Invited Talks Abstracts

Session #1: Tumor Heterogeneity and Metastasis, Thursday, October 20th

Towards cancer resistance: A biochemical model of necroptosis explains cell type-specific responses to cell-death cues

Presenter: Ildefonso, Geena

Geena V. Ildefonso, Marie Oliver Metzig, Alexander Hoffmann, Leonard A. Harris, Carlos F. Lopez

University of Southern California

A central question in cancer research is understanding how trillions of cells in the human body process signaling cues to make decisions every day to commit to a given cellular fate. Challenges arise when cells are improperly triggered by outside signals, resulting in mistaken cell fates progressing to cancer and diseases. Cell death is a crucial process in maintaining this balance in the body, however, cancer cells can evade the "normal" programmed route of cell death, apoptosis. A backup form of regulated cell death, necroptosis, has been recently discovered, which can crosstalk with the apoptosis pathway, yet yields significantly different outcomes. Although many of the primary molecular species involved in necroptosis have been identified, including receptor interacting protein kinase-1 (RIP1), RIP3, and mixed lineage kinase domain-like protein (MLKL), efforts to target necroptosis dysregulation or leverage it therapeutically are hindered by the lack of a detailed, mechanistic understanding of the biochemical pathways driving necroptosis execution.

Numerous published experimental studies have shown that RIP1 deubiquitination in complex I is driven by A20, CYLD, or both, depending on cell type. These varying reports have led to unresolved controversies about the specific molecular mechanisms driving necroptotic cell death. Here, we present a detailed biochemical model of TNF-induced necroptosis derived from decades' worth of published experimental studies. The model was calibrated using Bayesian parameter inference to experimental protein time course data for phosphorylated mixed lineage kinase domain-like protein (pMLKL), an established necroptosis reporter, from a well-established necroptosis-executing cell line. A subsequent dynamical systems analysis identifies four distinct modes of necroptosis signal execution, which can be distinguished based on rate constant values and the roles of the deubiquitinating enzymes A20 and CYLD in the regulation of RIP1 ubiquitination. In one case, A20 and CYLD both contribute to RIP1 deubiquitination, in another RIP1 deubiquitination is driven exclusively by CYLD, and in two modes either A20 or CYLD acts as the driver with the other enzyme, counterintuitively, inhibiting necroptosis.

We also performed sensitivity analyses of initial protein concentrations and rate constants and identified potential targets for modulating necroptosis sensitivity among the biochemical events involved in RIP1 ubiquitination regulation and the decision between complex II degradation and necrosome formation. We conclude by associating numerous contrasting and, in some cases, counterintuitive experimental results reported in the literature with one or more of the model-predicted modes of necroptosis execution. Overall, we demonstrate that a consensus pathway model of TNF-induced necroptosis can provide insights into unresolved controversies regarding the molecular mechanisms driving necroptosis execution for various cell types under different experimental conditions.

Preexisting resistance and its spatial allocation modulate therapy outcome

Presenter: Masud, M.A.

