons (IFNs) are among the broadest acting antivirals known, but, when delivered post-infection, IFN effects are limited by viral subversion. Furthermore, IFN is limited by major toxicities.

We hypothesized that optimized delivery of IFN to DENV-infected cells could enhance IFN antiviral effects.

Together with the Laboratory of Jeffrey Way, we have developed an optimized IFN directly fused to a human anti-DENV antibody, mIFN-antiDENV. We here show that mIFN-antiDENV effectively suppresses DENV NGC2 in a post-infection treatment model, as measured by

percent infected cells (Huh7.5.1). mIFN-antiDENV also suppresses DENV viral loads, as measured using qRT PCR.

Future directions include immunofluorescence studies of mIFN-antiDENV localization to DENV infected cells and testing of mIFN-antiDENV in a relatively immunocompetent mouse model for improved toxicity profile compared to conventional IFN.

15. 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. To this end, the Langenau lab developed an unbiased transplantation screen in zebrafish to identify molecular targets that promote T-ALL relapse. In T-ALL, aberrant MYC activity is a dominant oncogenic lesion. To identify MYC-collaborating genes, we overexpressed MYC to generate T-ALL that recapitulated the most common and treatment resistant subtype of T-ALL by morphology and microarray expression. Matched relapsed leukemias were analyzed by comparative genomic hybridization array to identify genome amplifications. Phosphatase of regenerating liver 3 (PRL3), a protein tyrosine phosphatase, was identified as a focal amplification in the genome of tumors with high relapse potential. In humans, PRL3 is not expressed by normal hematopoietic progenitor cells, but is highly expressed in >65% of primary T-ALL samples. Of note, the most aggressive and hard to treat human T-ALL samples have the highest expression of PRL3. Additionally it was determined that genetic and enzymatic inhibition of PRL3 in human T-ALL cell lines rapidly kill those lines via T-cell signaling induction. By studying the mechanisms by which PRL3 promotes tumor survival, a novel therapeutic approach to block relapse in T-ALL may be generated.

16. Functional TRIM24 degraders via conjugation of ineffectual bromodomain and VHL ligands

Lara Gechijian

The addressable pocket of a target protein is often not functionally relevant in disease. This is particularly true for multidomain gene regulatory proteins, such as the bromodomain-containing transcriptional regulator TRIM24. TRIM24 has been posited as a dependency in numerous human cancers, yet potent and selective ligands for the TRIM24 bromodomain do not exert effective anti-proliferative responses. We therefore repositioned these probes as

targeting features for heterobifunctional protein degraders. Recruitment of the VHL E3 ubiquitin ligase by a lead molecule, dTRIM24, elicits rapid, potent, and selective degradation of TRIM24. Using dTRIM24 as a probe of TRIM24 function, we characterize the dynamic genome-wide consequences of TRIM24 loss on chromatin localization and target gene control. Further, we identify TRIM24 as a novel dependency in acute myeloid leukemia. Pairwise study of TRIM24 degradation versus bromodomain inhibition reveals enhanced anti-proliferative response from target protein degradation. We offer dTRIM24 as a chemical probe of an emerging cancer dependency, and establish a path forward for numerous selective yet ineffectual ligands for proteins of therapeutic interest.

17. Upregulation of microRNA-132 following kidney injury promotes tubular cell sensitivity and fibrosis development in mice

Cory Gerlach

MicroRNAs (miRNAs) are increasingly shown to regulate multiple signaling pathways that can either promote or resolve disease progression. We previously identified that miR-132 expression positively correlated with kidney tubular injury in mice and humans. To investigate the functional role of miR-132 in kidney damage, we subjected miR-132 knockout (KO) mice to folic acid-induced nephropathy. As a result, we found that wild-type and miR-132 KO mice had similar kidney damage on day 1 but that by day 2 miR-132 KO mice had significantly less tubular injury (indicated by histopathology and expression of Kidney Injury Molecule-1 [KIM-1]) and improved kidney function (indicated by measuring levels of blood urea nitrogen). Importantly, by day 14, when evidence of fibrosis was clear in wild-type mice, miR-132 KO mice had less expression of extracellular matrix proteins, less myofibroblasts and less inflammation. This suggests that miR-132 KO mice had improved kidney repair that resulted in the amelioration of chronic disease. To understand the mechanism of miR-132 in the kidney, we conducted proteomics using tandem mass tags in Human Proximal Tubular Epithelial Cells (HPTECs) transfected with miR-132 mimic or scrambled RNA. Out of the 70 proteins ≥1.5-fold downregulated by miR-132, nine proteins overlapped as miR-132 targets using five open-source miRNA target prediction algorithms. We confirmed the interaction of miR-132 with RASA1 (inhibitor of Ras) and SOD2 (detoxifier of mitochondrial ROS). Next, we hypothesized that the miR-132-mediated decrease in RASA1 would lead to increased HPTEC proliferation as a result of decreased cell cycle arrest. Therefore, we transfected HPTECs with miR-132 mimic and treated them with TGF-β, a known inducer of p21-dependent cell cycle arrest. Using quantitative imaging, we found that TGF-β reduced HPTEC proliferation by 62% and increased p21-positive cells by 32% ($p < 0.05$). Interestingly, miR-132 mimic in the presence of TGF-β decreased p21 to control levels and rescued the TGF-β-mediated cell cycle arrest. Conversely, we hypothesized that the miR-132-mediated decrease in SOD2 could increase HPTEC susceptibility to toxicity. We treated HPTECs with cisplatin and found that miR-132 mimic resulted in ~2.5-fold increase

in cell death ($p < 0.05$). Taken together, we conclude that miR-132 increases tubular epithelial cell sensitivity by: 1) downregulating RASA1 and promoting proliferation upon injury, and 2) downregulating SOD2 thereby decreasing the ability to respond to toxic stress. This suggests that miR-132 inhibition following kidney injury could improve tubular repair and prevent the development of fibrosis.

