National Cancer Institute

PHYSICAL SCIENCES in ONCOLOGY PERSPECTIVES

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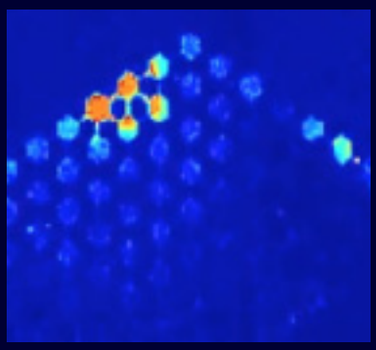
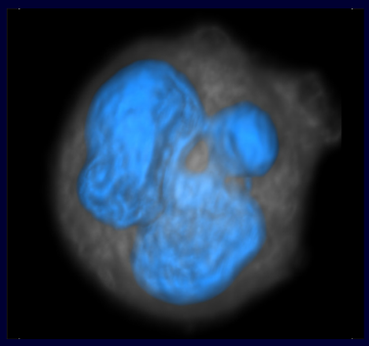
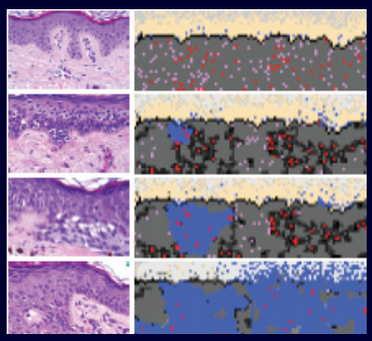
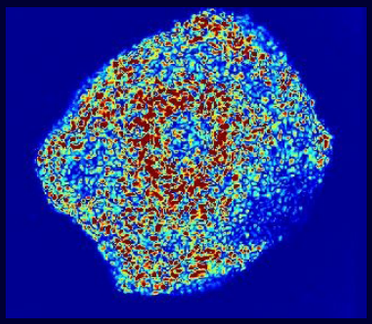
News from the Collaborative Network of Physical Sciences-Oncology Centers | Spring 2012









NCI Perspective: Evolution and Evolutionary Theory

An interesting theme area that emerged as critical in the view of physicists, engineers, and mathematicians and cancer biologists, was the importance of evolution and evolutionary theory in understanding all aspects of the origin and behavior of cancer cells at multiple scales. Cancer, as viewed by the physical sciences, should be considered a complex adaptive system that is most appropriately studied in the context of evolution and evolutionary theory. A major foundational aspect of this focus area includes experimentation and theoretical models that support the development of an evolutionary construct to understand, predict and control the cancer process. These constructs include the accommodation of "omics" data for evaluating and testing robust theoretical constructs and ways to measure physical science parameters.

The PS-OC Network

Arizona State University	Northwestern University
Cornell University	Princeton University
Dana-Farber Cancer Institute	Scripps Research Institute
H. Lee Moffitt Cancer Center & Research Institute	University of California - Berkeley
Massachussetts Institute of Technology	University of Southern California
Johns Hopkins University	The Methodist Hospital Research Institute

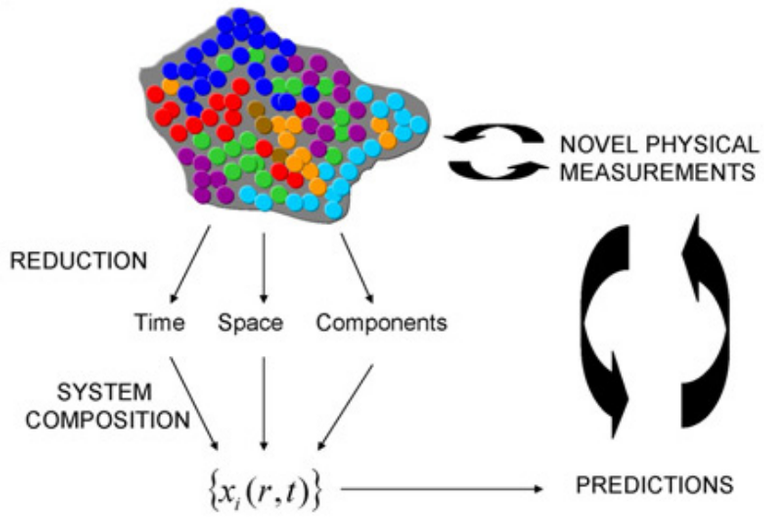
The Physical Sciences – Oncology Center (PS-OC) program funds teams of trans-disciplinary scientists to facilitate the convergence of physical sciences with cancer research and bring a fresh perspective to cancer research. This issue of the PS-OC Perspectives highlights experimental and theoretical approaches from the PS-OCs (Dana-Farber Cancer Institute, H. Lee Moffitt Cancer Institute, & Princeton University) that use evolutionary theory to further our understanding of cancer at all lengths scales. The issue emphasizes the team science that is abundant within each Center and across the PS-OC Network.

Trans-Network Perspective

Evolutionary Dynamics of Brain, Lung, and Hematopoietic Tumors: The Dana-Farber Cancer Institute PS-OC

By Sara Payton-Stone

Led by Franziska Michor, PhD, and Eric Holland, MD, PhD, the Dana-Farber Cancer Institute PS-OC's principal mission is to promote the understanding of cancer evolution, progression, treatment response, and the emergence of resistance by utilizing approaches from the physical sciences. The members of our PS-OC include computational biologists, physicists, mathematicians and chemists from the Dana-Farber Cancer Institute, Memorial Sloan-Kettering Cancer Center, City College of New York, Duke University, University of Minnesota, and Yale University, and scientists from Vanderbilt University and the Memorial Sloan-Kettering Cancer Center. The intended impact of our PS-OC is that its operations – the investigation of mutations arising during tumorigenesis, their cellular origin, and strategies to prevent the emergence of resistance – are carried out in close collaboration between theoretical and experimental scientists and clinical investigators to bridge the divide between the physical sciences and oncology, and eventually lead to theoretical investigations in conjunction with clinical trials. This paradigm shift from segregated research environments to integrated analyses of physicists, engineers, mathematicians and clinical oncologists, cancer biologists and surgeons is the ultimate goal of our PS-OC – as we envision that our detailed theoretical investigations of the evolutionary processes leading to cancer will eventually lead to rationally designed molecular profiling and interventions in clinical settings.



The approach of the PS-OC.

The PS-OC adheres to the following general approach: tumors in their temporal, spatial and compositional heterogeneity are being decomposed to various aspects such as time (when do events happen?), space (where do events happen?), and components (what is the distribution of events across all components of the system?).

Research Projects

Project 1: DEVELOPING A MATHEMATICAL FRAMEWORK TO UNCOVER THE SEQUENCE OF GENETIC EVENTS DURING TUMOR DEVELOPMENT is led by Ingo Mellinghoff, MD, and Chris Sander, PhD, both of Memorial Sloan Kettering Cancer Center. The recognition of cancer as a disease caused by the accumulation of genetic alterations has motivated large-scale efforts to annotate the cancer genome for all human cancers. When combined with computational approaches that can distinguish statistically significant, recurrent events from the background "noise" in high-resolution datasets, these cancer genome surveys yield molecular portraits which are specific for each cancer type and highly consistent across

"The intended impact of our PS-OC is that its operations - - are carried out in close collaboration between theoretical and experimental scientists and clinical investigators to bridge the divide between the physical sciences and oncology."

multiple sample sets. In this project, we are linking these emerging, large cross-sectional datasets with a novel mathematical model to predict the sequence of genetic events during tumorigenesis using an evolutionary model of the dynamics within a network of possible mutations. Our project leverages our expertise in computational biology, glioma, and leukemia - and through an iterative process of mathematical predictions and physical measurements, works to develop a tool that will be broadly applicable to other human cancer types.