M. A. Masud, Kim Jae-Young, Kim Eunjung

Korea Institute of Science and Technology

Drug resistance is one of the leading causes of treatment failure in cancer therapy. We explore the impact of preexisting resistance using a deterministic competition model and an agent-based model of the tumor cell population. Analyzing the ordinary differential equation (ODE) model that explains competition between sensitive and preexisting resistant cell populations, we showed that there might exist an effective dose window (EDW) that can contain the tumor indefinitely [1]. Next, we investigated the identifiability of the model parameters, fitted the model to clinical data of melanoma patients using the maximum likelihood approach, and estimated patient-specific EDW. Simulation of our model shows that doses belonging to EDW can contain tumor progression indefinitely using either continuous or adaptive dose scheduling. Using optimal control theory, we showed that the lower bound of the EDW approximates the minimum effective dose (MED) [1]. However, the ODE model assumes homogeneous well-mixing of sensitive and resistant cells. In reality, cell configuration diverges from this homogeneity. To understand the impact of the resistant cell distribution, we develop an agent-based model and simulate it for three tailor-made initial cell configurations: clumped, random, and uniform resistant cell distribution [2]. We formulate our model using the Hybrid Agent-Based Model Library (HAL) [3]. The random and uniform cell distribution is initially close to the homogenous mixing assumption of the ODE model and does not produce a significantly different result from the ODE model. The clumped distribution assumes a clump of resistant cells in the middle of the domain. Our analysis shows that under a continuous maximum tolerated dose (CT-MTD), tumors with clumped resistant cells end up with a longer progression time than tumors with random and uniform resistant cell distributions due to intra-species competition. When treated with adaptive therapy (AT), inter-species competition comes into effect in addition to intra-species competition and delays the progression further. So, the higher benefit of AT is associated with clumped initial cell configuration [2]. As cancer-associated fibroblast (CAF) is known to increase cell fitness, we simulated the impact of fibroblast location on treatment outcomes. We consider five different fibroblast locations relative to the preexisting resistant cells, such as fibroblasts overlapping, partially overlapping, surrounding, and partially surrounding the resistant cells, as well as fibroblasts randomly distributed in equal clumps. According to our analysis, the fibroblast-mediated advantage of tumor progression is not significant if CAF overlaps with the clump of resistant cells. If CAF and resistant cells occupy nonintersecting regions, progression is accelerated under both CT-MTD and AT in a likely fashion, which is correlated with the area of fibroblast region nonintersecting with the resistant cells. Hence, the existence of fibroblast does not modulate the preference of AT over CT-MTD [2]. Taken together, patient-specific calibration of doses may delay the progression under the homogeneity assumption. However, tumor microenvironment heterogeneity may modulate the progression.

References

[1] M A Masud, et al., (2022) doi.org/10.1101/2022.03.28.486150.

[2] M A Masud, et al., (2022) doi.org/10.1371/journal.pcbi.1009919.

[3] Bravo RR, et al. (2020) doi.org/10.1371/journal.pcbi.1007635.

Session #3: Translational Systems Biology, Thursday, October 20th

Mapping the molecular landscape of Acute Myeloid Leukemia enables prediction of drug response from proteogenomic data

Presenter: Pino, James C.

James C. Pino, Camilo Posso, Michael Nestor, Jamie Moon, Joshua R. Hansen, Chelsea Hutchinson-Bunch, Marina A. Gritsenko, Karl K. Weitz, Elie Traer, Cristina Tognon, Jeffrey Tyner, Brian Druker, Anupriya Agarwal, Tao Liu, Paul Piehowski, Jason E. McDermott, Sara J. C. Gosline, Karin Rodland

Pacific Northwest National Laboratory

Acute Myeloid Leukemia (AML) is diagnosed in ~20,000 patients in the US annually and despite extensive genomic characterization of the disease, the five-year survival rate remains at nearly 25%. Though there are some biomarkers with clinical utility, they generally do not always translate to a therapeutic benefit across all patients, likely due to the heterogenous molecular landscape across AML patients. This landscape, comprised of distinct mutational, transcriptomic, proteomic, and metabolic markers, represents a multidimensional view of the patient tumor and therefore provides a more in-depth summary of factors that give rise to individual drug response.

To understand the landscape, the Beat AML research program prospectively collected genomic and transcriptomic data from over 1000 AML patients and carried out ex vivo drug sensitivity assays to identify the genomic and transcriptomic aspects of the molecular landscape that could predict patient-specific drug responses. However, there are inherent weaknesses in using only genetic and transcriptomic measurements as surrogates of drug response, particularly the absence of direct information about protein signaling activity that represents an important component of the cellular signaling landscape. In this work, we extended the molecular landscape through the collection of global and phosphoproteomic data from 210 of the BeatAML patient samples to evaluate the impact that signaling activity has on drug response. We leveraged the proteogenomic data to cluster the samples into four distinct subtypes that capture the gene and protein expression differences across patients. Here we were able to identify patients that had distinct functional and biological differences despite having similar mutational profiles.

Specifically, the proteomic subtypes identified distinct patient populations that were uniquely sensitive to Venetoclax, Panobinostat, and Sorafenib, which were not identifiable from mutational data alone. We found complementary drug sensitivity profiles between Venetoclax and Panobinostat, suggesting potential synergistic behavior between these two drugs. We then screened the patient samples for both drugs and found that the two were able to reduce cell growth in all patient samples. To delve into the potential signaling pathways that predict drug synergy, we created a gradient boosted regression model to identify specific proteins that predict quantitative drug response metrics for various drugs. The molecular features identified from this model provide mechanistically interpretable targets, including proteins involved with regulation of cell cycle stress, mitochondrial metabolism, and MTOR signaling.