18. Stem Cell-Targeted Small Molecule Therapy for Spinal Muscular Atrophy

Rebecca Gibbs

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by insufficient expression of the Survival of Motor Neuron protein. SMA is the most common genetic cause of infant mortality, with an incidence of 1/6000 live births, and there is currently no cure. SMA is primarily characterized by motor neuron death in the spinal cord, and most therapies in clinical development focus on preventing nervous system defects. However, we have shown that SMN deficiency directly causes failures in muscle growth, independent of neurodegeneration, suggesting that correction of muscle pathology may be a critical approach for restoring longevity and quality of life to patients.

We recently devised a novel therapeutic strategy that leverages the intrinsic regenerative capacity of skeletal muscle to increase muscle growth. In adults, skeletal muscle stem cells known as satellite cells reside in a niche surrounding the muscle fiber. Satellite cells are usually quiescent, but in response to muscle damage they activate and proliferate to repair muscle fibers. We performed a high throughput chemical screen and identified small molecules that promote wild type satellite cell proliferation and stimulate muscle growth in vivo. Ongoing work aims to determine if small molecule-based modulation of endogenous satellite cells can promote functional muscle growth and ameliorate motor deficits in SMA models.

19. Tethered transcription system for cytoplasmic RNA production

Richard Guerra

Ribonucleic acids (RNA) are currently being explored for a number of immunotherapeutic applications, including antigen-encoding mRNAs, immuno-regulatory shRNAs/miRNAs, and RNA-based genetic circuits. As with all RNA-based therapies, their efficacy depends on the efficient delivery of RNA into the cell cytoplasm. Existing strategies have sought to improve RNA delivery by packaging them within formulations that mimic the shape and size of natural viruses. A complementary strategy is to recapitulate the replicative capacity of viruses, by making it possible to rapidly manufacture large quantities of RNA directly within the target cell.

20. A Novel Selectivity Determinant Informs the Development of Next-Generation Stapled Peptide Inhibitors of Anti-apoptotic BFL-1/A1

Edward Harvey

The development of selective inhibitors for the individual anti-apoptotic BCL-2 family proteins implicated in oncogenesis and chemoresistance remains a formidable but pressing challenge. Precisely tailored compounds would serve as ideal molecular probes and targeted therapies to respectively study and treat human cancers driven by discrete apoptotic blockades. The remarkable potential of this strategy is exemplified by ABT-199, a potent and selective small molecule BCL-2 inhibitor that is proving effective at reactivating apoptosis in BCL-2-dependent cancers. The BCL-2 protein homologue, BFL-1/A1, has emerged as a resistance factor in a series of human cancers, including melanoma, leukemia, and lymphoma, but remains undrugged. To identify binding and specificity determinants for targeting BFL-1/A1, we screened a library of hydrocarbon-stapled BH3 domain helices and identified a subclass of compounds that engage BFL-1/A1 with exquisite selectivity in vitro and in situ. The mechanistic basis for this specificity revealed a unique topographic feature of the BFL-1/A1 binding groove. Leveraging this natural BFL-1/A1 selectivity factor, we designed next-generation stapled peptide inhibitors that block BFL-1/A1's capacity to both bind BH3 domains and suppress BAX-mediated membrane poration. Our studies inform a new pharmacologic strategy for potent and selective inhibition of anti-apoptotic BFL-1/A1 in human cancer.

21. Application of Hydrogen-Deuterium Exchange Mass Spectrometry to Interrogate the Conformational Regulation of BCL-2 Family Proteins

Zachary Hauseman

BCL-2 family proteins are critical apoptotic regulators, whose essential functions are mediated by protein-protein interaction and conformational alteration. For example, BAX is a multidomain pro-apoptotic member that translocates from cytosol to the outer mitochondrial membrane in response to cellular stress, and undergoes a series of ligand-induced conformational changes that ultimately lead to pore formation. Defining the structural alterations induced by activating and inhibitory interactions, in real-time and in the membrane environment, poses a significant challenge for traditional NMR and X-ray crystallography approaches. Here, we describe the application of hydrogen-deuterium exchange mass spectrometry (HXMS) to interrogate the dynamic interactions of BCL-2 family proteins with BCL-2 homology domain ligands, and the structural and functional consequences. By tracking the solvent accessibility of backbone amide hydrogens, we can determine the structural requirements for ligand engagement of BCL-2 family proteins and the conformational changes induced in the protein target upon ligand interaction, both in solution and in a membrane environment. Thus, we find that HXMS is a powerful approach for identifying ligand binding

sites, allosteric mechanisms, and time-dependent conformational changes, yielding fresh insights into the structure and function of BCL-2 family proteins.

