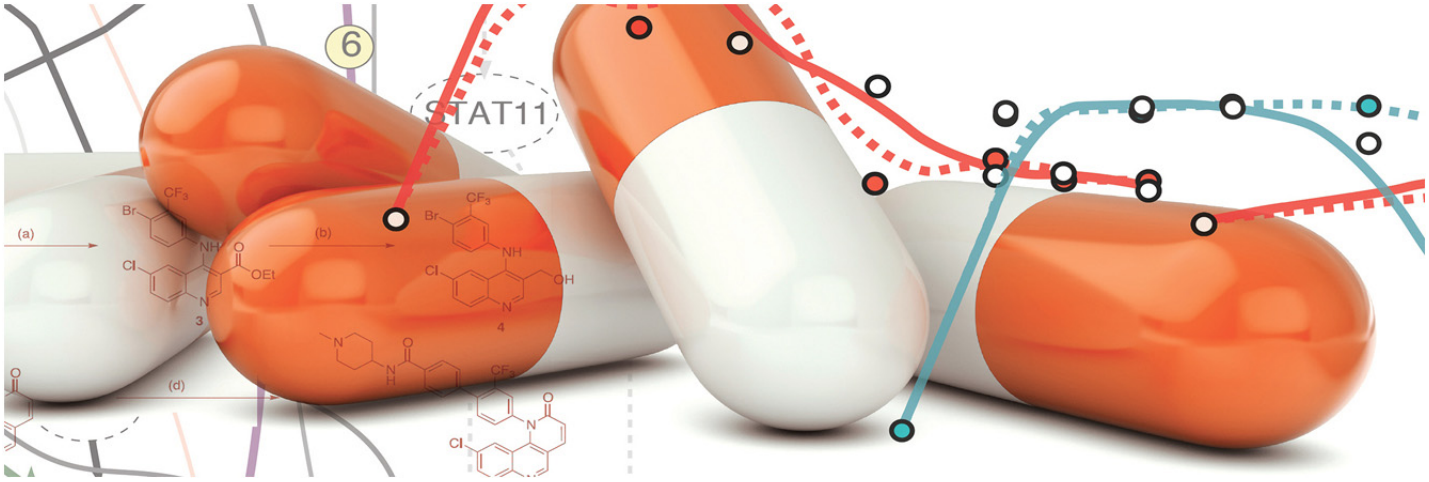

HiTS

Harvard Program
in Therapeutic Science

# HiTS Spring Symposium 2015

## Friday, June 5, 2015

**Harvard Medical School**
**TMEC Atrium/Amphitheater**



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| **Check In and Breakfast** | **TMEC Atrium** | **10:00 - 10:30am** |
| **LSP Platforms and Capabilities** | **TMEC Amphitheater** | **10:30 - 12:30pm** |
| Antibody Resources | *Noel Peters, Liz Williams* | |
| Small Molecule Libraries and QC | *Caroline Shamu* | |
| LINCS Database | *Jeremy Muhlich, Liz Williams* | |
| Mass Spectometry and You | *Robert Everley* | |
| Imaging Tools and Applications | *Jagesh Shah* | |
| RNAseq Optimization and Use | *Mike Springer* | |
| **BBQ Lunch** | **TMEC Atrium** | **12:30 - 1:00pm** |
| **IDP Workshop** | **TMEC Amphitheater** | **1:00 - 2:20pm** |
| | *Laura Maliszewski, Galit Lahav, Catherine Dubreuil* | |
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| | *Jeremy Duffield, Vice President of Research Biogen Idec* | |
| **HiTS Postdocs** | **TMEC 250** | **3:30 - 4:30pm** |
| Finding Fellowships | *Peter Sorger* | |
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| TGP Feedback Session | *David Golan, Catherine Dubreuil, EC Members* | |
| **Poster Session** | **TMEC Atrium** | **4:30 - 6:30pm** |
| Reception and Awards | | |

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# Poster Abstracts

## #1: Chromosomes, cancer, and the poster under my desk

Lee A. Albacker

We induced chromosome instability in adult mouse tissues by knocking out the spindle checkpoint protein Mad2 in T cells or hepatocytes. Surprisingly, mice developed tumors that were highly aneuploid and had cell type specific karyotypes. These karyotypes were maintained in the presence of ongoing chromosomal instability suggesting a selective advantage to maintaining an aberrant karyotype. In human cancers, specific chromosomal aberrations are often more frequent than point mutations in any one driver gene, a fact that has gone unnoticed in recent genomic profiling efforts.

## #2: A multiscale statistical mechanical framework integrates biophysical and genomic data to assemble cancer networks

Mohammed AlQuraishi

Functional interpretation of genomic variation is critical to understanding human disease, but it remains difficult to predict the effects of specific mutations on protein interaction networks and the phenotypes they regulate. We describe an analytical framework based on multiscale statistical mechanics that integrates genomic and biophysical data to model the human SH2-phosphoprotein network in normal and cancer cells. We apply our approach to data in The Cancer Genome Atlas (TCGA) and test model predictions experimentally. We find that mutations mapping to phosphoproteins often create new interactions but that mutations altering SH2 domains result almost exclusively in loss of interactions. Some of these mutations eliminate all interactions, but many cause more selective loss, thereby rewiring specific edges in highly connected subnetworks. Moreover, idiosyncratic mutations appear to be as functionally consequential as recurrent mutations. By synthesizing genomic, structural and biochemical data, our framework represents a new approach to the interpretation of genetic variation.

## #3: Identifying the insertion kinetics, stability, and stoichiometry of the apoptotic pore from fluorescence measurements and mathematical modeling

John Bachman

Mitochondrial outer membrane permeabilization, or MOMP, is believed to represent the “point of no return” in the pathways of both intrinsic and receptor-mediated apoptosis. The Bcl-2 family proteins constitute the core regulatory machinery for MOMP, and as such their structure, function, and interactions have been the subject of intense scrutiny. We attempt to develop a rigorous understanding of the dynamic properties of the apoptotic Bcl-2 protein Bax by coupling an in vitro biochemical model system with quantitative measurement and systematic mathematical modeling. We measure the pore formation kinetics of synthetic lipid vesicles incubated with purified cBid and Bax and use these measurements to rigorously distinguish between competing mechanistic hypotheses for Bcl-2 protein function. Mechanistic models are enumerated systematically, calibrated to data, and discriminated by Bayesian statistical methods. Results from this approach highlight several previously unappreciated kinetic and stoichiometric barriers to pore formation by Bax, provide an estimate of the minimal stoichiometry of the Bax pore, and identify the likely causes of discrepancies in previous mechanistic studies.