Project 2: THE CELL OF ORIGIN OF HUMAN CANCERS is led by Eric Holland, MD, PhD, and Franziska Michor, PhD, of Memorial Sloan Kettering Cancer Center and the Dana-Farber Cancer Institute, respectively. This project recognizes that although knowledge of the target of transformation is important for an understanding of the natural history of cancers, the cell of origin of most cancers is still unknown. As preliminary data, we designed a stochastic mathematical model of stem and progenitor cells to study the evolutionary dynamics of initiation of JAK2V617F-positive myeloproliferative neoplasms (MPN). In this project, we are aiming to 1) test the predictions of the mathematical framework in mouse models of JAK2-positive MPN and use this data to further refine the model; 2) design a mathematical framework to investigate the cell of origin of glioma stem cells, considering three different mutations leading to glioma formation: a mutation causing overexpression of PDGF and genetic alterations inactivating both alleles of certain tumor suppressors; and 3) validate the predictions and further refine the mathematical model with data derived from murine models of glioma which allow for expression of the known pathogenic mutations in the appropriate stem/progenitor cell compartments. These interdisciplinary approaches will enable us to identify the cell of origin of MPN and glioma and will also have relevance to other tumor types arising in tissues that are organized as a differentiation hierarchy.

Project 3: NOVEL TOOLS TO PREDICT AND PREVENT THE EMERGENCE OF RESISTANCE TO TARGETED DRUGS AND RADIATION THERAPY is led by William Pao, MD, PhD, and Franziska Michor, PhD, of Vanderbilt University and the Dana-Farber Cancer Institute, respectively. Some cancers are exquisitely sensitive to an-

Project 1 links the emerging, large cross-sectional datasets with a novel mathematical model to predict the sequence of genetic events during tumorigenesis.

ti-cancer treatment. For example, patients whose lung adenocarcinomas harbor specific mutations in the epidermal growth factor receptor (EGFR) tyrosine kinase domain frequently experience clinical and radiographic responses to the selective EGFR tyrosine kinase inhibitors (TKIs) gefitinib (Iressa) and erlotinib (Tarceva). Medulloblastomas analogously are extremely sensitive to radiation treatment. However, in both instances, the disease returns after an initial response to treatment. In half of such lung cancer patients, the Pao Lab has demonstrated that tumor cells harbor a second mutation in the EGFR kinase domain, which alters a 'gatekeeper' residue in the ATP-binding pocket. Another 20% of patients develop tumors with amplification of the gene encoding kinase, MET.

"....we've proposed an interdisciplinary approach utilizing mathematical modeling and unique experimental systems to predict and prevent the emergence of resistance against targeted drugs and radiation therapy....."

In patients with medulloblastoma, data from the Holland Lab has suggested that radiation-resistant cells in the perivascular niche undergo G1 arrest in response to treatment and then self-renew, giving rise to recurrence. Since acquired resistance to either targeted or radiation therapies represent severe limitations of therapy, and since existing treatment schedules were established empirically, we've proposed an interdisciplinary approach utilizing mathematical modeling and unique experimental systems to predict and prevent the emergence of resistance against targeted drugs and radiation therapy by developing a mathematical framework for the general scenario of drug resistance emerging during therapy with targeted drugs, based upon simple growth and death rates.

Research Core

Lastly, the Single Cell Analysis Core, equipped with the state-of-the-art single cell analysis tools, is developing innovative technologies for quantitative single-cell assays and facilitates the research proposed in Projects 1-3. More specifically, this core takes advantage of the most recent advances in microfluidic single-cell handling to establish five single-cell analysis platforms, including: single-cell secretomic profiling, single-cell phospho-protein profiling, single-cell whole genome amplification for genome-wide functional analysis, single-cell epigenomic analysis, and laser scanning cytometry for high-content single-cell analysis. These platforms enable a suite of functional evaluation ranging from genomics, epigenomics to proteomics of single primary tumor cells and examine phenotypic variability, functional state of activation and/or the response to drugs in heterogeneous populations of cells. To achieve these objectives, we are working to establish validated quantitative measurements of proliferation and death rates of primary tumors and associated molecular signatures, provide single-cell resolution of the variability of cellular signaling response during varying conditions, and establish systematic protocols to test the drug response of primary tumor cells.

Outreach and Education

The PSOC Education/Training and Outreach/Dissemination Units have developed into dynamic, informational, and rewarding aspects of our PS-OC. Each has sought to provide integrative training opportunities to develop a knowledge base relevant to cancer biology and the physical sciences as well as to disseminate information, PS-OC capabilities, and related projects to cancer biology/physical science communities. Two notable accomplishments include coordinating regularly scheduled informational seminars for the Dana-Farber community as well as our PS-OC collaborators to foster successful and meaningful partnerships. Lastly, members of our PS-OC met with 30 area students from local schools in an effort to hold a discussions regarding the mission of the PS-OCs, the importance of integration between physical sciences and oncology research.

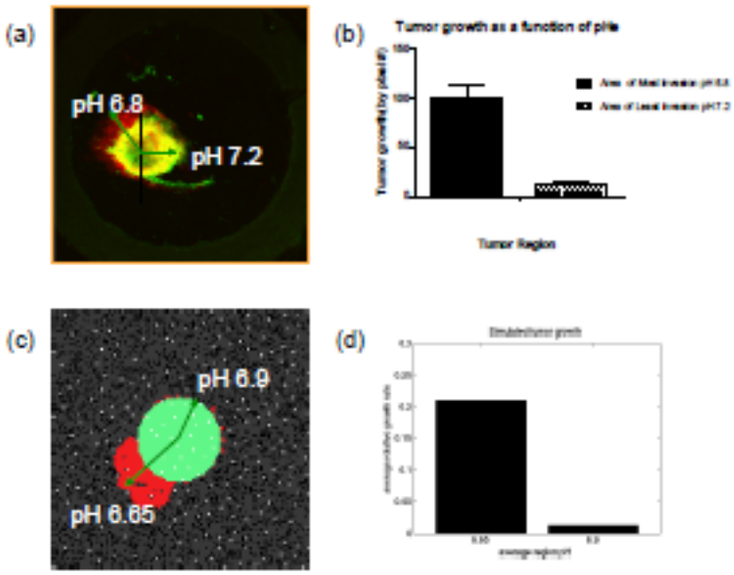
We have already developed a mathematical framework for the general scenario of drug resistance emerging during therapy.

Sara Payton-Stone is the leader of the Outreach and Dissemination Unit for the DFCI PS-OC.