22. High-dimensional single cell analysis of drug mechanism and toxicity with cyclic immunofluorescence (CycIF)

Connor Jacobson

23. Characterization of the Notch Pathway Regulator NRARP

Sanchez Jarrett

Notch signaling plays a vital role in a wide range of cell fate decisions in animals ranging from sea urchins to humans. The importance of Notch signaling is evident from Notch loss-of-function in model organisms, and from the developmental anomalies in humans that result from mutations that disable Notch signaling. In addition, aberrant or dysregulated Notch signaling is associated with several different human cancers, most notably T cell acute lymphocytic leukemia, where activating mutations of the Notch1 receptor are found in more than half of all cases. Thus, understanding how Notch signals are modulated by regulatory proteins is of fundamental significance in both normal development and disease states. The Notch regulated ankyrin repeat protein (NRARP) acts as a negative feedback regulator of Notch signaling in higher organisms. The importance of NRARP function is evident from the observed phenotypes in knockout models such as mice, which include skeletal defects and vessel regression. Using an unbiased proteomics approach, we have established that NRARP directly interacts with the core Notch transcription activation complex in T-ALL cells. Additionally, with mapping studies we have identified the molecular determinants for this interaction. We are now poised to elucidate the structural basis for NRARP recruitment into Notch transcription complexes and determine the underlying molecular mechanism for assembly of NRARP inhibitory complexes. Together with cellular studies we will fill a fundamental gap in our understanding of a central mechanism of Notch signal modulation.

24. Protein targeting from the endoplasmic reticulum to lipid droplets: probing the function of Rab18

Christina Jayson

Lipid droplets (LDs) are dynamic organelles composed of neutral lipids (i.e. triacylglycerols [TG] and cholesterol esters) bound by a phospholipid monolayer in which TG synthesis and lipolysis enzymes are embedded. Proteins that localize to LDs from the endoplasmic reticulum (ER) and

cytosol play key roles in catalyzing and regulating energy storage and metabolism of LDs to meet the cell's energy needs. Dysregulation of LD metabolism has been implicated in metabolic diseases including obesity and dyslipidemia. Despite the fundamental importance of protein targeting to LDs for the regulation of LD homeostasis, the mechanism by which proteins gain access to LDs is incompletely understood. One hypothesis is that proteins mediate the formation of membrane bridges between the ER and LDs, giving ER resident proteins access to the LD monolayer. A candidate that potentially mediates this process is the LD Rab GTPase, Rab18. Numerous studies have been conducted in knockdown cell models to test the effect of Rab18 on LD expansion and catabolism, however, a comprehensive Rab18 knockout study of LD metabolism is lacking. To address this, we generated a Rab18 knockout mammalian cell line using CRISPR/Cas9 to probe the effects of Rab18 on LD metabolism. Our studies indicate that Rab18 does not play a key role in regulating LD expansion, lipolysis or effect protein targeting to LDs from the ER.

25. Development of in situ BH3 profiling to predict apoptotic sensitivity of various cells within an intact tissue

Gaurav Joshi

BH3 profiling has become a valuable tool to predict apoptotic sensitivities of various cell lines and cells from healthy and tumor derived tissues. Current methods, though very powerful, are disruptive as they use cell suspension. In order to understand any heterogeneity amongst various cells or cell types within its native environment we have developed mitochondria based in situ profiling and cytochrome c based in situ (cis) BH3 profiling. Using cis BH3 profiling in lung tissue slices we find a heterogeneous response to Bim peptide from different cells. Various cell types need to be further characterized to understand this diversity.

26. Systems and Chemical Biology Approaches for the Study of Innate Immune Signaling Pathways

Peter Koch

27. Which biochemical functions of O-GlcNAc Transferase are required for cell viability?

Zebulon Levine

O-GlcNAc Transferase (OGT) is the only enzyme responsible for installing the monosaccharide GlcNAc onto hundreds of nuclear and cytoplasmic proteins. It has also recently been show to catalyze the proteolytic maturation of the transcriptional coactivator Host Cel Factor 1 (HCF1). It is required for cell viability in dividing mammalian cells; however, it is not clear why these functions are necessary for cell survival. Combining genetic replacement in living cells with mutations that allow biochemical separation of functions, we have investigated the role of each of OGTs catalytic activities in cell proliferation and survival.

28. Study of different pik3ca mutations in HR+ breast cancer cell lines

Kyun Lim

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3Kinase) and its downstream Akt/mTOR pathway are abnormally activated in many cancers. PI3Kinase is composed of p85 regulatory subunit and p110 catalytic subunit. pik3ca encodes p110 alpha isoform, of which mutation has been found in diverse cancers. For example, in breast cancer, pik3ca mutation is one of the most highly mutated genes, especially in the hormone receptor positive (HR+) and HER2 amplified tumors.

Two hotspot mutation sites are reported in Helical domain (HD) and Kinase domain (KD), both of which can induce the abnormal Akt activation as well as the cellular transformation. Beyond these common effects, there are also evidence supporting that the underlying mechanism of these oncogenic mutations are different.

We compared these mutations in the context of drug treatment in HR+ breast cancer cell lines. In the single cell analysis with cyclic immunofluorescence (cycIF), we found ERK activity remained high, specifically in the proliferating subpopulation after Fulvestrant (estrogen antagonist) and Alpelisib/BYL719 (PI3Kinase alpha inhibitor) treatment. This was observed only in the cell lines with HD mutation, not in the lines with KD mutation. In the combination with Fulvestrant and Alpelisib/BYL719, Trametinib (MEK inhibitor) made substantial effect in HD-mutation cell lines, indicating that ERK activity is functionally important in the cells with HD-mutation. Trametinib made strong synergistic effect with Fulvestrant and Alpelisib/BYL719 treatment, suggesting that patients with HR+ and pik3ca HD mutation may benefit from it.

