FIND THE CAUSE
Breast Cancer Foundation
Research. Education. Prevention.

Scientific Progress Report

Learn more at www.FindTheCauseBCF.org

October 2018

General considerations of progress in 2019: **Primary prevention, secondary prevention, and the immune system.** With emerging technologies, the Find the Cause Breast Cancer Foundation Consortium has been able to advance studies into a new area of breast cancer prevention that falls under the category of secondary prevention (see Figure). As opposed to primary cancer prevention, which includes identification and elimination of carcinogenic environmental chemicals, secondary prevention is the science of identifying people who have been exposed to environmental chemicals and who show molecular signs that predict development of cancer well before the cancer itself forms. Accurately predicting who may be at high risk for developing cancer may enable us to intervene and prevent cancers from occurring. Consortium work, particularly that conducted in Dr. Sherr's laboratory and with Dr. Monti's help, is now migrating to studies on how malignant cells suppress the immune system, the one biological system flexible and adaptable enough to kill rapidly changing cancer cells. The expectation is that these studies will lead to approaches to reverse the effects of environmental chemicals on both the cells that have become cancerous and on the immune system.

[1]Primary Prevention [2]Secondary Prevention [3]Immunity, [4]Basic Biology

Primary prevention: Identify what environmental chemicals are carcinogens and eliminate them**; Secondary prevention:** Identify who has been exposed, predict impending cancers, intervene**; Immunity:** Reverse immunosuppression that prevents elimination of developing cancers**; Basic biology:** Understand how cancers happen and extrapolate how environmental chemicals contribute.

Monti Lab. In efforts to advance primary cancer prevention approaches, Dr. Monti's laboratory has applied an emerging technology (Sparse Full Length DNA sequencing) to determine which of the 20,000 human genes are activated by environmental chemicals and doing so at a price that makes technologies for screening thousands of chemicals more practical and less dependent on outside suppliers of robotic platforms (1). In collaboration with Dr. Sherr's laboratory, Dr. Monti's laboratory published an important manuscript in a journal published by the National Institute of Environmental Health Sciences, the highest ranking journal dedicated to environmental causes of cancer and other diseases (2). The data in the article demonstrated a technology through which hundreds of chemicals can be screened for their potential to induce cancer. The work involved computational analysis of literally millions of bits of data generated with a robotic version of a laboratory technician. Comparison of the results with those produced over decades with approximately 150 known carcinogens indicated that Dr. Monti's new approach correctly predicted carcinogens with about an 85% accuracy. As opposed to the standard assay for carcinogenicity, which costs about $2,000,000 per chemical tested and takes two years, this new technology costs about $15 per chemical and takes only a couple of weeks. Associated with the work and the project, the Monti team developed an on-line database portal and search tool (https://carcinogenome.org/) with which environmental scientists can query all of the data generated in the study and analyze the data for hints of carcinogenicity in their respective systems. As of August, 2019, this manuscript had been downloaded 3,085 times, referenced in 62 papers, reached the top 14% of publications in similar area of expertise (environmental carcinogens) and the top 3% of all publications of similar age, been picked up by 7 news outlets, and tweeted by users in the US, Canada, France, UK, Germany, Australia, Brunei, and Sri Lanka.

Furthermore, expanding on the types of computational analyses used in this new platform, Dr. Monti has been able to assist Dr. Sherr's work in identifying genetic changes that appear to occur prior to overt cancer formation and in determining how emerging cancer cells suppress the immune system (see below).

Leveraging the data generated in these studies, Dr. Monti was able to secure $123,750 in funding from the Superfund Research Program within the National Institute of Environmental Health Sciences (NIESH) to generate a similar database for chemical toxicity in addition to carcinogenicity. Drs. Monti and Sherr have submitted a grant application to the Department of Defense to evaluate the carcinogenicity of environmental chemicals to which military personnel are routinely exposed. The context of these studies is the environmental causes of brain cancer.