The H. Lee Moffitt Cancer Center & Research Institute PS-OC

By Alexander Anderson

The Moffitt/UW PSOC is focused on understanding the role of the physical microenvironment in cancer biology and treatment. Our research paradigm investigates the complex interactions of tumor cells and their microenvironment through a multidisciplinary approach that includes mathematical modelers, clinical oncologists, and tumor biologists. The integrated mathematical oncology department plays essential role in the Moffitt/UW PSOC by building models that allow us to have a comprehensive coarse grain views of carcinogenesis, cancer invasion and cancer therapy as dynamical systems. Imaging continues to be a key tool that enables the integration of biology with mathematics. We have three projects, which integrate each of these key elements and the mathematics core as well as our education and outreach.



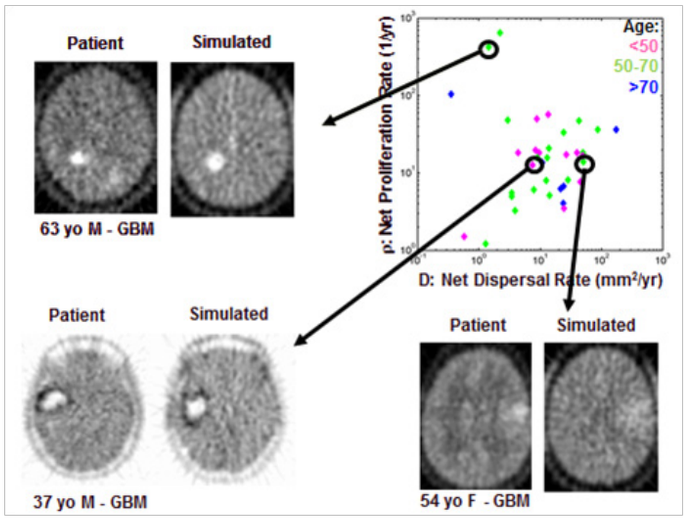
Lower panel shows multiscale model simulation and quantification of tumor growth as a function of pH. Upper panel shows in vitro imaging and quantification that directly validates this result.

Research Projects

Project 1: Project 1 is examining the importance of tumor-stromal interactions in driving melanoma progression. Utilizing a combination of 3D cell culture, pathology and an in silico virtual skin model we have begun to unravel new routes of melanoma initiation. Critically, we developed a multiscale mathematical model that captures the essence of normal skin development and maintenance and have systematically perturbed it to find the most likely routes tumors can propagate down once initiated from a spectrum of mutated melanocytes. Intriguingly, our integrated approach has been able to show that minimally transformed melanocytes combined with senescent stroma are just as effective at promoting melanoma progression as very aggressive melanocytes are on their own. This connects well with the observation that melanoma is a disease of the aged – as we age normal regulatory mechanisms may begin to malfunction and stroma may become senescent. We are now working closely with our pathologist colleagues to validate this hypothesis from patient-derived biopsies. In other work we are building upon emerging knowledge about how different initiating melanoma oncogenes dictate underlying biological behavior and incorporating this into our model.

Project 2: Project 2 has focused on testing the "Acid-Mediated-Invasion" (A-M-I) hypothesis. The A-M-I hypothesis fundamentally states that tumors, by virtue of their high glycolytic rates, produce high amounts of acid, which is then ex-

"Our research paradigm investigates the complex interactions of tumor cells and their microenvironment through a multidisciplinary approach..."



Inter- and intra-tumor heterogeneity of hypoxia and necrosis in glioma brain tumors is revealed by FMISO-PET imaging and further quantified and predicted by a patient-specific mathematical model calibrated using routine pre-treatment MRI.

ported into the surrounding parenchyma. This acid is toxic to the surrounding stromal cells, thus facilitating local cancer invasion. To this end, we observed that oral buffers prevented metastases, but did not affect primary tumor growth, in mice. The effect of buffers is a highly dynamic, and a fundamental understanding of their behavior has been achieved through the development of multiscale mathematical models. Under the auspices of the PS-OC, we have been further testing the A-M-I hypothesis by investigating buffer therapy and have determined that a variety of other buffers can inhibit metastasis formation as well as prevent the eruption and development of spontaneous prostate cancer. Experiments are ongoing to identify how some tumors are resistant to buffer therapy (with the aid of the mathematics core). The dorsal window chamber system, in which tumor cells are implanted in a gel matrix in the presence or absence of GFP-expressing microvascular buds within a dorsal window chamber, has been a core technology for quantifying A-M-I in vivo. In appropriate matrixes, tumor growth has been spatially compared to external pH (measured using SNARF-1 fluorescence) and has revealed a remarkable relationship between extratumoral acidity and the rate of growth, which can be abrogated by buffers. Notably, our multiscale mathematical model has been able to faithfully recapitulate this behavior, and has provided important clues as to the mechanisms underlying the acid-mediated invasion process.

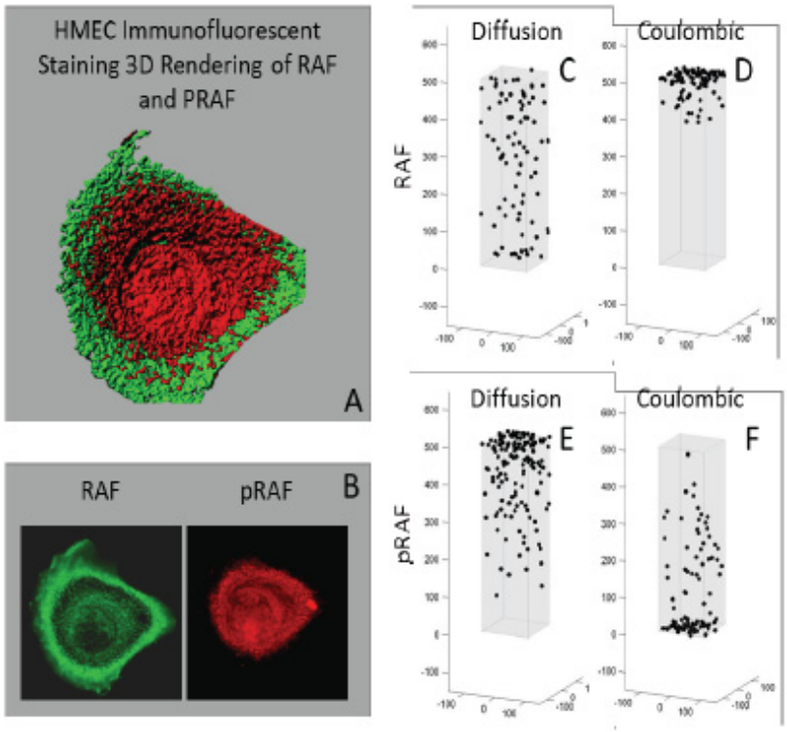
Project 3: Project 3 aims to see beyond the "tip of the iceberg" revealed by medical imaging and to utilize multi-modality imaging to investigate malignant progression of glioblastoma. Swanson and colleagues have shown that the heterogeneity in radiographic and genetic phenotypes of gliomas can be connected to routinely available pre-treatment imaging via a reaction-diffusion mathematical model which quantifies net rates of proliferation (ρ) and invasion (D). The model provides a patient-specific framework to investigate the clinical presentation of glioma brain tumors on a wide variety of orthogonal data types, from radiographic to histologic features. We have demonstrated the net rates of proliferation and invasion are prognostically significant and have a strong correlation with response to radiation treatment, effectiveness of resections, and overall tumor growth behavior. These parameters can be used to simulate hypoxia imageable on 18F-fluoromisonidazole (FMISO) PET images in a patient-specific

"....tumor growth has been spatially compared to external pH ... and has revealed a remarkable relationship between extratumoral acidity and the rate of growth."

manner. Such a tool provides a means to see beyond the clinical obscuring lens of PET imaging as well as investigate patient-specific predictions of malignant progression via quantifications of hypoxia and necrotic tissue within the tumor. These model-predicted synthetic PET images have value for treatment planning, as hypoxia is known to be correlated with resistance to radiation therapy.