29. A simple open-source method for highly multiplexed imaging of single cells in tissues and tumours

Jia-Ren Lin

Intratumoural heterogeneity strongly influences the development and progression of cancer as well as responsiveness and resistance to therapy. To improve our ability to measure and analyze such heterogeneity we have developed an open source method for fluorescence imaging of up to 60 protein antigens at subcellular resolution using formalin-fixed, paraffin-embedded (FFPE) tissue samples mounted on glass slides, the most widely used specimens for the diagnosis of cancer and other diseases. As described here, tissue-based cyclic immunofluorescence (t-CyCIF) creates high-dimensional imaging data through successive acquisition of four-color images and requires no specialized instruments or reagents. We apply t-CyCIF to 14 cancer and healthy tissue types and quantify the extent of cell to cell variability in signal transduction cascades, tumor antigens and stromal markers. By imaging immune cell lineage markers we enumerate classes of tumour-infiltrating lymphocytes (TILs) and their spatial relationships to the tumor microenvironment (TME). The simplicity and adaptability of t-CyCIF makes it a powerful method for pre-clinical and clinical research and a natural complement to single-cell genomics.

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

Mark Lipstein

The next generation of drug discovery may identify therapeutics targeting non-enzymatic moieties currently considered “undruggable". Protein-protein and protein-DNA interactions alongside allosteric modulators represent new pharmacological modalities with unique specificity and the ability to drug targets etiological to complex diseases3-5. Though for potential therapeutics to be discovered, the underlying assays and detection methods must also be adaptable and capable. DNA nanoswitches present novel opportunities to advance therapeutic science. Constructed from a linear single-stranded backbone, oligonucleotides functionalized with putative interactors (i.e. biotin) are hybridized to it. Detection and quantitation of molecular contacts occur as a binding partner (i.e. streptavidin) is added, and an interaction is converted into the formation of loops visualized by gel electrophoresis. Because oligos can be chemically functionalized with small molecules, nucleic acids, and proteins, and topological changes are robust over a large scale of concentration and temperature conditions, the nanoswitch platform is highly adaptable. Particularly important to this application, and unique to the nanoswitch, is the ability to discern individual binding modes within multi-component systems1.

31. A machine learning approach to predict binidng affinity between kinases and kinase inhibitors

Changchang Liu

Identification of the targets of a kinase inhibitor at the systems level is time and cost intensive. A computational model which predicts the binding affinity between a small molecule and a kinase can accelerate the experimental quest. Using machine learning, such a model can be trained with small molecule-kinase complex structures and the corresponding binding affinities. Additionally, the data-driven machine learning approach can synergize the wealth of biochemical data for kinases and known biophysics of ligand-kinase interaction. Here we used linear regression and neural networks to train models based on crystal structures of kinase-inhibitor complexes and obtained comparable performance from the two approaches. We also explored different atom typing schemes and found that a fine-grained atom typing scheme can provide important chemical information for binding affinity prediction.

32. Deep phenotyping of idiopathic and drug-induced skin disease with Cyclic Immunofluorescence

Zoltan Maliga

Immune checkpoint inhibitors (ICIs) are an effective, new therapeutic modality for previously untreatable cancers but can induce severe adverse reactions (ARs). Dermal ARs are common effects of ICI therapy that resemble idiopathic, auto-immune skin disorders and may provide clinical insights to improve outcome. Skin is also a readily accessible for routine punch biopsies and histologic analysis to provide deeper insights into systemic effects of new ICI mono and combiantion therapies in clinical trials. To complement clinical H&E or single-biomarker immunohistochemistry (IHC) workflows for a single molecular marker per tissue section, we previously developed a method for repeated staining and imaging of the same tissue section as a discovery tool for deep phenotyping of tissues at single-cell resolution. Cyclic immunofluorescence (CycIF) consists of iterative rounds of tissue staining with fluorescent antibodies, imaging and chemical fluorophor inactivation. A common DNA stain in each cycle permits registration of each imaging cycle into a high-dimensional, single-cell profile to characterize cell state in a tissue context. In this study, we applied CycIF to compare the immune infiltrates in ICI-associated and idiopathic skin disease. We collected punch biopsies of normal skin or patients presenting with idiopathic (n=9) or ICI-therapy associated(n=9) skin disease to identify biomarkers for anti-CTLA4, PD1 and/or PDL1

33. Identifying new therapeutic combinations for NRAS-mutant melanoma

Haley Manchester

Cutaneous melanoma is a highly metastatic and treatment refractory skin cancer, with ~90,000 new cases estimated to occur in 2017 in the US alone. 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 melanoma.

34. SSX-mediated chromatin engagement and targeting of BAF complexes activates oncogenic transcription in synovial sarcoma

Matthew McBride

Synovial sarcoma (SS) is a soft-tissue malignancy driven by a recurrent chromosomal translocation (t(X;18)) that uniformly produces the SS18-SSX oncogenic fusion protein. SS18 is a core subunit of the mammalian SWI/SNF (BAF) complexes, which remodel nucleosomes in an ATP-dependent manner and antagonistically oppose gene-silencing activity of polycomb repressive complexes to maintain transcriptional control throughout development and differentiation. We previously discovered that in SS, incorporation of the oncogenic SS18-SSX fusion into BAF complexes leads to eviction of the tumor-suppressor BAF47 (INI1/SMARCB1) subunit, and aberrant activation of polycomb target genes by displacement of H3K27me3-mediated repression. However, uncoupling the oncogenic consequences of two co-occurrent BAF complex perturbations, gain of 78- amino acids of SSX to SS18 and loss of BAF47, has remained a challenge for the field. To identify effective targeted therapeutics for this patient population, it is critical that we understand the contribution of the gain- versus loss-of-function properties of these molecular events in this malignancy.