Finally, we posited that we could map un-explored samples to the molecular landscape to predict drug response. To test this, we applied our models to a set of MOLM14 cell lines exposed to Quizartinib in a graduated fashion (Joshi et al, Cancer Cell 2021) to lead to cell lines with drug-naïve, early resistant, and late resistant phenotypes. We found these cells to transition across our subtype molecular landscape, predicted to switch from a sensitive to Venetoclax/resistant to Panobinostat to be resistant to Venetoclax/sensitive to Panobinostat. We are currently testing this hypothesis via drug treatment. In summary, we show that proteogenomic characterization of AML patient samples enables both individualized care and targeted selection of drug combinations.

Application of State-Transition theory and treatment modeling for chemotherapy

Presenter: Uechi, Lisa

Lisa Uechi, David E. Frankhouser, Denis O'Meally, Sergio Branciamore, Guido Marcucci, Ya-Huei Kuo, Russell C. Rockne

City of Hope

We have shown that state transition theory is applicable to acute myeloid leukemia (AML) to predict disease progression from health to leukemia in our previous research. This mathematical model represents AML evolution from health to disease as a state-transition of the transcriptome state represented as a particle undergoing Brownian motion in a double-well quasi-potential, where critical points define states of perturbed hematopoiesis (c1), transition to AML (c2), and overt AML (c3). Here we test the applicability of AML state-transition model to predict disease response to chemotherapy.

To this end, we performed a time-series experiment using the conditional Cbfb-MYH11 (CM) knock-in (Cbfb+/56M/Mx1Cre) mouse model recapitulating inv(16) AML. CM leukemic mice were treated with a combination of cytarabine (50mg/kg/day; 5 days) and daunorubicin (1.5mg/kg/day; 3 days) after detection of overt leukemia which is monitored by circulating leukemia blast (cKit+ > 20%) to model the 7+3 standard of care treatment for newly diagnosed AML. A total of 143 peripheral blood samples from 9 CM mice were collected weekly before, during, and following chemotherapy and subjected to RNA-sequencing. Data from 6 of the mice were used to train the mathematical model, and 3 mice were used to test the model prediction. Singular value decomposition was used to construct the transcriptome state-space and prediction of chemotherapy effects on the transcriptome state. To model the effects of the chemotherapy treatment, we introduce a treatment force to the equation which modifies the gradient of the double-well potential according to the pharmacodynamics of the treatment.

The mathematical model predicted dynamics of the peripheral blood gene expression profiles following chemotherapy treatment in the state-space relative to the critical points. We observed the effects of treatment as the transcriptome particle moving from leukemia (c3) towards a state of perturbed hematopoiesis (c1), before eventual relapse, re-crossing the transition point (c2) back to overt AML (c3). All 9 CM mice achieved a partial response, defined as the transcriptome particle crossing the c2 critical point, with a mean time to relapse of 5 weeks, defined as the time of the first observation after the particle crosses back over c2 towards the leukemic state c3. We performed mean arrival time analysis using monte carlo simulations of the state-transition treatment model. Mean arrival time analysis accurately predicted the time to relapse in the state-space with a prediction error of 6.6 days for the 3 mice in the test data set.

We successfully applied state-transition mathematical modeling and proposed a corresponding treatment model to predict response to chemotherapy and the time to relapse in all 9 CM mice, confirming the applicability of the model to post-chemotherapy disease dynamics. This predictive model has implications to improve therapeutic strategies by targeting transcriptome state-transition critical points in human AML.