## #4: CNV-SABRE: A length-adaptive binning strategy to discover copy number variants

Samuel Bandara

Copy number variations (CNVs) are ubiquitous in human cancer and promote tumorigenesis through the amplification of oncogenes and the loss of tumor suppressors. Next generation sequencing (NGS) is rapidly emerging as the preferred method of CNV detection. However, variability of read density across the genome poses a statistics challenge that has bred a large number of empirical and inferential approaches. Here, we develop a first-principle statistical model of the distribution of read events in a short genomic interval to derive a length-adaptive non-heuristic binning strategy that operates uniformly throughout the genome at precisely defined specificity. We implement CNV-SABRE as an open-source software package and test it on resampled NGS read data and on spontaneous tumors arising in a mouse model of liver cancer. We show that CNV-SABRE is significantly superior to binning read data over fixed-lengths and that it provides a superior means to detect CNVs in tumor DNA.

## #5: Multiplexed Exchange-PAINT imaging of RTKs reveals ligand-dependent EGFR and Met interactions in the plasma membrane

Verena Becker

Signal transduction by transmembrane receptors involves complex ligand- and time-dependent changes in conformation and modification state that lead to assembly of homo- and hetero-oligomers and larger aggregates (receptor clusters). In the case of receptor tyrosine kinases (RTKs) structural studies provide detailed data on individual receptor dimers but do not reveal how different RTKs are distributed or interact in the membrane. Studying such interactions is complicated by variable and often low receptor affinities and the inability of conventional microscopy to resolve nanoscale structures. We report the use of multiplexed super-resolution imaging (Exchange-PAINT) followed by mean-shift clustering and random forest analysis to measure the precise distribution of five receptor tyrosine kinases (RTKs) from the ErbB, IGF-1R and Met families that interact in breast cancer cells. Interestingly, these receptors are normally intermixed in a non-homogenous manner on the plasma membrane. Stimulation by EGF does not appear to induce a change in the density of EGFR in local clusters, implying that such clusters are largely pre-formed. Instead, ligand addition results in formation of non-canonical receptor pairs previously associated in drug resistance. These findings are supported by biochemical analysis, which demonstrates that our combined approach can find meaningful patterns in heterogeneous protein distributions on the nanoscale.

## #6: aRrayLasso: a network-based approach to microarray interconversion

Adam Brown

Robust conversion between microarray platforms is needed to leverage the wide variety of microarray expression studies that have been conducted to date. Currently available conversion methods rely on manufacturer annotations, which are often incomplete, or on direct alignment of probes from different platforms, which often fail to yield acceptable gene-wise correlation. Here, we describe aRrayLasso, which uses the Lasso-penalized generalized linear model to model the relationships between individual probes in different probe sets. We have implemented aRrayLasso in a set of five open-source R functions that allow the user to acquire data from public sources such as GEO, train a set of Lasso models on that data, and directly map one microarray platform to another. aRrayLasso significantly predicts expression levels with higher fidelity than technical replicates of the same RNA pool, demonstrating its utility in the integration of data sets from different platforms.

## #7: Screening for kidney toxicity in Mexican children exposed to environmental toxicants

Mariana Cardenas

Chronic kidney disease (CKD) is a public health problem with a prevalence estimated to be 8-16% worldwide. Diabetes mellitus, hypertension, obesity and exposure to some xenobiotics are considered risk factors for CKD. Environmental exposure to naturally occurring heavy metals and fluoride in drinking water has been associated with an increased prevalence of CKD. Children are considered a high-risk group, specifically because they have higher gastrointestinal absorption for metals than adults rendering them particularly susceptible to toxicity. In this study, we evaluated the association between exposure to arsenic (As), cadmium (Cd), lead (Pb), fluoride (F) exposure and the susceptibility to kidney toxicity in children. A cross sectional study was conducted with children aged 4-13 years living in the high-risk area of north-central Mexico (n=97) suspected to have exposure to high levels of environmental toxicants. The levels of exposure to As, Cd, Pb and F (32, 0.76 ppb, 6.8 µg/dl and 2.4 ppm, respectively) were found to be significantly above the normal range (recommended by the US Centers of Disease Control and Prevention) for children. Although kidney function was found to be normal (as measured by serum creatinine) urinary biomarkers of kidney injury like KIM-1 was significantly associated with blood levels of Pb (95% CI: 0.003, 0.019). Likewise, urinary KIM-1 (95% CI: 0.001, 0.006), NGAL (95% CI: 0.01, 0.005), miR_21 (95% CI: 0.5, 0.005), miR_423 (95% CI: 2.4, 1.2) and miR_200c (95% CI: 1.27, 2.9) were significantly associated with urinary levels of Cd. These results suggest that kids living in north central Mexico are exposed to higher levels of kidney toxic environmental contaminants such as Pb and Cd. Furthermore, we also shown that novel kidney injury biomarkers such as KIM-1, NGAL, miR-21, miR-423 and miR-200c can be considered as new and sensitive tools for environmental risk assessment screening. Funded by Harvard-Mexico Foundation and CONACyT (grant 234833).