Here we demonstrate that the SSX 78aa tail engages mononucleosomes and targeted, quantitative mass spectrometry proteomics reveals preferential engagement to nucleosomes decorated with histone modifications associated with transcriptional repression. Using biochemical affinity assays, we find that SSX dramatically increases the affinity of SS18-SSX-containing BAF complexes for chromatin, thereby decreasing the dynamic mobility of BAF complexes. Furthermore, we show that SS18-SSX-containing BAF complexes possess a broader genomic footprint and exhibit distinct chromatin localization in that expression of SS18-SSX drives a near complete retargeting of BAF complexes genome-wide. SS18-SSX directs BAF

complexes to polycomb-repressed sites to activate embryonic development and neuronal gene pathways hallmark to SS primary tumors. This targeting by SSX results in a transcriptional signature markedly distinct from sarcomas such as malignant rhabdoid tumors, which are driven solely by biallelic loss of BAF47. Moreover, using CRISPR/Cas9-mediated KO of BAF47 in SS cell lines, we show that the proliferative arrest of SS cell lines upon suppression of SS18-SSX is independent of BAF47 reassembly into BAF complexes, thereby demonstrating that SSX targeting of BAF complexes drives oncogenesis in a manner distinct from BAF47 loss. Taken together, these studies uncover a novel functionality of the SSX tail that is required for SS oncogenesis, and inform the selection of appropriate targeted therapeutic agents for this gain-of-function BAF complex-driven cancer.

35. A High-Throughput Screen Identifies Novel Targets for Kidney Tubular Regeneration

Maria Beatriz Monteiro

Acute kidney injury (AKI) is associated with substantial morbidity and mortality, and often serves as the precursor to chronic kidney disease, which can only be managed supportively, with no curative therapy. Kidney proximal tubular epithelial cells (PTECs) contribute the most towards tubule repair, playing a key role in regeneration after injury. To uncover new molecules that can promote tubular regeneration, we performed a high-throughput phenotypic screening of 1902 compounds at 11 µM, measuring increases in proliferation of primary human PTECs. After 48h of treatment, 129 compounds promoted an average increase in Normalized Cell Count (NCC) >1.1 compared to the initial count (0h). Clear separation of positive, negative and toxic controls showed sensitivity (Z-factor >0.3) and a correlation coefficient of 0.86 amongst duplicates demonstrating reproducibility. A secondary screening with 129 positive hits at 1uM, 3uM, 10uM and 30uM confirmed eight compounds as pro-proliferative based on an increase in NCC >1.1 and an increase in actively cycling EdU-positive cells >6.5%. Furthermore, we determined the impact of these compounds on tubular cell proliferation under basal conditions and after three different types of injury: hypoxia (1% O2 for 24h); drug-induced toxicity (Cyclosporin A, 7.5uM or CdCl2, 15uM) and physical injury (scratch assay). The compound that demonstrated strongest proliferative potential based on NCC increase was an inhibitor of the dual specificity tyrosine-phosphorylation-regulated kinase (DYRK). After testing nine different functional and structural analogues of this DYRK inhibitor we found that ID-8, a DYRK2/4 inhibitor and Harmine, a DYRK1a inhibitor promoted cell proliferation after acute injury in human PTECs (1µM). RNAseq analysis revealed upregulation of genes related to cell cycle such as CDK1, PCNA and PI3K revealing potential mechanisms by which these compounds can promote cell proliferation. In summary we have identified potential first-in-class compounds that stimulate kidney epithelial cell regeneration following acute kidney injury in vitro.

36. Creating a map of genomic redundancy within druggable genome as guide for functional polypharmacology

Nienke Moret

Polypharmacology of small molecule drugs has frequently been reported with the average number of targets per drug varying from 5 to 201. Although these additional targets have classically been used to explain toxicity of compounds, there is an emerging notion that polypharmacology also plays into the efficacy of small molecules.

A rational explanation of this polypharmacology need is functional gene redundancy; the concept that the function of a single gene-product can be performed by several other gene-products. Functional redundancy is most cleanly studied in yeast, where duplicated genes from the whole genome duplication (WGD) have resulted in many well-defined paralogs. The aim of this study is to organize the human genome into functionally redundant units and hence identify instances of desired polypharmacology.

37. Drug independence explains the curability of childhood acute lymphoblastic leukemia

Adam Palmer

38. Targeting the Prolyl Isomerase Pin1 with Covalent Peptidomimetic 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, and is frequently overexpressed in cancer. In triple-negative breast cancer (TNBC), Pin1 mediates oncogenic signaling networks to drive the epithelial-mesenchymal transition (EMT) and cell migration, suggesting that Pin1 inhibition could address the critical need for targeted TNBC therapy. However, despite previous efforts, there are currently no Pin1 inhibitors that can serve as informative cellular probes. Through structure-based design and in silico docking studies, we developed and characterized peptidomimetic inhibitors that act via the formation of a covalent adduct with a critical cysteine residue, C113, in the Pin1 active site. Such covalent inhibitors overcome the limitations of existing Pin1 inhibitors by achieving added specificity and longer lasting biochemical effects. In parallel to inhibitor development, we employed a chemical genetic strategy to achieve targeted Pin1 degradation in TNBC MDA-MB-231 cells, thereby providing a tool with which to assess the expected phenotype of Pin1 loss. Overall, this work offers the first Pin1 inhibitors with the requisite potency, selectivity, and cell permeability to interrogate the potential of Pin1 as a therapeutic target in TNBC.