Session #4: Immunology & Microenvironment, Friday, October 21st

Mathematical modeling of fibroblast-mediated drug resistance in HER2+ breast cancer

Presenter: Poskus, Matthew

Matthew D. Poskus, Thomas O. McDonald, Ioannis K. Zervantonakis

University of Pittsburgh

Introduction: Drug resistance is a major challenge for patients with HER2 amplified (HER2+) breast cancer, which accounts for 20% of all breast cancer cases. Fibroblasts are a prominent cell type in the breast tumor microenvironment and are associated with drug resistance and disease progression. Recent studies have found tumor cells cocultured with fibroblasts have reduced sensitivity to the HER2-targeted therapy lapatinib via reactivation of the PI3K/Akt/mTOR signaling axis in tumor cells. The number of fibroblasts, or fibroblast density, in the tumor microenvironment can vary patient-to-patient; however, the role of fibroblast density on tumor growth and survival has yet to be quantitatively explored.

Methods: Four HER2+ breast cancer cell lines (BT474, EFM192, HCC202, HCC1954) were cocultured with AR22 stroma fibroblasts and subsequently treated with lapatinib. The number of live/dead tumor cells were measured every 4 hours for 96 hours using time-lapse microscopy at varying seeding densities of tumor cells and fibroblasts. The effects of fibroblasts on tumor cell growth dynamics were analyzed. An ordinary differential equation (ODE) model was implemented and calibrated using this experimental data to 1) identify equations that best describe the tumor-fibroblast system and 2) predict under what conditions tumor cells may stop responding to treatment and 3) simulate alternative treatments to target these drug-insensitive tumor cells. Cell line-specific sensitivity to lapatinib and fibroblasts was quantitatively compared using estimated model parameters. The model was used to predict a threshold at which tumor cells no longer respond to lapatinib due to fibroblast presence. Other treatments (longer duration, greater dose) are explored to target these surviving tumor cells.

Results: Each cell line exhibited different sensitivity to lapatinib concentration and fibroblast density. Greater fibroblast densities increased the lapatinib IC50 for BT474 and EFM192 compared to monoculture; however, HCC1954 sensitivity to lapatinib was unaffected by fibroblast density in coculture. Tumor growth dynamics analysis revealed fibroblasts can reduce lapatinib sensitivity by 1) decreasing the growth-inhibitory effects of low doses of lapatinib or 2) decreasing cytotoxic effects of high doses of lapatinib. Furthermore, coculture with fibroblasts increases the delay in response to lapatinib ("lag time") for several cell lines. An ODE-based mathematical model is used to predict tumor cell growth and death over time for given lapatinib concentration and tumor/fibroblast seeding density. Model selection was performed using Akaike Information Criterion to determine the equations that best describe the effects of fibroblasts on cancer cells treated with lapatinib. Simulations predict targeting surviving tumor cells by treating for longer durations is ineffective, indicating alternative treatments may be necessary.

Conclusions: Mathematical modeling may be useful in understanding of the range of fibroblast densities that limit drug responsiveness and will provide insights into how drug resistance may emerge in fibroblast-rich vs. fibroblast-poor HER2+ tumors. An ongoing area of investigation is the development of models that include time-dependent state transitions (e.g., signaling pathway rewiring in tumor cells or drug-induced microenvironment adaptation via secretion of protective factors). A comprehensive understanding of how fibroblast density influences drug sensitivity may inform adaptive drug treatment schedules to combat drug resistance.

Perturbations of cellular interaction networks in the melanoma tumor microenvironment as a result of immune checkpoint blockade

Presenter: Talkington, Anne

Anne M. Talkington, Sepideh Dolatshahi

University of Virginia

The success of immune checkpoint inhibitor (ICI) therapy is dependent on its ability to disrupt tumor-immune and immune-immune interactions that contribute to an immunosuppressive tumor microenvironment (TME). Existing ICI strategies include disrupting programmed cell death protein-1 (PD-1)/ programmed cell death-ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), and more recently, T-cell immunoglobulin mucin-3 (TIM-3). While immune checkpoint blockade has been shown to hold greater efficacy in combination than via targeting individual receptors, it has nevertheless been met with mixed success for advanced stage melanomas. We apply de novo network inference methods to predict which mechanisms of cellular crosstalk are active in the melanoma TME prior to and after anti-PD-1 treatment. Immunosuppressive interactions that appear or immunostimulatory interactions that disappear following ICI are of particular interest as potential mechanisms for treatment resistance. Our results from a publicly available single cell RNA-sequence (scRNA-seq) database of 32 melanoma biopsies, split into pre- and post- ICI treatment, suggest that macrophage-cytotoxic T cell interactions contribute substantially to the immunosuppressive TME. Specifically, the interaction GAL9 (macrophage) -> TIM3 (CD8+ T cell) appears in several patients who failed to respond to ICI, suggesting that it may be a mechanism by which the melanoma is able to evade PD-1 blockade. Additionally, we present evidence that the MHCI complex is heavily implicated in both tumor-immune and immune-immune interactions involving pairwise combinations of CD4+ T cells, CD8+ T cells, NK cells, macrophages, and malignant cells. Finally, we demonstrate the potential for intercellular interactions in the TME to be mediated by post-translational modification, namely, glycosylation. We demonstrate the upregulation of sialylation via ST6GAL1 post-ICI and further show correlation between TIM-3 on CD8+ T cells with core fucosylation and sialylation genes. Our models hold the potential to suggest avenues of complementary therapy based on our understanding of perturbations to the TME interaction network following administration of ICI.