Research Core

The mathematics core provides support for the existing projects and for innovative new areas that emerge from the continuous interactions among the Moffitt PS-OC investigators. Of specific note are: (i) Evolutionary dynamics of metastases. (ii) Evolutionary dynamics of cancer therapy. Mathematical models applying evolutionary principles to cancer therapy have demonstrated that high dose density (maximum tolerated dose given over the shortest possible time period) is not optimal. (iii) Intracellular communication. We propose messenger protein localization and movements are highly regulated and governed by Coulomb interactions: The model has been applied to the RAF-MEK-ERK pathway and scaffolding protein KSR1 using computer simulations and in-vitro experiments.



Predicted and measured steady state distribution of RAF and pRAF under typical culture conditions. Left panels demonstrate pRAF and RAF distribution in HMEK cells. In the right panels, computer simulations demonstrate that if protein movement is by diffusion alone, pRAF will exhibit a concentration gradient with highest levels near the CM where it is generated by interactions with RAS and lowest levels near the nucleus.

Outreach and Education

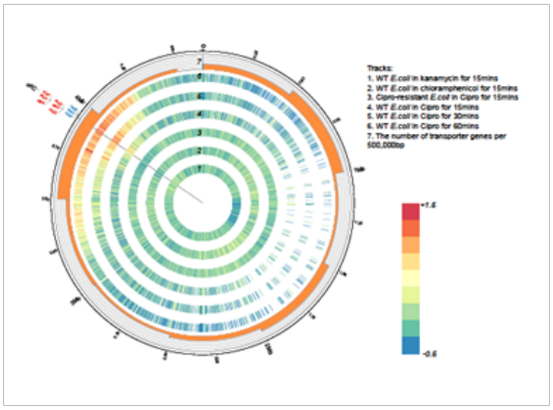
The IMO-PSOC seminar series serves as a primary venue for our education and outreach activities. Presentations, by a wide range of biological, computational and clinical experts covering the gamut in cancer research, are recorded and placed online for the greater PSOC community. We also have a discussion group joint with the Cornell PS-OC, the Cancer Brainstorming Club (CBC), designed to promote creativity and critical thinking among early stage scientists and foster dialogue across physical sciences and biology. The CBC hosts a website to highlight their activities (http://www.cmm.cornell.edu/brainstorming-club.html).

Alexander (Sandy) Anderson is a Project Leader in the H. Lee Moffitt PS-OC.

Cancer as an Evolutionary Phenomena: The Princeton University PS-OC

By Melissa Aranzamendez

The Princeton PS-OC is led by Robert Austin (Princeton University) and Thea Tlsty (University of California San Francisco). It examines the evolutionary dynamics of cancer growth and metastasis from a fundamental perspective of Darwinian natural selection. The Center remains focused on several provocative questions: (1) Does cancer start due to the presence of pre-existing mutant cells in the body which slowly evolve until some sort of transition to full uncontrolled growth occurs due to the build-up of several critical mutations, or can cancer begin in a community of healthy cells due to the response of cells to high stress conditions? (2) Do the physical parameters of a tumor (i.e. pressure, temperature, metabolic state, nutrient feeds) lead in the evolutionary progression of a tumor to an uncontrollable state? (3) What is the role that network dynamics play in the development of cancer from an evolution perspective? (4) What is the role that heterogeneity plays in the evolution of resistance to chemotherapy in cancers?



Summary of custon microarray experiments. Centering of gene expression multiple copies at the oriC.

Research Projects

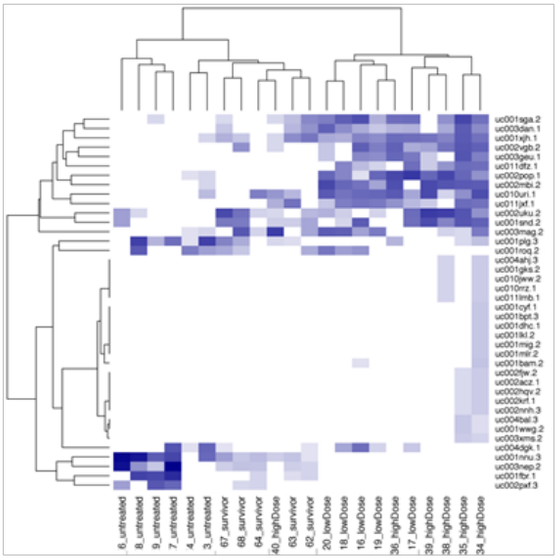
Project 1: Cells in vivo have to adapt and evolve in a complex and heterogeneous world. Probably the first line of defense to external toxins is to over-express membrane associated pumps expressed by the rbsA gene which lower the internal concentration of toxins if possible to a level where replication is possible in the case of a genotoxic toxin. A similar response is often seen in cancer cells when they are treated with a mutagenic chemotherapy agent. Since expression of the rbsA gene lowers the internal concentration of external antibiotics, an efficient and quick way to maintain DNA replication when challenged by cipro would be for the organism to quickly make multiple copies of the rbsA gene. In order for this to happen, the rbsA gene would need to be located near the origin of DNA replication (oriC) on the E. coli genome. We developed a custom microarray using 60 basepair probes, these spots tile the entire 4.6 MBp E.coli genome, and in particular, all the known E. coli genes had a complementary probe spot. There is a clear increase in gene copy observed around the oriC that increases with the duration of cipro exposure, while E. coli exposed to chloramphenicol and kanyamycin did not show a significant difference compared to the wild type strains. Multiple copy number also gives those transporter genes a higher chance to mutate.

Project 2: Cell-to-cell gene expression differences stem from the stochastic nature of transcriptional dynamics and cell-cycle dependence. Thus, adjacent cells from the same tissue could have dramatically different gene expression profiles. We have done a pilot study by performing single-cell RNA-Seq using human breast cancer cell line MDA-MB-231. We treated a fraction of these cells with an anti-cancer chemotherapy drug, paclitaxel (Taxol).

"It [the Princeton PS-OC] examines the evolutionary dynamics of cancer growth and metastasis from a fundamental perspective of Darwinian natural selection."

In the preliminary results from this single-cell RNA-Seq study, Fig. 2 shows that it is possible to perform single-cell whole-genome gene expression analysis with good sensitivity. Our data show that there is heterogeneity in the single cells that we cannot detect in pools or populations. We identified 40 significantly differentially expressed genes. Gene ontology enrichment analysis reveals that genes that are expressed in single cells treated with Taxol are involved in regulation of apoptosis, and proteolysis.