## #8: Molecular dynamics of oncogenic BRaf-induced senescence

Jia-Yun Chen

Oncogene-induced senescence (OIS) is a safeguard mechanism that suppresses proliferation upon aberrant oncogene activation in normal cells. Studies have shown that the OIS response is heterogeneous within a population with some cells undergoing senescence while others remain proliferative, eventually proceeding to malignancy. It is therefore crucial to identify the source of heterogeneity and the mechanism by which oncogenic activity is translated by individual cells into distinct cell fates. Here, we use high-content imaging and quantitative time-lapse microscopy to dissect the molecular dynamics of oncogenic BRafV600E-induced senescence in primary human epithelial cells. We show that BRafV600E induction leads to substantial heterogeneity in cell fates with a fraction of cells entering into a prolonged G1-phase arrest while others kept proliferating. Interestingly, in single cells, a preceding burst of proliferation was not observed and is not required for the subsequent long-term arrest. We further show that ERK activity but not BRafV600E expression levels strongly correlate with the growth and divisions of individual cells. We show that a moderate induction of ERK activity accelerates proliferation whereas beyond a certain threshold activity, proliferation is inhibited. Epistasis analysis suggests that the BRafV600E-induced senescence is independent of the canonical p16INK4A-pRB and p53 tumor suppressor pathways. We further identify p38 pathway as an essential component leading to BRafV600E-induced senescence. We are currently examining whether pre-existing differences in ERK activity, cell cycle history, and the temporal dynamics of tumor suppressors determine future cell fates. This knowledge will allow us to understand how cell-to-cell variability at various levels contributes to the establishment of OIS, and explains how OIS is escaped in some cells.

## #9: Therapeutic induction of FOXO transcription factors is associated with cell cycle arrest and apoptosis in TP53-mutant triple negative breast cancer cell lines

Sameer Chopra

Triple negative breast cancer (TNBC) is an aggressive subset of invasive ductal carcinoma that disproportionately affects young women, confers a poor prognosis, and is ineffectively treated with conventional chemotherapy. Genetic inactivation of the p53 tumor suppressor (TP53) is a ubiquitous feature of TNBC that confers resistance to drug-induced cell cycle arrest and apoptosis. It is uncertain to what extent redundant transcriptional programs exist that may functionally compensate for reduced p53 activity. Using cell lines with concomitant genetic lesions in TP53 and components of the PI3K/Akt signaling pathway, we are testing the hypothesis that induction of Forkhead box (FOXO) transcription factors can functionally compensate for loss of p53 in TNBC. By screening a large panel of PI3K/Akt/mTOR inhibitors, we found that drug-induced cell cycle arrest and apoptosis strongly correlates with the extent of FOXO3a nuclear translocation. Based on these findings, we are developing FOXO3A-deficient cell lines using CRISPR/Cas9. We are also using a fluorescent reporter of FOXO3a nuclear-cytoplasmic shuttling to study the dynamic relationship between FOXO3a nuclear translocation and pro-apoptotic gene expression in live cells. Finally, we are developing strategies for combination targeted therapy that potentiate the nuclear translocation and induction of FOXO3a, with the ultimate goal of augmenting drug-induced cell cycle arrest and apoptosis in this aggressive subset of breast cancer.

## #10: Systematic analysis of drug-induced adaptive responses in melanoma

Mohammad Fallahi-Sichani

Treatment of BRAF(V600E) melanomas with drugs, such as vemurafenib, that inhibit RAF/MEK signaling is effective in the short term, but remission is not durable. Acquired drug resistance is thought to involve short-term adaptive responses that compensate for RAF/MEK inhibition via up-regulation of other pro-growth mechanisms. Thus, understanding and ultimately preventing adaptive responses is key to durable therapy. Systematic data comparing BRAF(V600E) tumor cells is generally lacking and it is not known whether adaptation is fundamentally similar across cell types or among individual cells within a cell population.

We apply a systematic approach to studying the responses of human melanoma cell lines to five drugs, RAF and MEK inhibitors, with the overall goal of (i) characterizing variability in adaptation with time, dose, cell type and across individual cells, (ii) discovering new or poorly characterized adaptive mechanisms, and (iii) demonstrating the effectiveness of a high-throughput approach involving multiplex measurement, single-cell analysis and computational modeling. The data involves time-course measurement of total level and activity of signaling proteins and cell state markers using array-based methods and single-cell immunofluorescence assays as well as measurement of apoptosis and cell viability under the same conditions. Statistical modeling using partial least squares regression (PLSR) revealed which of the changes in the ~2×105 point dataset were phenotypically consequential.

We found that responses to RAF inhibitors are remarkably diverse and involve multiple pathways that can be up or down-regulated over time, with significant variability across cell types and individual cells. We identified a role for JNK/c-Jun signaling in altering the cell-cycle distribution of melanoma cells, causing apoptosis-resistant cells to accumulate and drug maximal effect (Emax) to fall; co-drugging with RAF and JNK inhibitors or JUN knockdown reverse this effect. The primary effect of JNK inhibitors is to minimize the cell-to-cell variability in pS6 suppression, promoting the induction of apoptosis.

Our study shows that a systems-level approach (combining high density time-dependent measurements, quantitative modeling and single-cell analysis) may provide a general framework for evaluating new drugs with adaptive and paradoxical response, and identifying potentially useful combination therapies.

## #11: Investigation of MYC Collaborating Oncogenes in T-cell Acute Lymphoblastic Leukemia Progression and Relapse

Elaine Garcia

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymocytes, with less than 30% of children and 8% of adults able to survive relapsed disease. In T-ALL, aberrant MYC activity is a dominant oncogenic lesion. The MYC pathway can be activated or overexpressed by NOTCH1 activation or trisomy 8 (+8) respectively. However to evolve T-ALL and accelerate its progression, additional mutations are likely required. To identify MYC collaborating genes, we generated MYC-overexpressing T-ALL in zebrafish that recapitulates the most common and treatment resistant subtype of human T-ALL. Through an unbiased transplantation screen, we have been able to assess functional differences between individual cells and use array comparative genomic hybridization to identify focally amplified genes associated with high self-renewal capacity, short latency, and/or increased aggression. Through this analysis, we identified 16 commonly amplified genes, 10 of which are required for continued tumor growth in human T-ALL cell lines. Interestingly, 3 of those 16 genes are co-amplified with MYC on +8 in human T-ALL – including phosphatase of regenerating liver 3 (PRL3), TatD DNase Domain Containing Protein 1 (TATDN1), and cleavage and polyadenylation specificity factor 1 (CPSF1). Moreover, PRL3, TATDN1, and CPSF1 were each independently amplified in zebrafish T-ALL, suggesting that they are not merely passenger genes in the context of human +8 T-ALL. To date, I have confirmed that each gene collaborates with MYC to accelerate time to tumor onset in the zebrafish model and assessed their role in human T-ALL maintenance in vitro and in mouse xenograft studies. I hypothesize that each gene has distinct roles in progression and relapse in T-ALL. My early studies suggest that TATDN1 enhances LPC frequency and PRL3 suppresses apoptosis. The long-term goal of this work is to identify novel molecular pathways that can be exploited for therapeutic innovation.