Session #5: Systems Pharmacology, Friday, October 21st

A model of clinical drug additivity accurately predicts the efficacy of most FDA-approved drug combinations for advanced cancer

Presenter: Hwangbo, Haeun

Haeun Hwangbo, Adam Palmer

University of North Carolina at Chapel Hill

BACKGROUND: The benefits of combination therapy are often attributed to synergy, that is, drug interactions resulting in an anti-cancer effect that is more than the sum of its parts. Accordingly, the rationale for new drug combinations is often based on synergistic drug interactions. However, preclinical metrics of drug interaction are not applicable to clinical trial data, and there has been no established quantitative method to assess synergy versus additivity in clinical settings. We recently showed that because of extensive patient-to-patient heterogeneity in single drug responsiveness, increasing the chance of a good response to at least one drug was a quantitatively sufficient explanation for the clinical efficacy of many approved combination therapies. Some combinations surpass this "highest single-agent" model, which could be due to either drug additivity or synergy. Here we propose and test a model of drug additivity for Progression-Free Survival (PFS) data from clinical trials, to assess if the efficacies of approved drug combinations are more than, or equal to, the sum of their parts.

METHODS: For all drug combinations that were FDA approved in the past 25 years for advanced cancers, we searched for constituent monotherapy data in the same disease setting with matching dosage. We identified 39 approved combinations with appropriate PFS data. We defined "clinical drug additivity" as the sum of PFS times of each drug in a combination. This calculation was applied to a joint distribution of the clinically observed single drug PFS distributions to compute the expected combination PFS. The clinically observed PFS distribution was compared to the new additivity model or the highest single-agent model (Palmer & Sorger, 2017). We used Cox Proportional Hazard Model to test if an observed PFS distribution is significantly different from expected.

RESULTS: 27 out of 39 combinations (69%) were consistent with drug additivity (i.e., no significant difference; mean R2= 0.95). 9 (23%) were significantly less than additive, but consistent with a model of highest single agent (mean R2= 0.96). 2 (5%) were superior to additivity, i.e., synergistic. One combination (3%) was inferior to highest single agent. Importantly, all approved combinations would have been expected to succeed (significantly superior to control arm) according to this model of additivity. Clinical efficacy, relative to additivity, was significantly associated with the inclusion of immune checkpoint inhibitor, monotherapy approval, and lower hazard ratio versus control arm in the original trial.

CONCLUSIONS: A straightforward definition of drug additivity accurately matched the clinical efficacy of most approved drug combinations, and few drug combinations are 'more than the sum of their parts'. Although most claims of synergy are not supported by clinical data, this does not mean these combinations are ineffective; it means they are predictably effective. The predictable efficacy of most FDA-approved drug combinations suggests that additivity can be used as a design principle for novel drug combinations and clinical trials. Additivity also suggests that single-agent efficacy by each drug is usually required for the clinical success of combination therapy.