Project 3: It is clear that changes in cellular structure along with the microenvironment can be important modulators of the evolution of therapeutic resistance in cancer. Among the goals of Project 3 are to utilize quantitative nuclear and cellular morphometric features to evaluate evolutionary changes; to examine the effects of the development of resistance on nuclear structure by proteomics as well as digital image analysis of nuclear morphometry and to examine micro-ecologies as physically-induced stressors. In order to examine some potential mechanisms of drug resistance, cellular models have been evaluated. Prostate cancer cells that are resistant to the chemotherapy, paclitaxel, elicited a discrete loss of epithelial cell markers and had a significantly lower expression of keratins, while at the same time these paclitaxel-resistant cells gained expression of mesenchymal markers typically implicated in an epithelial mesenchymal transition (EMT). Consistent with these molecular manifestations of EMT, biophysical studies revealed that paclitaxel resistance also conferred the cells with the ability to exercise higher cell traction forces, invade the surrounding extracellular matrix (ECM), and form colonies in soft agar. Strikingly, the internal network of the cytoskeleton in paclitaxel-resistant cells showed an unanticipated fluid-like behavior and displayed faster remodeling dynamics than the parental cells. While these resistant cells appear to demonstrate an EMT like phenotype, the development of resistance to chemotherapeutic agents results in their being more sensitive to other microenvironmental stresses such as temperature, pH and glucose deprivation. In this regard, combinations using classical therapeutic approaches for cancer such as chemotherapy and radiation therapy, and approaches which modulate microenvironmental stress may decrease the ability of cancer cells to develop resistantce and therefore increase the efficacy of these currently used therapies. Overall, this project is revealing novel informa-



Preliminary results from this single-cell RNA-Seq study. Top 40 significantly differentially expressed genes.

"We will compare the genetic difference between the rapid resistant myeloma cells from our chip and the chronic resistant myeloma cells from tradition protocols."

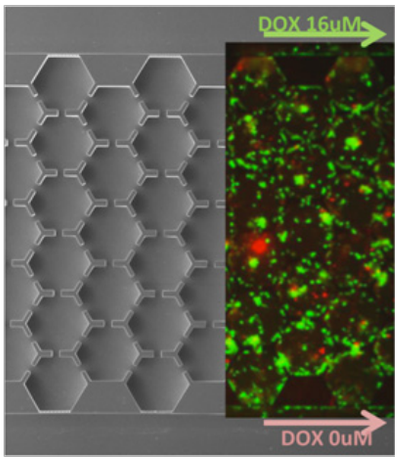
tion about the evolution of resistance and developing concepts and tools that might attack cancer at its Achilles heel.

Project 4: The tumor microenvironment plays a crucial role in the cancer evolution of drug resistance. To address this issue, we are performing in vitro experiments which mimic the tumor microenvironment, in which cells are exposed to not uniform concentrations but rather gradients of drugs, nutrients, and other factors. Compared to traditional in-vitro methods, microfluidic structures enable better control of the temporal and spatial profile of gradients. We have developed a microfluidic platform with a stable doxorubicin (genotoxic drug) gradient and heterogeneous landscapes to mimic the tumor microenvironment. We have observed that the multiple myeloma cells are more sensitive to doxorubicin than bone marrow stroma. Further, we have seen emergent colonies forming in high doxorubicin regions above their minimal inhibitory concentrations in 1 week (see Figure). We will compare the genetic difference between the rapid resistant myeloma cells from our chip and the chronic resistant myeloma cells from tradition protocols.

Outreach and Education

The Princeton PS-OC has maintained a strong program of both intellectual outreach to the wider community via two workshops, and has also initiated a series of "Boot Camps for Microfluidics" to reach out to the community of biologists who would like to learn the techniques we are developing. In addition to this work, we have been a major presence at the American Physical Society March Meeting over the past 3 years. In 2012 we organized four Focus sessions on the physics of cancer and ran a tutorial on "An Introduction to the Physics of Cancer". This tutorial was attended by graduate students, post-docs, university faculty and industrial researchers interested in a broad introduction to the current state of our understanding of cancer from a physics perspective.

The Microfluidics facility is one of the three core facilities of the Princeton PS-OC . Last year, for our inaugural camp, we hosted 16 students for a week-long course. The course consisted of a series of lectures and hands-on lab work, constituting a broad introduction to microfluidics. Participants successfully built their own microfluidic devices from start to finish and performed several experiments using



Left: SEM image of array of microhabitats; Right: +144 hours after inoculation RFP expressing myeloma cells are still propagating in the Doxorubicin gradient.

them. The material from this course is posted on our website.

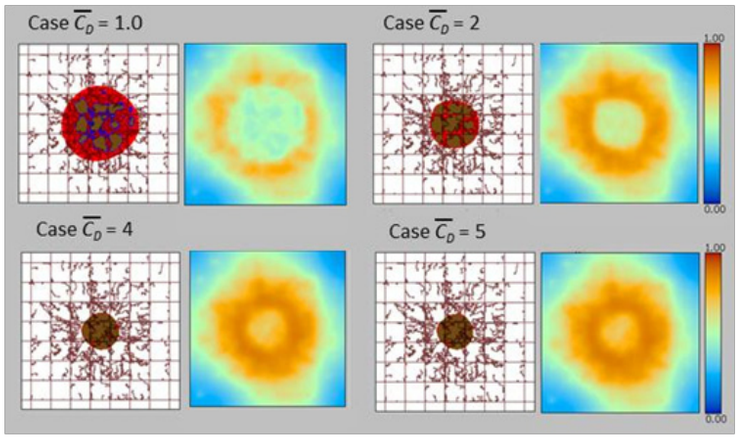
We have run two workshops. One of them was a theoretical workshop organized by Professor Sal Torquato on "Understanding Cancer via Theoretical Science", and hosted by the Princeton Center for Theoretical Sciences on April 15-16, 2010. The second one was an experimental/clinical "Workshop on the Physics of Tumor Heterogeneity" which was held at Princeton on June 5-7, 2011.

Melissa Aranzamendez leads the Outreach and Dissemination Unit for the Princeton University PS-OC.

Physical Oncology: Building a Bridge from the Cell to the Tumor

By Hermann Frieboes

Molecular and cellular events may not completely account for cancer behavior in patients, since this behavior involves multifaceted interactions in a diverse microenvironment with effects across a wide range of time and length scales. In order to study cancer at a system level, engineering and physical sciences approaches tightly integrated with experimental data and clinical observations have proven useful. Collaborating with colleagues at the USC and TMHRI PS-OCs, and together with the biomathematical team led by Professor Vittorio Cristini (UNM), I have worked to develop and integrate experimental biology, mathematical modeling, and computational simulation approaches to study cancer growth and treatment response. The aim of this work is to predict tumor growth and invasion from the molecular and cellular scale events, with the ultimate goal to help analyze and treat tumors of specific patients [1].