## #12: Systems Biology of Polycystic Kidney Disease

Kelly George

Polycystic kidney disease (PKD) is a group of hereditary disease states characterized by cystic kidneys and often accompanied by other manifestations such as cystic liver and hypertension. PKD occurs in 1 in 500 people, usually resulting from a dominant mutation in one of two genes, PKD1 or PKD2. There are currently no treatments for PKD and it is the leading genetic cause of renal failure. Numerous studies have identified many pathways that are misregulated in PKD but targeting these individual pathways has not led to a successful therapeutic intervention or molecular biomarker. The only biomarkers for PKD are total kidney volume and the functional glomerular filtration rate, which are lagging indicators of disease status.

To identify potential biomarkers and develop a systems-level understanding of PKD, we are using transcriptomics, and quantitative proteomics and phospho-proteomics to evaluate the state of cystic and normal kidneys in a mouse model of PKD. Kidney cysts also induce injury to the surrounding tissues, confounding any analysis of the "cystic state". In an effort to separate the injury signature from the cystic signature, we are also evaluating the state of the injured kidney using a folate model of acute kidney injury and fibrosis. From the transcriptomic experiments, we have a list of potential biomarker candidates that are currently being tested by qPCR in tissue at various stages of disease progression in a mouse model of PKD.

The multi-kinase inhibitor Roscovitine has been shown to prevent cystogenesis in multiple mouse models of PKD but its mechanism of action remains unknown. To gain insight into the mechanism(s) of Roscovitine and to identify biomarkers that change with treatment, we are also using the above "omic" strategies using kidneys from mice following treatment. Phosphorylation at motifs for CDKs, CK1, CaMK, and others are reduced in kidneys treated with Roscovitine. The primary transcription factor family found to be suppressed with Roscovitine treatment is the E2Fs, likely a result in the loss of CDK signaling. These experiments will not only provide candidate biomarkers for PKD progression, but will also yield a deeper understanding of the state of the cystic kidney.

## #13: Bayesian analysis of uncertainty in pathway models

Benjamin M. Gyori

Dynamical models of signaling pathways contain a large number of parameters whose values can only be inferred to a limited extent from noisy experimental data.

Consequently, all predictions made with such a model are subject to uncertainty. We present a framework

that allows us to make intuitive, qualitative statements using temporal logic about uncertain model predictions and verify whether they are satisfied in a statistical sense.

On a small model of JAK-STAT signaling and a large model of extrinsically-triggered apoptosis with many unknown parameters, we show

that even though some parameters cannot be identified from experiments, the model can reliably predict quantities of interest.

## #14: Transcriptional landscape of drug response guides the design of specific and potent drug combinations

Marc Hafner

Characterizing the molecular effects of targeted therapies is an important step towards understanding and predicting drug efficacy in cancer. In this work, we used the L1000 assay developed at the Broad Institute to measure the transcriptional response of six breast cancer cell lines to more than 100 different targeted drugs, many of whom are in clinical trials. We focused on inhibitors targeting the most important signaling kinases such as PI3K, AKT or MAPK, as well as receptor tyrosine kinases (RTKs) and cyclin-dependent kinases (CDKs). With two time points and six doses, the dataset contains more than 8000 unique perturbations.

We clustered the perturbations that elicited a significant response (37% of measured perturbations when using $p<0.05$) according to their gene expression profile and obtained 23 clusters. The perturbation significance was correlated with inhibitor dose, but no cluster was biased toward a particular concentration. Clusters were generally time point specific: the transcriptional responses at 3 hours differed significantly from the 24 hour ones. Some clusters contained perturbations from multiple cell lines whereas others were cell line specific. In particular, responses to CDK inhibitors were similar across most cell lines and showed a down-regulation of genes related to the cell cycle. On the other hand, cell lines responded differently to PI3K/AKT and MAPK inhibitors as illustrated by clusters specific to each cell line and pathway. Interestingly, the perturbations induced by RTK (e.g. EGFR, MET, ALK) and non-RTK (e.g. SRC, ABL, BTK) inhibitors, clustered with either the PI3K/AKT or the MAPK inhibitors depending on the cell line. Thus the transcriptional response allowed us to identify differences in pathway connectivity between cell lines, in particular which RTK connects to the PI3K/AKT pathway or the MAPK one.

In parallel to the transcriptional response, we measured the growth inhibition after three days of treatment. We found diverse phenotypic responses that are not necessarily related to the strength of the transcriptional signature. In particular, we identified cases where inhibitors had little effect on growth, yet induced a significant transcriptional response. These cases suggest possible adaptation mechanisms to the inhibitors that may lead to drug resistance. We followed these cases experimentally and identified cell line-specific resistance mechanisms.

Finally, we used the directionality of the transcriptional response in the mRNA space to guide co-drugging strategies. By testing if targeting parallel pathways is more potent than inhibiting twice the same pathway, we derived a systematic approach to design synergetic combinations. Because of the differential responses across cell lines, we were able to find combinations that are potent only in a specific cell line. This approach is a step toward the design of co-drugging strategies with differential effect and large therapeutic windows.

In conclusion, our measurements of expression signatures and cellular phenotype across thousands of perturbations and six breast cancer cell lines allowed differential analyses that complement the C-MAP strategy based on consensus signatures. With our approach, we found connections between drugs based on transcriptional responses and paved the way for systematic design and analyses of drug synergies.