Sherr Lab. Towards the end of 2018, Dr. Sherr's team, including a Ph.D. student (Supraja Narasimhan) supported by a Find the Cause Seed the Scientist grant, published a manuscript in an international journal that demonstrated how a class of common environmental pollutants induces cancer and drives cancer progression to a lethal form (3). In early 2019 the Sherr lab published an article demonstrating, for the first time, that the most aggressive form of breast cancer, i.e., "inflammatory breast cancer", is driven by a receptor that recognizes a variety of environmental chemicals (4). The paper, published with collaborators from Cairo, Egypt, where these types of cancers are much more common, showed how environmental chemicals may contribute to a highly aggressive cancer for which the average survival period is about 2 years. Using information generated in these two studies, Dr. Sherr's laboratory, in collaboration with colleagues at Harvard Medical School and Brigham and Women's hospital, published a manuscript in an extremely high profile journal, *Nature Neuroscience*, demonstrating that, in addition to driving cancer formation and aggression, environmental chemicals, through their cellular receptor,

suppress the immune system responsible for killing cancers (5). This study extends previous Find the Cause-supported studies in breast and oral cancers to demonstrate that environmental chemicals have similar effects in brain cancers. Indeed, the laboratory has now begun to investigate the dual role of environmental chemicals in driving cancer aggression and suppressing the immune system to lung cancers. A common element in these studies appears to be environmental chemical suppression of the immune system. Although not yet published, the lab has shown that they can identify early molecular markers of environmental chemical exposure that may be used to predict who will get cancer (secondary prevention) enabling intervention before cancers form.

The work described above, particularly the studies that evaluated how environmental chemicals suppress cancer immunity, led to acquisition, in 2019, of $776,000 in support through two NIH grants and one from the Johnson and Johnson Foundation. One of the two NIH grants will continue through 2020 (~$225,000/year) and the other NIH grant will continue through 2024 (~$190,000/year). A renewal for the Johnson and Johnson grant will be submitted in December of 2019.

Sonenshein Lab. Dr. Sonenshein' s laboratory has begun to develop cutting-edge technologies to detect the presence of cancer using a blood sample. Although currently directed towards early detection of breast cancer, Dr. Sonenshein believes that the technology might be used to determine who has been exposed to environmental chemicals and the likelihood that they will develop cancer (secondary prevention). She will be working with Dr. Sherr to test this theory using a mouse model of environmental chemical-induced cancer that Dr. Sherr's laboratory has adopted. Dr. Sonenshein's laboratory has been awarded $165,000 from the Ellison Foundation to evaluate a potential intervention in people predicted to develop cancer or showing early signs of cancer.

Kuperwasser Lab. In 2019, Dr. Kuperwasser's lab published three manuscripts that address the root causes of breast cancer (6-8). Specifically, the data demonstrate the role of specific genes in blocking the inhibitors of cancer (referred to as "tumor suppressors"). Dr. Kuperwasser had previously demonstrated deletion of one of them, called "Slug", rendered mice nearly completely resistant to breast cancer formation. One of the manuscripts (8) was an invited review in a high profile publication (*Cell Stem Cell)* cancer-causing genes and their role in generating cancer "stem cells" and cancer stem cell resistance to therapy.

Publications cited above:

1. Reed, E., E. Moses, X. Xiao, G. Liu, J. Campbell, C. Perdomo, and S. Monti. 2019. Assessment of a Highly Multiplexed RNA Sequencing Platform and Comparison to Existing High-Throughput Gene Expression Profiling Techniques. *Front Genet* 10: 150.
2. Li, A., X. Lu, T. Natoli, J. Bittker, N. S. Sipes, A. Subramanian, S. Auerbach, D. H. Sherr, and S. Monti. 2019. The Carcinogenome Project: In Vitro Gene Expression Profiling of Chemical Perturbations to Predict Long-Term Carcinogenicity. *Environ Health Perspect* 127: 47002.
3. Narasimhan, S., E. Stanford Zulick, O. Novikov, A. J. Parks, J. J. Schlezinger, Z. Wang, F. Laroche, H. Feng, F. Mulas, S. Monti, and D. H. Sherr. 2018. Towards Resolving the Pro- and Anti-Tumor Effects of the Aryl Hydrocarbon Receptor. *Int J Mol Sci* 19.
4. Mohamed, H. T., R. Gadalla, N. El-Husseiny, H. Hassan, Z. Wang, S. A. Ibrahim, M. El-Shinawi, D. H. Sherr, and M. M. Mohamed. 2019. Inflammatory breast cancer: Activation of the aryl hydrocarbon receptor and its target CYP1B1 correlates closely with Wnt5a/b-beta-catenin signalling, the stem cell phenotype and disease progression. *J Adv Res* 16: 75-86.