Optogenetic dissection of how oncogenic protein condensates modulate signal perception and drug tolerance

Presenter: Bugaj, Lukasz

David Gonzalez-Martinez, Lee Roth, Thomas Mumford, Juan Guan, Bo Huang, Asmin Tulpule, Trever Bivona, Lukasz Bugaj

University of Pennsylvania

Drug resistance remains a significant obstacle in the successful treatment of cancer, highlighting the critical need to understand how oncogenes and cancer drugs impact cell physiology and resistance development. EML4-ALK is a receptor tyrosine kinase (RTK) fusion oncogene that drives 3-7% of lung cancer. Despite potent ALK inhibitors, EML4-ALK+ cancers frequently develop resistance to targeted therapy. Recently, it was discovered that EML4-ALK and other RTK fusions form cytoplasmic protein condensates, and that condensate formation was required for oncogenic signaling. However, it remains unclear whether oncogenic condensates play a role in drug responses. In this study, we applied optogenetics to precisely probe growth signaling networks to ask whether EML4-ALK condensates impact cell signal transmission and drug response. Using light-stimulated RTKs, we found that EML4-ALK condensates strongly suppress signaling through transmembrane RTKs, including through EGFR, a central receptor in resistance development. Strikingly, treatment with ALK inhibitors (ALKi) rapidly restored and hypersensitized RTK signaling. Through optogenetic mapping of feedback interactions, we found that EML4-ALK condensates suppress RTK signals through sequestration of downstream adapters including Grb2, which is essential for signaling through EGFR and other RTKs. Drug treatment caused the release of Grb2 from condensates and re-sensitized RTKs within 10s of minutes of ALKi addition. Re-sensitized RTKs, in turn, caused sporadic RTK activation pulses within 10s of minutes of treatment due to paracrine RTK signals released by apoptotic neighbors. We found that these paracrine signals counteracted ALK inhibitor therapy and promoted survival and drug tolerance. Blocking paracrine signals through co-treatment of ALKi with inhibitors of either EGFR or matrix metalloproteases enhanced cell killing and minimized long-term drug tolerance. Our study uncovers a new role for how oncogenic condensates impact cancer cell persistence during therapy, reveals a novel mechanism for oncogene-induced suppression of RTK signaling, and suggests novel co-therapies to more effectively treat cancers driven by EML4-ALK and possibly other RTK fusions. Our work also demonstrates the potential of optogenetics to diagnose signal dysregulation in cancer cells and to promote drug discovery for enhanced therapy.

Session #6: Cancer Evolution and Mechanisms of Resistance, Friday, October 21st

A systems approach to elucidate basic science questions and clinical translation in multiple myeloma

Presenter: Basanta, David

Anna K. Miller, Ryan Bishop, Conor C. Lynch, David Basanta

Moffitt Cancer Center

Multiple myeloma (MM) is a plasma cell cancer that despite being responsive to therapies such as proteasome inhibitors, frequently relapses. Understanding the mechanism and the niches where resistance disease evolves remains of major clinical importance. In addition to acquired intrinsic resistance mechanisms, bone cells such as osteoclasts and mesenchymal stems cells can protect MM during treatment via the generation of growth factors that facilitate the emergence of resistant clones. How precisely these evolutionary and ecosystem effects contribute to the outgrowth of resistant MM is difficult to accomplish with existing experimental models.

Computational models that incorporate first principles and integrate biological data can allow for unique insights into the spatiotemporal aspects of cancer evolution and how treatment impacts the evolutionary dynamics of the disease. We will describe a novel biology-driven agent-based model that incorporates key cellular species of the bone ecosystem that control normal bone remodeling and, in MM, yields a protective environment under therapy. We used the model to examine how the bone ecosystem contributes to the evolutionary dynamics of resistant MM under control and proteasome inhibitor treatment. Our data demonstrates that resistant disease cannot develop without myeloma intrinsic mechanisms. However, protection from the bone microenvironment dramatically increases the likelihood of intrinsic resistance developing and ultimately tumor relapse. The spatial nature of the model also demonstrates how the bone ecosystem provides a protective niche for drug sensitive cells, thus enhancing the likelihood of multiple resistant subclones arising under treatment and increased tumor heterogeneity upon relapse. In conclusion, we have developed an *in silico* model that demonstrates a significant role for the bone ecosystem in myeloma survival and tumor heterogeneity that can be modified to generate novel treatment strategies to prevent the emergence of resistant disease.

Characterizing Clonal Hematopoiesis to Assess Cancer Risk in People with Down Syndrome

Presenter: Evans Jr., Edward J.