Keywords: kinase inhibitors, breast cancer cell lines, drug synergies, signaling networks

## #15: Single-Cell Dynamics of caspase-8 Activation by Death Receptor Agonists

Yvonne Hua

Since its discovery in 1995, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) - induced extrinsic apoptosis has been studied for years and almost all the major molecules involved are known. However, despite the seemingly simple model that binding of the death receptor (DR) on cell membrane leads to cell death, it remains unclear why TRAIL sensitivity is different across different cell types, and more interestingly, even among the individual cells in the same population. One explanation for the heterogeneous cell-to-cell sensitivity is the abundance of known positive and negative regulators associated with the apoptotic pathway. Yet in order to understand the biology and to predict the fate decision of individual cells, in addition to individual regulator's function, the knowledge of their relative activity in the whole pathway is of critical importance. Thus I am using TRAIL and varieties of synthetic ligands to study the kinetics and mechanisms of caspases-8 activation at a single-cell level, with the goal to answer (i) what determines cellular sensitivity to the death ligand (ii) what is the relative activity of the positive and negative regulators in extrinsic apoptosis pathway (iii) what are the kinetics of caspase-8 activation induced by death receptor

agonists of different valency. My approach includes single-cell assays of caspase-8 activation and computational modeling of molecular mechanisms.

## #16: Characterization of the Notch Pathway Regulator NRARP

Sanchez Jarrett

Notch signaling is a conserved pathway in multicellular organisms which influences critical cell fate decisions in a variety of cell types. As such this signaling pathway is important in a host of fundamental cell processes in a variety of tissues and dysregulation of components has been linked with a wide range of human diseases from neurodegeneration to a number of cancers, most notably T-cell acute lymphoblastic leukemia/lymphoma (T-ALL). There remain important gaps in existing knowledge regarding the biochemistry of signal regulation following pathway activation. The objective of this study is to improve our molecular understanding of the function of the Notch pathway feedback inhibitor, Notch regulated ankyrin repeat protein (NRARP), using biochemical and cell-based approaches. Improved understanding of Notch pathway regulation following activation may lead to identification of new drug targets, which could have significant implications in immune system modulation, anticancer therapies and control of a myriad of cell fate decisions whether in vitro or in vivo.

## #17: Elucidating and Inhibiting Inflammatory Pathways in Synovial Fibroblasts

Annie Jenney

Rheumatoid arthritis (RA) is a prevalent, chronic, debilitating disease that causes inflammation of the synovium surrounding joints, eventually causing the joint to mount an immune response against itself. The most common drugs prescribed for treating the painful and chronic symptoms of RA are anti-inflammatory medications and some biologics. In order to find a more appropriate biological target in synovial fibroblasts (SF), which are the cells that make up the synovium of joints, and to halt the inflammatory loop that these cells become stuck in, the pathways that are activated in the inflammatory response in SF must be elucidated. The largest signaling and secreted response in these cells occurs when the cells and pathways are 'activated' with inflammatory ligands and cytokines. Many clinical trials evaluated targeted inhibition of the p38 MAP kinase to alleviate RA symptoms. Treating primary human synovial fibroblasts with activating ligands and inhibitors in a high throughput manner allows us to closely view the specific cytokine and pathway response through cellular signaling and secretion data. Using many tested clinical compounds and other biological inhibitors, we have discovered that inhibiting p38 in synovial fibroblasts can potentiate the secretion of several cytokines including RANTES, IL-16 and MIG. The ability of these drugs to induce context-dependent secretion of cytokines and their ability to change the signaling occurring in some pathways contributed to their poor performance in clinical trials. Drugs should be designed for RA to inhibit certain targets more selectively. Mapping the pathway response in synovial fibroblasts will allow for more targeted therapies that can contribute to stopping this inflammatory cycle of RA.

## #18: Analysis of 8800 cancer patients' germline genome reveals frequent mutations in tumor suppressor genes

Ruomu Jiang

## #19: A framework for identifying context-dependent inhibitor effects enables systems-level evaluation of kinase inhibitors for rheumatoid arthritis

Doug Jones

A critical need in drug target identification and drug development is to rapidly assess efficacy in physiologically relevant contexts. Towards addressing this need, we are coupling high-through experimentation with computational analysis to identify context-dependent inhibitor effects in rheumatoid arthritis (RA). Synovial fibroblasts (SF) are resident cells of synovial joints that play a strong role in progression of RA. By quantifying levels of ~50 cytokines secreted by primary human normal or RA SF we identified a set of inflammatory stimuli that induce a secretion response in SF that is significantly correlated to the composition of synovial fluids from patients diagnosed with RA. This suggests the secretion landscape of SFs plays a key role in shaping the disease microenvironment of RA patients. To explore the ability of targeted kinase inhibitors to modulate this secretion landscape we developed a new modeling framework that enables inference of context-dependent effects from perturbation-response data. Application of this new framework to a newly generated perturbation-response compendium of data comprising >50,000 cytokine secretion data points uncovered wide context-dependent signal-response relationships underlying SF secretion. Our approach provides a systematic framework for exploration of the RA inflammatory environment and should be applicable to additional inflammatory diseases characterized by complex microenvironmental interactions.

## #20: Identification and functional dissection of lncRNAs in Tregs

Evgeny Kiner

## #21: Profiling the STING Pathway

Peter D. Koch

## #22: Dissecting the glycosyltransferase and proteolytic catalytic functions of O-linked N-acetylglucosamine transferase

Zeb Levine

The glycosyltransferase OGT is essential for viability of dividing mammalian cells; however, it remains unclear as to why. It is the sole glycosyltransferase responsible for the common addition of the monosaccharide O-GlcNAc to serine and threonine residues of cytoplasmic and nuclear proteins. Levels of this glycosylation are perturbed in type II diabetes, Alzheimer's disease, certain types of cancers, and other disease states. Recently, OGT has been found to have a second catalytic activity, the proteolytic cleavage of the cell-cycle associated nuclear scaffolding protein HCF-1. In this project, I seek to develop a cellular system with which to determine which of OGT's effects are due to this proteolytic activity and which are due to glycosyltransferase activity. Determining which activities are essential for which of OGT's pleiotropic effects may guide the development of chemical probes that selectively modulate individual phenotypic effects of OGT.