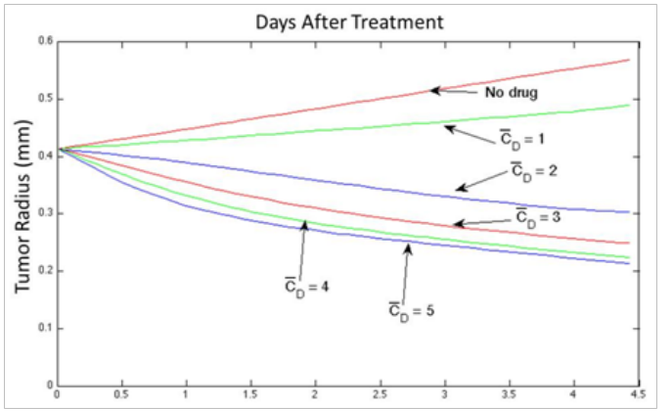
Tumor inhibition simulated after 4 days of doxorubicin release from nanoparticles lodged in the tumor neovasculature for various values of the initial drug concentration at the tumor site. Tumor tissue is shown in 2x2mm panels (viable regions in red, hypoxic in blue, and necrotic in brown). The corresponding drug distributions (orange to blue color) as a fraction of the maximum value in the vasculature are shown next to the tumor panels, except for the negative control case (= 0).

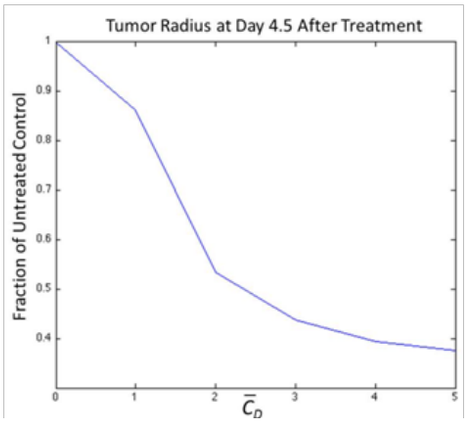
Inefficient vascularization hinders the optimal transport and uptake of cell nutrients, oxygen, and drugs by cancer cells in solid tumors. With the TMHRI PS-OC, we have integrated mathematical modeling of cancer with intravital microscopy to evaluate and optimize the behavior of nanoparticle-based drug delivery systems designed to circumvent these biophysical barriers. The complex interactions between blood flow, vasculature, and tumor growth were previously examined in Macklin et al. [2] by coupling an updated version of a continuum tumor model [3,4] with a detailed model of tumor-induced angiogenesis [5-7]. We recently extended this modeling framework to incorporate interstitial pressure and flow, lymphatic fluid drainage, and blood vessel leakage. The resulting system provides a realistic platform to simulate nanoparticle flow and distribution within tumor vessels.

Two key questions with nanotherapy are to determine how much drug to load per particle and how many particles to release within the tumor vasculature in order to achieve remission. Simulating the heterogeneous tumor microenvironment, we extended the cancer model to explore these questions by incorporating measurements obtained from intravital microscopy [8]. Interestingly, the model predicts a saturation phenomenon in which increasing the amount of drug leads to a correspondingly smaller increase in the death effect. This is well known from previous studies, where a linear increase in drug concentration

“.....timescale of drug diffusion and uptake is significantly shorter than cell death and removal, hence drug levels after an initial burst release would be insufficient to induce death for cells farther removed from the vasculature .”

does not necessarily correspond to a linear increase in the death effect. One reason is that the timescale of drug diffusion and uptake is significantly shorter than cell death and removal [9], hence drug levels after an initial burst release would be insufficient to induce death for cells farther removed from the vasculature [10,11]. This suggests the need for particles capable of sustained drug delivery. Even with a continuous release model, we find that tumor shrinkage begins to level off after several days after simulated treatment. This behavior is attributed to dynamic heterogeneities in the tumor and vascular structures limiting the drug transport, such as regions of poor drug penetration created when cells undergo apoptosis. Our studies suggest that smarter particles designed through a combination of mathematical modeling and empirical data collection may provide a more optimal treatment strategy than simply delivering more nanoparticles or more drug per particle. In particular, nanoparticles that can release drug in steady, regular bursts over a prolonged period of time may in some cases be more effective. We are currently further exploring this concept of “metronomic nanotherapy.”





Comparison of tumor inhibition based on different values of initial drug concentrations delivered via silicon nanoparticles at the tumor site (top). Plot of tumor size at the end of treatment as a function of initial drug concentration, showing that values of > 1.2 would lead to a tumor volume reduction of 50% or higher. (Bottom)

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Hermann Frieboes is an Assistant Professor at the University of Louisville. He is a member of the USC and TMHRI PS-OCs.

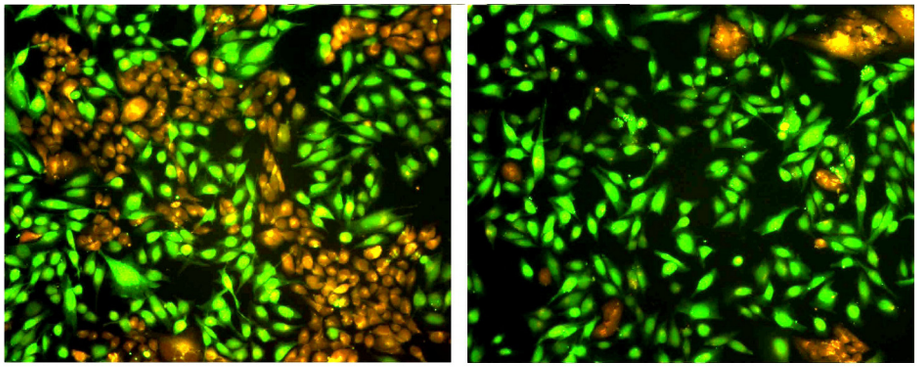
The Impact of the PS-OC Network on a Cell Biologist

By Shannon Mumenthaler

Three years ago I thought of myself as a cell biologist; I was familiar with hundreds of cellular pathways, and cellular phenotypes as well as diverse assay techniques for identifying and then characterizing a gene of interest. Unsurprisingly, ordinary differential equations were not part of my scientific vocabulary; I didn't typically think about how mathematical models could impact cancer biology. Today, I describe myself as a quantitative biologist and find myself debating the merits of diverse forms of mathematical models on a daily basis. I attribute this transformation to my mentors and to my experiences in the NCI Physical Sciences in Oncology initiative exposing me to new areas of biomedical research.

When I look back, I can remember the exact day that signifies the turning point in my career. It was the 1st Annual USC PS-OC Symposium in June 2010 and my first interaction with members from other PS-OC institutions. I recall my mentor, Parag Mallick, saying "there is someone you have to meet. She is a mathematical modeler from Dana Farber." My first thought was, 'what will a mathematician and cell biologist have to talk about?' It was like being set up on a scientific blind date: two people with completely distinct backgrounds from opposite parts of the country meet over coffee and pray it won't be awkward and painful. I soon found out we had common goals and complimentary skill sets. We were both interested in studying the challenges associated with therapeutic response and drug resistance. And thus was the beginning of a fruitful collaboration with Jasmine Foo, who at the time was a postdoc in Franziska Michor's group (Dana Farber PS-OC) and now is an Assistant Professor of Mathematics at the University of Minnesota.