Edward J. Evans Jr., Matthew Galbraith, Ross Granath, Keith Smith, Andrew Thorburn, Joaquin Espinosa, James DeGregori

University of Colorado Anschutz Medical Campus

In cancer-free tissues, somatic mutations accumulate in in all of us over time. In most instances, these mutations are non-consequential to cellular fitness or to overall health. However, some mutations can result in a cell having a fitness advantage over its surroundings, enabling proliferation and potentially cancer. In the blood, this proliferation, known as clonal hematopoiesis (CH), is associated with an increased risk of hematologic malignancies, most notably leukemia, and is commonly observed in the elderly and in people with Down syndrome (DS). People with DS have a markedly higher risk of leukemia (up to 400x for certain subsets). Therefore, I hypothesize that characterizing the CH in people with DS will help explain susceptibility to hematologic malignancies, especially cancer, and shed light on differences in disease outcome. To test this hypothesis, I used a rare-mutation detection technique called Duplex Seq, only requiring a blood draw to obtain the DNA from leukocytes. This targeted-sequencing tool uses a double-stranded tag to incorporate mutational data from both strands of DNA, enabling high sensitivity through the elimination of DNA processing errors.

Using bioinformatics and COSMIC's Cancer Gene Census, we have established differences in mutational patterns that enable us to distinguish people with DS from people without DS and to demonstrate that CH patterns are highly variable within the DS cohort. Notably, CEBPA and NPM1 harbor protein-altering mutations with high variant allele frequencies (VAFs) exclusively in people with DS. CEBPA is a part of a master transcription factor hub including RUNX1 and PU.1 that has been shown to be important for maintaining a balance of self-renewal and differentiation in the hematopoietic lineage, especially for granulomonocytic progenitors. CEBPA is frequently mutated or otherwise functionally disrupted in myeloid leukemias. Leukemia-associated mutations in NPM1 can also disrupt this transcription factor hub through dislocation of PU.1, leading to a differentiation block. We propose that somatic CEBPA and NPM1 mutations specifically in people with DS will enhance the maintenance of progenitors and the inhibition of terminal differentiation, leading to clonal expansion. The role of these clonal expansions in the increased disease risk for individuals with DS merits further investigation.

Ongoing work is centered around determining the gene expression and cytokine pathways that are dysregulated in hematopoietic cells from people with DS who harbor these high-VAF mutations using multi-omics analyses from the same individuals. Additionally, we will use mouse models of DS to investigate the selective advantage of the observed mutations in the DS context and how they impact differentiation choices, highlighting how cell intrinsic and extrinsic factors can influence clonal expansions. Overall, these studies offer a means by which leukemia risk in people with DS can be monitored through observing hematopoietic perturbations, with elucidation of potential mechanisms. By characterizing CH, early markers of perturbed hematopoiesis can be established to determine susceptibility of leukemia and be used to monitor those who are most at risk in vulnerable populations.

Session #7: Signaling Networks in Cancer, Saturday, October 22nd

Mechanistic neural network models of signaling predict drug treatment effects on cell viability

Presenter: Nilsson, Avlant

Avlant Nilsson, Joshua M. Peters, Nikolaos Meimetis, Bryan Bryson, Douglas A. Lauffenburger

Massachusetts Institute of Technology

Human cells are signal-processors; signals from ligands that bind receptors on the cell-surface are integrated via signaling proteins to transcription factor (TF) activity pattern that govern cell fate. Disruptions to this network is common in disease, e.g., cancer, where it can cause cells to rapidly multiply and to ignore termination signals. Simulations of this flow of information could help predict causative responses to mutations and identify suitable drug targets. However, the human signaling network consists of thousands of molecules, and it has been challenging to parametrize systems-wide models using traditional methods [1].

To address this, we developed LEMBAS (Large-scale knowledge EMBedded Artificial Signaling-networks) [2]. It represents these processes as a recurrent neural network with signaling molecules as hidden nodes connected by established protein-protein interactions. Applied to synthetic data of ligand stimulated cells, LEMBAS rapidly parameterizes models that predict unseen test-data (Pearson correlation r=0.98) and the effects of knocking out signaling nodes (r=0.8).