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

Chelsea Powell

Here we present the development of the first small molecule degraders that are able to induce anaplastic lymphoma kinase (ALK) degradation in cells, 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.

40. 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 on a small scale 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.

41. Computational repositioning and preclinical validation of mifepristone for human vestibular schwannoma

Jessica Sagers

The computational repositioning of existing drugs represents an appealing avenue for identifying effective compounds to treat diseases with no FDA-approved pharmacotherapies. Here we present the largest meta-analysis to date of differential gene expression in human

vestibular schwannoma (VS), a debilitating intracranial tumor, and use these data to inform the first application of algorithm-based drug repositioning for this tumor class. We apply an open-source computational drug repositioning platform to gene expression data from 80 patient tumors and identify eight promising FDA-approved drugs with potential for repurposing in VS. Of these eight, mifepristone, a progesterone and glucocorticoid receptor antagonist, consistently and adversely affects the morphology, metabolic activity, and proliferation of primary human VS cells and HEI-193 human schwannoma cells. Mifepristone treatment reduces VS cell viability more significantly than cells derived from patient meningiomas, while healthy human Schwann cells remain unaffected. Our data recommend an immediate Phase II clinical trial of mifepristone in VS.

42. Using the automated building of computational models to understand cardiotoxic drug responses

Bobby Sheehan

Sorafenib, a tyrosine kinase inhibitor used in the treatment of a number of cancers, including renal cell carcinoma, has been shown to cause cardiotoxic side effects. However, the mechanisms through which these side effects are triggered remains unclear. Here we attempt to understand the source of these side effects through a combination of experimental characterization of cardiomyocyte response to Sorafenib, and computational modeling of mechanisms downstream of Sorafenib activity. “Omics” level profiling of these cells showed significant changes in glycolysis and amino acid metabolism following treatment, however the mechanistic connection to Sorafenib is still unclear. We hope to better understand the mechanistic dynamics through modeling. Modeling such a broad hypothesis is a difficult modeling task, since such mechanistic models are typically less exploratory and focus on a single, well-defined pathway. Additionally, building such a far-reaching model would be a slow and error-prone process if done manually. To address these issues we are using the INDRA (Integrated Network and Dynamical Reasoning Assembler) software to automate the process. INDRA allows us to read hundreds of thousands of published papers, extract the mechanistic information relevant to our problem, and build a model with minimal human intervention. We have used INDRA to build a mechanistic model that connects known targets of Sorafenib to enzymes that participate in glycolysis and amino acid metabolism. This model, which focuses on events down stream of the FLT3, KDR, and PDGFRA tyrosine kinases, is able to recapture key qualitative trends in the data, notably showing that Sorafenib treatment can lead to an increase in PKM2 activity, leading to increased flux through glycolysis and reduced amino acid synthesis. Additional model calibration us to make quantitative predictions on how Sorafenib causes changes in glycolysis that are absent following treatment with similar drugs, and identify how we can moderate these changes to potentially reduce side effects.

43. Drug resistance as a general response of the liver to xenobiotics

Kenichi Shimada

Living organisms are constantly exposed by xenobiotics and they have developed a system to protect the body from them in the course of evolution. However, tissues involved in this system, particularly liver and kidney, are at high risk of xenobiotic-induced injury. While individual tissue injury phenotypes are have been studied extensively, these phenotypes were defined empirically and it is still not clear how many different disease states were induced by xenobiotics, or how the disease states interact with each other. In this study, we discovered nine disease states from physiology and histology of a large toxicogenomic data in a purely data-driven manner. Transcriptome analysis discovered that many of the disease states correspond to existing pathology. We also discovered many disease states ended up in a particular disease state at the end, which was characterized by acquisition of resistance to various xenobiotics. Further analysis highlighted a potentially critical role of the liver in acquiring body-level drug resistance via rewiring glucose distribution.

44. Computational prediction of new ligands for new targets

June Shin

Although computational methods have had some success, they do not routinely reduce the complex, expensive, and time-consuming bottlenecks in drug discovery. Specific drug-protein binding partners are hard to predict as even ligands very similar to each other may have very different known target binding affinities. In addition, more target genes are being identified through new screening approaches such as CRISPR, but even candidate ligands for the majority of these targets are unknown. I propose to build a method that predicts ligands for as much of the human genome as possible and associated binding specificity. I will incorporate an evolutionary couplings representation of target protein structure and variational autoencoder representation of ligand structure into a semi-supervised neural network.

45. A druggable dependency in treatment resistant melanoma

Whitney Silkworth

Though initially promising, advances in targeted and immune therapies for melanoma, are met with resistance and an inadequate response rate. Analysis of patient biopsies and cell lines revealed that both acquired and intrinsic resistance to these therapies is associated with an undifferentiated cell state characterized by low levels of MITF, the master regulator of melanocyte development. Using the Project Achilles data set, we identified a genetic dependency in low-MITF melanomas on LSD1, a lysine-specific histone demethylase that has

commercially available inhibitors. We validated the LSD1 dependency in multiple melanoma cell lines using both genetic and pharmacologic inhibition. To understand the mechanistic basis of this dependency, we have shown that LSD1 mediated cell death in low-MITF melanoma cells is dependent upon the function of NDRG1, a suppressor of metastasis, and is known to be mediated by LSD1 to affect the motility and invasiveness in neuroblastoma. Both our in vitro and in vivo results suggest that combination of pharmacologic inhibitors targeting LSD1 and BRAF is synergistic, extending xenograft survival. We will analyze the downstream mediators of LSD1, to identify potential pharmacodynamics markers and mediators of LSD1 dependency. We have identified a functional and targetable dependency of low-MITF melanoma cells, providing unique insight and a therapeutic strategy for treatment resistant melanoma.