HCC827: H1975 Admixture



0μM Erlotinib, 48H **1μM Erlotinib, 48H**

Visualization of tumor heterogeneity before and after treatment. Admixture of sensitive (orange) and resistant (green) non-small cell lung cancer cells treated with an EGFR tyrosine kinase inhibitor, erlotinib.

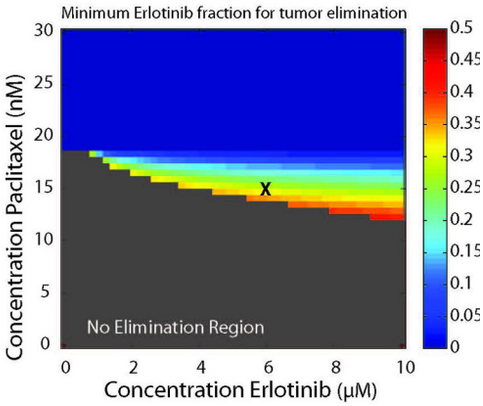
Early on Jasmine and I realized that in order for our collaboration to be successful we had to speak a common language. This was first tested in July 2010 when we developed a PS-OC trans-network proposal linking our group from USC, the Michor group at Dana Farber Cancer Center, the Tlsty Group at UCSF, and the LaBaer group at ASU. We defended our proposal "Development of Models of Penetrance of Resistance", during a two-day head-to-head proposal review meeting in Denver. The premise of our proposal was to develop an integrated computational and experimental platform to understand how evolutionary pressures (i.e. therapy, micro-environmental fac-

"There is something truly rewarding about bringing together minds from diverse backgrounds to work as a team, challenge ideas, and generate new approaches to fight the war on cancer."

tors) impact the penetrance of drug resistant cells throughout a tumor leading to poor clinical outcomes. Once the platform was developed, we proposed to use this model to develop treatment strategies for limiting the outgrowth of resistance cells, possibly through the use of treatment cycling or combination strategies. Developing and presenting a trans-network proposal was a thrilling experience that opened my eyes to the wealth of knowledge, ideas, and amazing talent that was to be found in academia. Having the ability to flush out ideas face-to-face with other group members and receiving real-time feedback on our proposal from the selection committee was a pivotal exercise for a young investigator venturing into a new realm of interdisciplinary research. Even more exciting was winning the trans-network award and receiving validation that our ideas and approaches were novel, plausible, and clinically relevant.

The next challenge came when we had to start transitioning our ideas into results. As we began discussing what data would be needed for developing the model, it became clear that we might have to develop a novel experimental platform, which we accomplished using the Cellomics arrayscan. This high-throughput automated fluorescence microscopy platform allowed us to monitor cell heterogeneity in an extremely detailed, accurate and reproducible manner and generate truly quantitative cell growth data to serve as calibrants for the model. Since receiving the trans-network award, we have published some of our initial work, have used the preliminary data to write other grants, initiated new projects, and cultivated other collaborations.

The PS-OC has given me a new perspective on biomedical sciences and a new career direction. The encouragement of my mentors, Parag Mallick and David Agus, the collaborations that I have formed through the PS-OC, and the promise of multidisciplinary projects impacting cancer research, has motivated me to pursue a career in academia. There is something truly rewarding about bringing together minds from diverse backgrounds to work as a team, challenge ideas, and generate new approaches to fight the war on cancer. I believe that mathematical modeling is one such tool that will have tremendous utility in the clinic by improving current treatment strategies and proposing novel treatment paradigms. Science is changing and it is an exciting time for young investigators from all fields to come together to challenge the status quo.



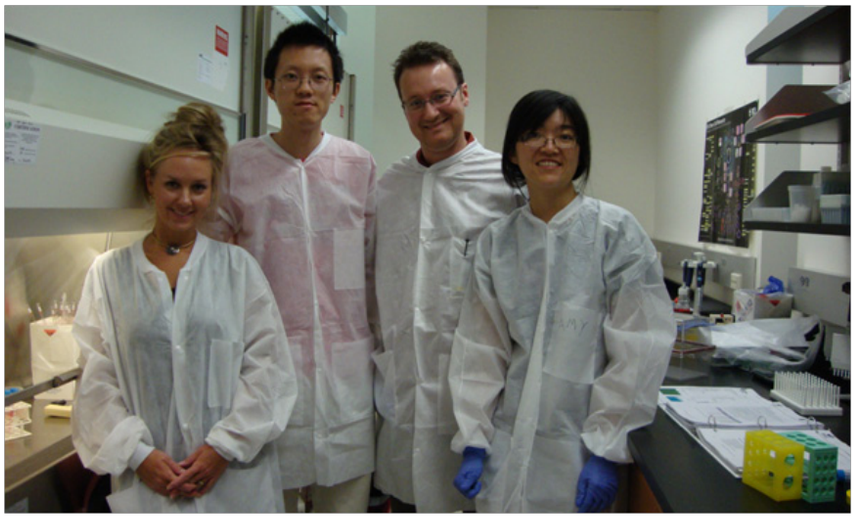
USC PS-OC postdoc, Shannon Mumenthaler (top). The image below, derived from model predictions, is a map of the sequential paclitaxel–erlotinib combination treatment space highlighting schedules that can achieve elimination of a NSCLC cell population.

Shannon Mumenthaler is a Postdoctoral trainee in the University of Southern California PS-OC.

ATCC Cell Cuture Workshop Experience

By Amy Wu

Before I headed for the workshop in Summer 2011, I suffered a lot from dealing with the cancer cell lines in my lab. I just could not repeat what I had done a couple of weeks before and did not know why. Lots of biological labs provide tissue culture training and we, biological physicists, sort of know how to do it. However, in many cases we relied on the biologists to tell us how the cells should look based on their experience and the qualitative description bothered us very much.



Participants in the 2011 PBCF ATCC Cell Culture Workshop. Left to right: Heather Branscome (ATCC instructor), John Qiucen Zhang (Austin lab; Princeton PS-OC), John Foulke (ATCC instructor), and Amy Wu (Austin lab; Princeton PS-OC)

The ATCC cell culture workshop came just in time to solve the puzzle in our lab. The lecturers, especially Dr. Yvonne Reid, gave excellent talks to explain the fundamentals of cell culture, cell cryopreservation, and cell contamination from the perspectives of microbiology, chemistry, and physics.

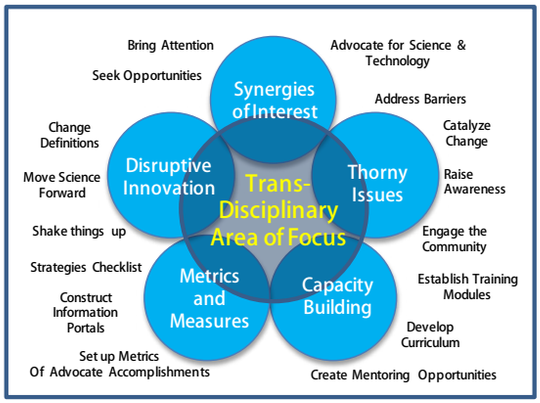
In four days, we were trained to use the strictest practice to culture both suspension and adherent mammalian cell lines. Most importantly, we learned how to manage the contamination issues. I realized that there are actually a lot of assays and resources to scientifically examine "if the cells are still the same." Cell line authentication is challenged frequently in most biomedical researches. If we find something new or unusual, how do we know it is a great discovery or just an artifact? We could not just satisfy ourselves if the experiment is not reproducible.