To test LEMBAS performance on data from living cells, we trained a model to predict cell viability in response to drugs, using a dataset assembled from literature sources. These data contained viability for 120 different cell lines under the influence of 7 different anti-cancer drugs in 8 different concentrations (around 6720 observations in total). Additionally, they contained the basal gene expression in each cell line and presence of prominent mutations (e.g., in KRAS and BRAF), that we used to contextualize cell type specific responses. The model contained 1262 signaling proteins and 6594 interactions and additionally included a network layer connecting drugs to their known intracellular targets, as well as network layers connecting mutations and basal gene expression to their corresponding signaling proteins. Following an approach pioneered by Fröhlich et al. [1] we predicted viability as a weighted sum of activities of viability-related transcription factors, e.g., MYC and the FOXO and STAT families. With this framework we rapidly trained models that had both high expressiveness (r=0.87±0.01 fit to training data) and generalization performance (r=0.70±0.06 under cross-validation). Through a combination of sensitivity analysis and perturbations on the trained models we inferred causative signaling cascades, which could mechanistically explain the viability effects under the specific conditions. Using LEMBAS, we have also predicted the transcriptional response to ligand stimulations in macrophages (r=0.8 under cross validation) and modeled the interaction effect of VCAM1 and DLL4 in T-cell development. Demonstrating the feasibility and utility of genome-scale simulations of intracellular signaling. We are currently expanding the framework to directly predict gene expression from signaling events and in future work we are looking to integrate neural network modules of signaling, metabolism, and gene regulation for a more complete mechanistic description of cellular activities.

References:

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Rapid assembly and extension of network models in cancer from the information in literature

Presenter: Ahmed, Yasmine

Yasmine Ahmed, Natasa Miskov-Zivanov

University of Pittsburgh

Creating or extending computational models of complex systems, such as intra- and intercellular biological networks, is a time and labor-intensive task, often limited by the knowledge and experience of modelers. Automating this process would enable rapid, consistent, comprehensive, and robust analysis and understanding of complex systems. In biology, model creation is often highly dependent on human input, it requires reading hundreds of papers to extract useful information, incorporating background and common-sense knowledge of domain experts, and conducting wet lab experiments.

Mechanistic models have been used to explain how biomolecular signaling pathways regulate cell functions. Usually, modelers start with a few seed components and their interactions to build a baseline model which summarizes the modeler's knowledge about the system. Depending on the questions to be answered by the model, the baseline model is often further extended with the information extracted from literature or obtained from the domain experts. Several machine reading engines have been developed recently focusing on biomedical literature and extracting hundreds of thousands of events from thousands of papers within hours. In order to add this information to existing models, or to build new models from it, one needs methods and tools for systematic selection of most useful information from this large machine reading output.

We present CLARINET (CLARIfying NETworks), a novel methodology and a tool for automatically and efficiently expanding models by selecting most relevant and useful information extracted from literature by machine reading. CLARINET creates an Event CoLlaboration Graph (ECLG) from the events extracted by machine readers namely Extracted Event Set (EES). Each node within the ECLG represents a distinct event extracted from literature and each edge indicates a co-occurrence in the same paper of the two events corresponding to its adjacent nodes. CLARINET then uses several novel metrics for evaluating these events individually, in pairs, and in groups. These metrics are based on the frequency of occurrence and co-occurrence of events in literature, and their connectivity to the baseline model.

We tested CLARINET on three previously published biological networks of different sizes namely the naive T cell differentiation (T-cell) model, discrete dynamic T cell large granular lymphocyte (T-LGL) leukemia model and the pancreatic cancer cell (PCC) model with different machine reading outputs that varied in size from hundreds to tens of thousands. CLARINET was able to reproduce these manually built networks with an average recall of 0.8 and average precision of 0.7, while also identifying new interactions with high confidence, all within several seconds. By automatically extending models with the information published in literature, CLARINET allows for rapid collection of the existing information in a consistent and comprehensive way, while facilitating information reuse and data reproducibility, and replacing hundreds or thousands of manual experiments, thereby reducing the time needed for the advancement of knowledge. CLARINET is parametrizable, it allows users to select different extension criteria, depending on the context, focus and goals of their models. CLARINET is highly scalable, its average runtime is at the order of ten seconds when processing several thousand interactions, and it outperforms previously proposed methods.