## #23: Highly multiplexed high-throughput imaging of single-cell using CycIF cyclic immunofluorescence

Jerry (Jia-Ren) Lin

Single cell analysis reveals aspects of cellular physiology not evident from population-based studies, particularly in the case of highly multiplexed methods such as mass cytometry (CyTOF) that measure the levels of multiple signaling, differentiation and cell fate markers simultaneously. Immunofluorescence (IF) microscopy adds information on cell morphology and the microenvironment that are not easily obtained using flow-based techniques but the multiplicity of conventional IF is limited. This has led to the development of imaging methods that require specialized instrumentation, exotic reagents or proprietary protocols that are difficult if not impossible to recreate in most laboratories. Here we report a public-domain method for achieving high multiplicity single-cell IF using cyclic immunofluorescence (CycIF), a simple and versatile procedure in which four-color staining alternates with chemical inactivation of fluorphores to progressively build a multi-channel image. Because the method uses standard commercial reagents and instrumentation and is no more expensive than conventional IF, it is both easy to perform and suitable for high-throughput and high-content assays.

## #24: Toward small molecule tools and therapeutic targets in human myeloid cells

Zoltan Maliga

## #25: ARID1A Loss Impairs Enhancer-Mediated Gene Regulation & Drives Colon Cancer in Mice

Radhika Mathur

The SWI/SNF subunit ARID1A has been identified as a candidate tumor suppressor gene in colorectal cancer, as recurrent inactivating mutations across the length of the gene occur in 10% of all colorectal cancers and 37% of colorectal cancers of the microsatellite-instable (MSI) subtype. Although ARID1A is the subunit of the SWI/SNF chromatin remodeling complex that is most widely and frequently mutated in human cancer, little is known of its contribution to SWI/SNF chromatin remodeling and of the mechanism by which its inactivation contributes to oncogenesis. Here, we demonstrate that conditional inactivation of ARID1A across many mouse tissues leads to the formation of aggressive, invasive colon adenocarcinomas. Mechanistically, we find that loss of ARID1A in colon epithelial cells impairs the ability of SWI/SNF complexes to target to and activate enhancers, resulting in dysregulation of gene expression. Collectively, given the close histological match to human colon adenocarcinoma and the exclusivity of origin to the colon, these results represent a substantial advance in colon cancer mouse modeling and further establish the tumor suppressor function of SWI/SNF subunits to be via enhancer activation and maintenance.

## #26: Single cell imaging of kinase inhibitor-induced effects in breast cancer cells

Caitlin Mills

Six breast cancer cell lines representing triple negative, hormone receptor positive, and Her2 amplified disease were treated with a panel of 108 kinase inhibitors. Cells were stained with DRAQ5 (DNA) and TMRE (mitochondrial membrane potential), and images were acquired using an Opera microscope 24 hours after treatment. Cell segmentation and feature extraction (intensity, morphology, and texture) was performed with Acapella software. Over 300 features were extracted for ~1.5 million cells. Supervised and unsupervised clustering approaches have been applied to identify those kinase inhibitors that induce significant changes to cell phenotype, as well as to identify cell line and pathway specific effects.

## #27: Drug-induced Death Signaling Measured by Dynamic BH3 Profiling Predicts Clinical Response to Targeted Agents.

Joan Montero

J. Montero1, K.A. Sarosiek1, J.D. DeAngelo1, J. Ryan1, D. Ercan1, H Piao1, N. S. Horowitz1,3, R. S. Berkowitz1,3, U. Matulonis1, P.A. Jänne1,3, P.C. Amrein2, R. Drapkin1,3, A. Letai1.

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When effective death signaling is initiated by a targeted therapy, an increase in mitochondrial apoptotic 'priming for death' can be observed within hours. We developed a new technique, Dynamic BH3 Profiling (DBP), that measures changes in priming induced by chemotherapy in cancer cells, without the requirement for prolonged ex vivo culture, and we assessed if it could be used as predictive assay to personalize cancer therapy in clinic.

Our first test was whether Dynamic BH3 Profiling performed following only 16 hours of drug exposure could predict cytotoxicity at a much later (72-96 hours) time point. We initiated our studies on a diverse panel of human cancer cell lines (solid tumors and hematological malignancies) treated with a wide range of kinase inhibitors. We observed a significant correlation between the increase in priming following short-term exposure to the agents and cell death at 72-96 hours, demonstrating the predicting capacity of DBP in different cancer cells. We then used samples from CML and ovarian adenocarcinoma patients of known clinical outcome to imatinib or carboplatin treatment respectively. In both cases we found that the induction of mitochondrial priming caused by short term (16 hr) ex vivo exposure to treatment predicted clinical response. Moreover, the ROC curve analysis demonstrated that DBP is an excellent binary predictor.

Our experiments demonstrate the potential for Dynamic BH3 Profiling to be used as a powerful real-time tool to predict chemotherapy and recognize the best agent of possible therapies for an individual tumor and improve cancer patients' therapy.

## #28: A small molecule screen for compounds that inhibit growth of Staphylococcus aureus.

Heidi Morris

Staphylococcus aureus is a highly adaptable pathogen, and methicillin-resistant S. aureus (MRSA), is one of the leading causes of community- and hospital-acquired infections with over 11,000 fatalities every year. MRSA is resistant to most β-lactam antibiotics, which target peptidoglycan in the cell wall, and has become an imminent public health threat. The cell envelope of S. aureus is complex and partially composed of peptidoglycan, as well as two other polymers, wall teichoic acids (WTAs) and lipoteichoic acids (LTAs). In this study we screened a small-molecule library to identify compounds that target the cell envelope and inhibit growth of S. aureus. Target validation and follow up on our top hits is currently ongoing in order to determine the specific mode of action of these compounds.

## #29: Quantifying Cell cycle deformations and its implications in Systems Biology and systemPharmacology of cancer

Satabhisa Mukhopadhyay

Cell cycle, as retrieved from the mutual information flow in fixed asynchronous population of cells, exhibits characteristic topology in the multidimensional protein-protein scatter plane specific to the cell types and conditions such as normal vs cancer spectra. Landscape of drug perturbations with respect to various doses and incubation time gives rise to characteristic deformations of the known cell cycle topology, a direct consequence of context averaged drug response. From a differential mutual information flow study on such cell cycle deformations we quantitatively predict the branching of cell cycle fluxes into proliferating and arresting subpopulations as a function of cell type, drug type, drug doses and incubation time on normal vs cancer cells. We use this framework in quantitative understanding of adaptive resistance in cancer.