After I went back to my institution, our lab had a revolution to abandon all the sloppy practices. I conveyed what I have learned from the workshop and passed the 8 mm thick lecture notes around. Since then, our lab has been operating very well. I cannot imagine how much time my colleague and I would have wasted if I did not attend the workshop. One thing that impressed me a lot is that there were more trainers than trainees. They shared their great experiences with us and they kindly answered our questions. It was a very useful and cheerful experience. As a biophysicist, I am glad I learned these practical biological techniques and will try to make our microfluidic capabilities function in a more compatible way.

Amy Wu is a graduate student at Princeton University where she works in the laboratory of PS-OC principal investigator Dr. Robert Austin.

Bay Area Physical-Sciences in Oncology Center (PS-OC) Advocacy Forward: Move It!

By Susan Samson



Vision Moving Forward... Integrating emerging physical science and adocacy trans-disciplinary engagement into clinical practice will require stakeholders to promote new collaboration paradigms. Collectively we should consider new approaches that point to five areas.

Transdisciplinary Collaboration: Innovation of the Future?

Mention passion, innovation, and a vision for what's next in science collaboration, and the culture of the PS-OC comes to mind. Fostering novel transdisciplinary interactions between physicists, mathematicians, biomedical engineers, molecular/ evolutionary biologists, clinicians, and advocates, the PS-OC promotes "convergence" to make physical science compatible with oncology.

Advocacy Forward

Reflecting savvy leadership, since its inception the Bay Area PS-OC has been an exemplary role model in the promotion and development of advocacy engaged programs. Researchers have been involving advocates within a complex scientific setting that investigates the mechanical properties of breast tumors and how changes in the tumor microenvironment influence cancer cell behavior.

Representing the collective voice of the patient, advocates share the drive of the Bay Area PS-OC forward focused philosophy, its trajectory of values, and its maverick identity as a mover and shaker. As 'lay knowledge brokers', advocates offer perspectives on the translational impact of research focus, peer review practices, message delivery, and the expansion of patient advocacy initiatives with government research funding entities. Drawing upon the legacy of the Bay Area SPORE, I SPY2, and TBCRP advocacy programs, the Bay Area PS-OC's advocacy partnerships focus on strategic innovation, collaborative execution, evidence-based decision-making, and ethical codes of conduct.

Breaking Down the 'Silos'

A key challenge for these collaborations has been the programmatic 'silos' that hinder interactions among different stakeholders. Our integrative model (see figure) breaks down programmatic silos by identifying common themes, thorny issues, capacity building strategies, metrics and measures, and new engagement models.

To incentivize cross sector collaboration in the areas of research and support, education and communication, policy and strategy, advocates:

- Partner with the trans-disciplinary research team to identify, develop, and implement new tools, resources, program direction, and scientific areas.
- Provide technical assistance informing stakeholders within the broader PS-OC Network about hot button issues in establishing advocacy integration within programs.
- Partake in programmatic committees/teleconferences, PS-OC lectures, monthly dinner meetings, book club meetings, and ad hoc email requests for advocate involvement in research projects.
- Present perspectives of Bay Area PS-OC program activities at local

"As vital catalysts for transdisciplinary innovation, advocates will provide researchers with new perspectives on problem solving ..."

"During my three years as an advocate member of the Bay Area PS-OC, I have been impressed by how faithfully scientists push boundaries to change the status quo in cancer research. By integrating sophisticated molecular biology with the physics of the body, multi-scale imaging technologies, and mathematical models, they are providing a new organizing framework for relating mechanics, biology, genomics, and disease in a compelling and basic way towards calculating cancer and revolutionizing therapeutic paradigms.

Perhaps the most compelling part of the work to me is how integrating the physical sciences perspec-tive to open new frontiers in oncology will offer greater precision in the diagnosis and treatment of cancer as a heterogeneous disease. I am hopeful that these multi-scale approaches will bring more tissue based prediction models of breast cancer risk."

seminars, NCI external site review, and national PS-OC and academic meetings.

• Co-author articles on spurring advocacy collaboration within PS-OC settings.

What's next?

• Leverage new technology to engage, inform, and disseminate information and research results regarding the importance of physical sciences approaches to accelerate progress towards controlling the disease and ultimately finding a cure.

• Develop tangible metrics for cross-cutting PS-OC themes in the areas of education, training and capacity building that scientists and advocates can use for both planning and evaluation.

Capacity Highlight

Team work in science is a critical component of our success. To foster bidirectional collaboration and facilitate leadership development, stakeholders from the research and advocacy communities have sought to move out of their comfort zones to identify best practices for trans-disciplinary engagement.

For example, to build the capacity of young scholars to more effectively communicate their science and develop the translational potential of their projects, Valerie Weaver, the Bay Area PS-OC co-director, encouraged Irene Acerbi, a biomedical engineer and post-doctoral researcher studying the mechanical properties of human breast tissue, to assemble a mentor committee composed of senior researchers in biology and clinical sciences, along with trained members of the research advocacy community. The advocates, Linda Vincent, Hannah Klein Connolly, and I will help Irene in grantsmanship, discussing the significance and impact of the project from the patient's perspective. During the development of the project advo-cates will receive periodic progress reports and will be invited to lab meeting presentations and workshops about the nanotechnology techniques that Irene uses to study breast cancer tissue samples in the project. Advocates will participate in the discussion of the project progress and results. As vital catalysts for transdisciplinary innovation, advocates will provide researchers with new perspectives on problem solving, offer insights to guide the review of research proposals, and discuss strategies to mobilize science into sound translational and policy solutions.

Susan Samson is an advocate for the UCSF Breast Oncology Program and the UC Berkeley PS-OC.

On the Cover

Figure 1: Death Galaxy. Image courtesy of the Robert Austin Lab, Princeton PS-OC.

Figure 2: The image shows an aggressive, metastatic breast cancer cell (MDA-MB-231) exhibiting multilobular nuclear morphology with highly irregular nuclear membrane. The nuclear surface is colored blue and the cytoplasm gray. Image courtesy of Vivek Nandakumar, Laimonas Kelbauskas, Roger Johnson and Deirdre Meldrum, Center for Biosignatures Discovery Automation (ASU PSOC).

Figure 3: Left panel shows a series of histological sections highlighting malignant melanoma progression. Right panel shows an in silico progression of melanoma, driven by senescent stroma (red) and transformed melanocytes (blue). Image courtesy of Alexander Anderson, Moffitt PS-OC.

Figure 4: Disorder strength maps of squamous epithelial cells from histologically normal lining of proximal esophagus of a healthy patient and a patient harboring esophageal cancer." Image courtesy of Benjamin Keane, a member of the Backman Lab, Northwestern PS-OC.

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