46. Exploiting apoptotic priming and dependencies in nervous system tumors

Rumani Singh

Nervous system tumors including brain tumors and neuroblastomas comprise a diverse group of neoplasms and are a leading cause of cancer-related death in adults and children. In addition to surgical resection, postoperative radiotherapy and chemotherapy are the mainstays of treatment. Understanding the key determinants of radiation and chemotherapy responses may improve personalized treatment regimens and reduce tumor recurrence.

The mitochondrial apoptosis pathway, which is controlled by the BCL-2 family of proteins, is the most physiologically dominant form of cell death and plays a pivotal role in radiation- and chemotherapy-induced tumor cell killing. We have previously found that cancers that are "primed" for apoptosis respond more favorably to chemotherapy than those cancers that suppress apoptosis and are "unprimed" or completely block apoptosis and are "apoptosis refractory."

BH3 profiling is an assay that measures cellular proximity to the apoptotic threshold (apoptotic priming) as well as reliance on various pro-survival BCL-2 family proteins (apoptotic dependencies). Using BH3 profiling, we found that levels of apoptotic priming varied widely across different nervous system cancer cell lines and contributed to their therapy sensitivity or resistance. Specifically, we found glioblastomas are less primed for apoptosis than neuroblastomas and medulloblastomas and are also resistant to ionizing radiation or a wide range of chemotherapeutic agents. This is in agreement with clinical observations for these tumors. Nervous system tumors also displayed distinct differences in their antiapoptotic dependencies: medulloblastomas exhibit higher BCL-xL dependence and neuroblastomas rely on BCL-2 for survival. Glioblastomas didn't show any selective pattern. To build on these findings, we are optimizing treatment strategies combining apoptosis-targeting agents (BH3 mimetics) with front-line therapies in order to improve outcomes for patients with nervous system tumors.

47. Targeting phosphatidylinositol 5-phosphate 4-kinase (PIP4K2) using novel covalent inhibitors in cancer

Carmen Sivakumaren

Phosphoinositides are important signaling molecules known to localize in the nucleus, Golgi, endoplasmic reticulum and at the plasma membrane, regulating a wide variety of cellular functions. The phosphorylation of phosphatidylinositol 5-phosphate (PI5P) at the 4-position, yielding the product phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2), is catalyzed by the phosphatidylinositol 5-phosphate 4-kinases, PIP4K2α, β and γ. Recently, it was demonstrated that the kinase activity of PIP4K2α and β is crucial for the growth of cancers harboring TP53 (encoding p53) deletions. The alpha and beta isoforms of PIP4K2 have also been shown to be critical in driving cellular proliferation in leukemia and Her2-positive breast cancer respectively, while the gamma isoform regulates the immune system through mTORC1 signaling, potentially being a useful target in enhancing cancer immunotherapy. These studies provide a strong genetic rationale that targeting PIP4K2 using a potent and selective inhibitor may have anti-proliferative effects in various cancer types. Therefore, we aim to develop inhibitors which can be used as potential therapeutic candidates and tools to further uncover novel underlying biology surrounding these novel lipid kinases.

48. ICCB-Longwood Screening Facility

Jennifer Smith

49. Effects of Microtubule Drugs in Neurodevelopment and Injury

Yuyu Song

Microtubules (MTs) are structural components vital for important neuronal functions such as neurite outgrowth and maintenance, as well as for axonal trafficking and synaptic remodeling. Neuronal MTs are particularly stable compared with those in other cell types and such stability increases during development and maturation, but decreases with axonal injury and neurodegeneration. This leads to an intriguing question: can stabilizing MTs facilitate neurite outgrowth during early development or restore axonal integrity and function upon injury? To address these questions, we have characterized a group of MT stabilizing drugs (Epothilone D, Epothilone B, Ixabepilone, Taxol and Synstab) regarding their binding affinity to MTs in vitro using biochemical assays and fluorescent live imaging; and evaluated their effects on neurite growth during normal differentiation and regrowth after axotomy. We found that these drugs showed bimodal effects on initial neurite extension and regeneration after injury and we are currently studying the molecular pathways underlying these changes. These results may

provide useful information for understanding not only neuronal MT dynamics and stability in health and disease, but also for determining the therapeutic value of MT stabilizers in axonal injury and neurodegeneration where loss of neuronal MT integrity may exacerbate disease pathology.

50. Engineered proteins for targeted immunosuppression and activation

Emma Spady

Many diseases are caused by inappropriate immune system activation or inactivation. Inflammatory disorders, such as ulcerative colitis and rheumatoid arthritis, are caused by unnecessary immune activation. The glucocorticoid drugs commonly used to treat these diseases have detrimental global effects, including osteoporosis and diabetes. In contrast, activating the immune response in cancer cells could lead to a suppression of proliferation and inhibit tumor growth and spread. Due to ubiquitously expressed interferon receptors the administration of the immune response stimulating cytokine, interferon alpha leads to severe flu-like symptoms and diarrhea. Engineered fusion proteins can mitigate these side effects by localizing these therapeutics to specific cells. Our interferon fusion protein is designed to highly bind to mesothelin, a cell surface glycoprotein, overexpressed in mesothelioma, pancreatic cancer and ovarian cancer. Only after the fusion protein has bound to a target cell, the downstream innate immune response is activated. Our glucocorticoid-carrier fusion protein will bind to leukocyte markers, shepherding the steroid drug exclusively into the relevant cell types. We expressed a secreted anti-CD45 minibody fused to corticosteroid binding globulin (CBG), a carrier protein that transports glucocorticoids through the bloodstream. Affinity assays are underway to find designs that bind steroids tightly in the blood, but release them upon endocytosis. The resulting proteins will be assayed for triggering glucocorticoid receptor nuclear translocation, the first step in glucocorticoid action.