## #30: A new method to uncouple drug response measurement from growth rates.

Mario Niepel

Measuring the phenotypic responses of cell lines to small molecule inhibitors is a crucial tool to investigate drug action in a laboratory setting. Recently, two very large data sets were published that purported to be a general resource, listing sensitivities of hundreds of cell lines to small molecule inhibitors. However, the quality and consistency of these data has been called into question. Beyond this, measuring drug responses is not trivial when accounting for difference in division rate across cell lines and growth conditions.

Here we develop a method that accurately corrects drug response for variations in growth rate. With this method we uncover a complex relationship between cell density and drug sensitivity: Increasing cell density can lead to increasing or decreasing drug sensitivity or leave the sensitivity unchanged. In addition, this effect can be dependent on the specific combination of cell line and drug investigated or it can be a common feature of a drug across multiple cell lines.

We identify some of the underlying mechanistic drivers of this effect and suggest an effective way to minimize variability of drug response measurements with a new experimental and analytical protocol.

## #31: Integrating OMERO and cluster computing for high throughput image analysis?

Mario Niepel

This poster introduces the present and future workflow of image management and analysis at Harvard Medical School (HMS). HMS has an established LSF cluster called Orchestra and a large Isilon distributed parallel filesystem for use in scientific computing. Our goal is to improve the organisation and curation of image data and associated metadata, through the use of OMERO and also to improve the workflow for large scale parallel computing on that data and metadata using Orchestra and Isilon. In addition, this is sympathetic to our current strategy of using OMERO to store and organise image data for publication through the HMS LINCS website.

## #32: The roles of synergy and cross-resistance in combination chemotherapy

Adam Palmer

Monotherapies are often effective for only a short duration because of the evolution of drug resistance, while more durable, sometimes even curative outcomes can be obtained with combination themotherapies. Which interaction properties amongst the therapies in a combination are most conducive to clinical efficacy? A common goal of pre-clinical combination studies is 'synergy', indicating a combination whose potency is greater than the sum of its parts. Here we study an examplar of effective combination therapy, R-CHOP, which achieves curative outcomes for many patients with Non-Hodgkin Lymphoma. We quantified the interactions between all pairings of these different therapies and found that none showed synergy, and indeed many demonstrated antagonism, the opposite effect. However, simple theoretical considerations indicate that synergy is likely to be less important in the design of combinations than is finding combinations with non-overlapping mechanisms of drug resistance, such that mutants with resistance to one component in a combination remain sensitive to at least one other component. Previously measurements of such 'cross-resistance' interactions have been extremely low throughput, but here we describe a DNA-barcoding based approach to systematically measure cross-resistance interactions amongst therapies by tracing the survival of many drug resistant lineages in parallel when treated with a variety of single therapies. High-throughput measurement of cross-resistance interactions between anti-cancer therapies should provide critical data in the assessment of potential combinations and facilitates a novel perspective on the rational design of combination therapies.

## #33: Detection of Kidney Toxicity in Humans using Urinary microRNA-21, -200c and -423

Mira Pavkovic

Acute kidney injury (AKI) is often encountered in patients as a result of nephrotoxic drug administration. Since traditional biomarkers (BMs) like serum creatinine (SCr) and blood urea nitrogen (BUN), currently in practice, are insensitive and nonspecific there is an unmet need for more sensitive BMs allowing early detection of AKI. The objective of this study was to evaluate the performance characteristics of urinary microRNA (miR)-21, -200c and -423 as BMs following drug-induced AKI in humans. Urine samples were collected from 1) a cross sectional cohort of patients (n=136) with acetaminophen (APAP) overdose of which approximately 60% had developed AKI and 2) a longitudinal cohort of patients (n=42) with malignant mesothelioma receiving a cisplatin (Cp) therapy with samples collected over 10 time points before and after Cp administration (pre, post, 8, 12, 24, 48, 72, 96, 120 and 144h). All three miRs (measured by qRT-PCR and normalized to miR-489) were significantly ($p<0.01$) higher in urines from APAP or Cp treated patients with AKI as compared to patients without AKI. ROC-AUC analysis revealed miR-21 to be sensitive and specific for AKI (AUC: 0.70 for APAP- and 0.75 for Cp-study) outperforming miR-200c (AUC: 0.61 and 0.40, respectively), miR-423 (AUC: 0.65 and 0.40, respectively) and urinary kidney injury molecule 1 (KIM-1; AUC: 0.66 and 0.52, respectively). Logistic regression analysis showed that higher expression of all three miRs were significantly ($p<0.05$) associated with APAP-induced AKI, whereas only miR-21 was associated with Cp-induced AKI (odds ratio of 1.73, $p<0.05$). Our results suggest that urinary miR-21, -200c and -423 are promising BMs for detection of kidney toxicity in humans.

## #34: Development of TAF1 bromodomain inhibitors

Dave Remillard

Small molecule inhibitors are under development for the bromodomain of TBP-associated factor 1, a component of the general transcription factor TFIID. Through biochemical assay development and medicinal chemistry, lead compounds of high potency have been attained and are under optimization for improved selectivity. We hope to obtain quality probes that will enable us to interrogate the role of TAF1 acetyl-lysine recognition in transcription initiation.

## #35: NAD+ Regulates Hif-1α Stability and the NLRP3 Inflammasome

Michael Schultz

## #36: Targeting phosphatidylinositol 5-phosphate 4-kinase (PIP4K2) using novel covalent inhibitors in cancer characterized by p53 loss

Carmen Sivakumaren

## #37: Distinct roles for matrix metalloproteinases 2 and 9 in zebrafish hematopoietic stem cell emergence

Lindsay Theodore

Matrix-metalloproteinases (MMPs) are a group of extracellular matrix (ECM) proteins involved in regulating cell interactions, migration and signaling. Despite the importance of ECM remodeling in tissue homeostasis, the role of MMPs in hematopoietic stem cell (HSC) formation is poorly understood. A screen of MMP-inhibitors in the zebrafish identified MMP2 and MMP9 as modulators of hematopoiesis. HSCs are born in the aorta-gonad mesonephros (AGM) region and migrate to/expand in the caudal hematopoietic tissue (CHT) before colonizing the thymus and kidney. Chemical and genetic inhibition of MMP2 during HSC emergence retained runx1+ cells in the AGM, delaying their migration to the CHT. These findings are consistent with the broad expression of MMP2 during the onset of hematopoiesis and its enrichment in the vascular niche. In contrast, MMP9 expression is restricted to a subpopulation of myeloid cells, and its loss did not affect HSC migration, instead resulting in aberrant HSC expansion within the CHT. Additionally, MMP2/9 inhibition led to abnormal colonization of the thymus. Repression of MMP2/9 activity prior to thymus colonization by AGM-derived HSCs reduced the number of rag+ cells in the thymus; however, inhibition after the initial seeding of the thymus led to an increase in rag+ cells, indicating a temporal-dependence for MMP activity on lymphoid migration and maturation. We previously identified MMP9 as a downstream target of prostaglandin-E2 (PGE2). Co-treatment with PGE2 and an MMP9 inhibitor increased HSC numbers within the CHT compared to PGE2-only treatment. Conversely, co-treatment with MMP2 attenuated the PGE2-mediated enhancement of AGM HSC production, supporting the idea that MMP2 and MMP9 have distinct functions in hematopoiesis. In sum, our findings indicate that MMP2 is required for HSC budding and migration between the embryonic sites of hematopoiesis, whereas MMP9 is involved in HSC expansion in the CHT, and both MMP2/9 are required for colonization of the thymus.

## #38: Single cell network modeling of drug-induced cardiotoxicity

Huan (Sharon) Wang

Development of drugs targeted at specific oncogenes has revolutionized cancer care, but many patients suffer from drug-induced cardiovascular disease. Cardiovascular-related mortality is 7-fold higher in age matched pediatric cancer patients than normal individuals and ~12% of breast cancer survivors suffer heart failure within three years of treatment. Tyrosine kinase inhibitors (TKIs) are exemplars of promising anti-cancer drugs plagued by cardiotoxicity. Whether this reflects drug-mediated inhibition of the same signaling pathways as those involved in oncogenesis remains unclear. To tackle this question, we utilize human cardiomyocytes trans-differentiated from induced pluoripotent stem cells as a model system. As intracellular signaling pathways and cellular function are inter-connected spatially and temporally, we combines computational and experimental “systems biology” methods to investigate how cardiomyocytes are regulated by TKIs at single cell and cell population levels. We observed that different TKIs induce dose- and time-dependent effects on cell survival and calcium handling. To find explanation on these phenotypes, we measured signaling kinases downstream of tyrosine kinases (such as pERK1/2 and pp70S6K) using multiplexed imaging. The activation of these kinases were inhibited by TKIs and showed heterogeneous responses among individual cells. We also profiled gene expression using mRNA-seq and found distinct mRNA signatures across dose and time of TKI treatment. Signaling and gene signatures that are predictive of cellular phenotypes were defined based on statistical modeling and they may present clinically relevant biomarkers for early detection of cardiotoxicity. Understanding the mechanistic action of these TKIs on cardiac cells can help optimize their dosing regimen, facilitate the design of targeted secondary treatments to mitigate the adverse cardiac effects, or promote the development of new drugs with similar potency against cancer but reduced cardiotoxicity.

## #39: Mechanistic hypothesis for the anti-inflammatory drug Colchicine

Jui-Hsia Weng

Microtubule (MT) is one of the crucial features in cells and related to many cellular events. While roles of MT during cell division are well defined, it is not known how MT functions in non-dividing cells. Colchicine, a MT-targeting drug, rescues patients with inflammatory disorders via unclear mechanisms outside mitosis. Based on colchicine kinetic distribution, I propose that the non-proliferating liver hepatocyte is the main target of colchicine. To address if and how colchicine-blocked MT dynamics in liver cause whole-body anti-inflammatory effects, I will initially use primary cultured hepatocytes as a model to dissect signaling pathways, gene expression, and secreted proteins upon colchicine treatment. I will then study how perturbing MTs lead to these changes. I will then investigate how the communication between liver and white blood cells happens and identify the future implication for therapy improvement.

## #40: Integration of genome-wide datasets identifies SOX10 as a lineage-specific dependency in melanoma

Terence Wong

# Schedule

| | | |
|---|---|---|
| **Check In and Breakfast** | **TMEC Atrium** | **10:00 - 10:30am** |
| **LSP Platforms and Capabilities** | **TMEC Amphitheater** | **10:30 - 12:30pm** |
| Antibody Resources | *Noel Peters, Liz Williams* | |
| Small Molecule Libraries and QC | *Caroline Shamu* | |
| LINCS Database | *Jeremy Muhlich, Liz Williams* | |
| Mass Spectometry and You | *Robert Everley* | |
| Imaging Tools and Applications | *Jagesh Shah* | |
| RNAseq Optimization and Use | *Mike Springer* | |
| **BBQ Lunch** | **TMEC Atrium** | **12:30 - 1:00pm** |
| **IDP Workshop** | **TMEC Amphitheater** | **1:00 - 2:20pm** |
| | *Laura Maliszewski, Galit Lahav, Catherine Dubreuil* | |
| **Coffee Break** | **TMEC Atrium** | **2:20 - 2:30pm** |
| **Therapeutics Seminar** | **TMEC Amphitheater** | **2:30 - 3:30pm** |
| | *Jeremy Duffield, Vice President of Research Biogen Idec* | |
| **HiTS Postdocs** | **TMEC 250** | **3:30 - 4:30pm** |
| Finding Fellowships | *Peter Sorger* | |
| **Therapeutics Graduate Program Students** | **TMEC Amphitheater** | **3:30 - 4:30pm** |
| TGP Feedback Session | *David Golan, Catherine Dubreuil, EC Members* | |
| **Poster Session** | **TMEC Atrium** | **4:30 - 6:30pm** |
| Reception and Awards | | |