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51. Single-cell analysis of apoptotic priming in healthy tissues

Johan Spetz

Apoptosis is an evolutionarily-conserved process of programmed cell death which is vital for normal tissue development and homeostasis. Consequently, different forms of cell death are often implicated in the development, progression and treatment outcomes of many human diseases. Furthermore, current treatment options for e.g. cancer 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 are

especially prevalent in pediatric patients, and may be more or less toxic to different lineages of cells within the 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. Consequently, the level of apoptotic priming within healthy tissues governs their sensitivity to sources of damage or stress such as ionizing radiation or chemotherapy. Finally, apoptotic priming has been found to be dynamically regulated by lineage specialization programs throughout postnatal development, leading to a higher risk for treatment-associated toxicities in young tissues as compared to adults.

The aims of this project are to measure apoptotic priming at a single-cell level in mammalian tissues during normal development and aging, and subsequently identify the cellular mechanisms responsible for the regulation of apoptosis.

Preliminary results indicate cell type specific sensitivities dependent on lineage program and age, future studies will determine whether the level of apoptotic priming affects the cellular sensitivity to damage or stress by inducing genotoxic damage in various mouse models using ionizing radiation.

52. The decisive role of NF-κB during early stages of TB infection

Amy Thurber

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.

During infection of the human leukemic monocyte cell line Thp-1, we have observed variability at the single cell level with some cells maintaining a low bacteria burden, some having a high burden, and others dying. We propose that cell-to-cell differences in NF-κB signaling at least partially explain single-cell variability, and that the dynamics of NF-κB are predictive of later infection outcome. We are using live imaging of cells expressing NF-κB reporters during infection to monitor the dynamics of NF-κB activation and bacteria burden over time to identify correlations between NF-κB and bacteria killing. This data will be used to first adapt a mass-action kinetics model of the NF-κB pathway to Thp-1 cells and then perform sensitivity analysis to identify potential sources of variability.

Our single-cell dataset including both signaling dynamics and outcome will enable investigation of variability in NF-κB during Mtb infection and identification of NF-κB states most conducive for Mtb killing. This will improve our mechanistic understanding of disease progression allowing future advances in the development of new intervention strategies.

53. Long-term p53 dynamics following ionizing irradiation

Michael Tsabar

Following ionizing irradiation, the levels of p53 protein in MCF7 cells exhibit an oscillatory dynamics that is regulated by a feedback loop with MDM2. These oscillations are thought to persist as long as the DNA damage is not resolved. By monitoring MCF7 cells for 72 hours following irradiation using live cell imaging we find that a subset of the population switches from an oscillatory p53 dynamic to a persistent dynamic and that this switch is a result of divisions following irradiation. We show that p53 dynamic switch 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.

54. Can we predict single-cell effects of sequential drug treament?

CyCIF reveals predictive properties of unknown reaction networks

Shu Wang

Although CyCIF and other single-cell-omics methods contain a wealth of correlational information between observables, this information is often not fully used in data analysis. In principle, these correlations arise from the underlying chemical reaction networks shared by cells in the same population. In fact, key, predictive aspects of these networks are readily extracted from the data, assuming a general constraint on the hidden differential equations of the reaction network. On the side of theory, we lay out why, how, and what we can extract. On the side of data, we validate our framework by showing that although culture cells treated with different doses of the same drug vary as much as cells treated with different drugs, our extracted network property remains unchanged only when two populations have been treated with the same drug. As our next step, we seek collaborators to experimentally test an immediate consequence of our framework: we expect that we can predict the CyCIF single-cell distribution for sequential treatments (e.g. of first A, then B), provided CyCIF data of each singular treatment (e.g. just A, or just B).

55. Anti-inflammatory activity of Colchicine

Jui-Hsia Weng

56. Base editing in vivo alters the posttranslational modification of β-catenin and induces production of cochlear hair cells

Wei Hsi (Ariel) Yeh

Activation of the Wnt/β-catenin pathway plays a critical role in the self-renewal and homeostasis of mammalian tissues. We developed a base editing strategy to upregulate Wnt signaling by installation of a S33F mutation in β-catenin, which blocks its phosphorylation and reduces the rate of β-catenin degradation, allowing β-catenin-mediated transcription of Wnt-responsive genes. Base editing installed the S33F mutation in β-catenin in cultured human cells with much higher efficiency (13-32%) and precision than a current homology-directed repair (HDR) approach, achieving a 200-fold higher ratio of editing:indels than HDR. In vivo, lipid-mediated ribonucleoprotein (RNP) delivery of the β-catenin-targeting base editor:guide RNA complex resulted in the proliferation and transdifferentiation of post-mitotic supporting cells into new hair cells. Delivery of corresponding HDR agents did not induce these changes, consistent with the poor efficiency of HDR in post-mitotic cells. These results establish a novel strategy for regulating posttranslational modification states and complex signaling pathways, demonstrate the effectiveness of base editing in post-mitotic cells in vivo, and suggest an approach to cellular reprogramming in the postnatal mammalian inner ear.