5. Takenaka, M. C., G. Gabriely, V. Rothhammer, I. D. Mascanfroni, C. C. Chao, K. Alves de Lima, J. E. Kenison, E. C. Tjon, T. Vandeventer, S. Rothweiler, S. Ghannam, L. Healy, D. H. Sherr, A. Pratt, J. Antel, D. A. Reardon, S. C. Robson, G. Getz, H. L. Weiner, and F. J. Quintana. 2019. Control of tumor associated macrophages by the tumor microenvironment in glioblastoma. *Nature Neuroscience*.
6. Elman, J. S., T. K. Ni, K. E. Mengwasser, D. Jin, A. Wronski, S. J. Elledge, and C. Kuperwasser. 2019. Identification of FUBP1 as a Long Tail Cancer Driver and Widespread Regulator of Tumor Suppressor and Oncogene Alternative Splicing. *Cell Rep* 28: 3435-3449 e3435.
7. Gross, K. M., W. Zhou, J. L. Breindel, J. Ouyang, D. X. Jin, E. S. Sokol, P. B. Gupta, K. Huber, L. Zou, and C. Kuperwasser. 2019. Loss of Slug Compromises DNA Damage Repair and Accelerates Stem Cell Aging in Mammary Epithelium. *Cell Rep* 28: 394-407 e396.
8. Gupta, P. B., I. Pastushenko, A. Skibinski, C. Blanpain, and C. Kuperwasser. 2019. Phenotypic Plasticity: Driver of Cancer Initiation, Progression, and Therapy Resistance. *Cell Stem Cell* 24: 65-78.

Seminars/Lectures (August 2018-August 2019)

Project Leader	Title, Status, or Seminar Series	Location
D. Sherr	The AHR as a driver of cancer and cancer immunity (immunosuppression)	University of Paris, Descartes, Paris, FR
D. Sherr	**Keynote address**: The role of the aryl hydrocarbon receptor in cancer immuno-metabolism.	World Pharma Week, Small Molecules for Immuno-oncology, Boston, MA
D. Sherr	**Grand Rounds:** The Aryl Hydrocarbon Receptor: A Cancer Instigator and Immune Checkpoint Regulator	Dartmouth Hitchcock Medical Center
D. Sherr	The Cancer Interception Program, Boston, MA.	The Boston University Cancer Center Annual Symposium
D. Sherr	Intercepting the AHR in oral cancer	Dana-Farber Cancer Institute
D. Sherr	Plenary lecture: The AHR: A Major Player in Cancer Aggression and Immune Checkpoint Regulation	Dioxin 2019 Conference, Kyoto, Japan
D. Sherr	The AHR as a Novel Immune Checkpoint Regulator Influenced by Environmental AHR Ligands	Evans Research Foundation Area of Research Concentration
D. Sherr	**Plenary Lecture:** The AHR as a Driver of Cancer and Immunosuppression	American Association of Immunologists, San Diego
D. Sherr	Breast Cancer: A Panel on Causes and Prevention Possibilities	Boston University
C. Kuperwasser	UPENN Distinguished Lecture Series	U. Penn

C. Kuperwasser	Big Data in Environmental Science.	Boston University
C. Kuperwasser	Invited Speaker	NET Research Foundation, Boston
C. Kuperwasser	**Keynote Speaker**	Dana-Farber/Harvard Cancer Center Breast and Gynecologic
S. Monti	New Technologies and Their Impact on Environmental Health	Boston University; Environmental Health Seminar Series
S. Monti	**Keynote speaker**	BD2K-LINCS Data Science Symposium (DSS), Cincinnati, OH
S. Monti	Invited Speaker	3rd Annual Next Generation Sequencing Congress. Boston, MA, USA.
S. Monti	**Session Chair**	3rd Annual Next Generation Sequencing Congress. Boston,
S. Monti	Integrative Cancer Multi-Omics to advance prevention and therapy.	Novartis Institutes for BioMedical Research (NIBR), Cambridge, MA.
S. Monti	Big Data in Environmental Science	2018 Northeast Superfund Research Program Meeting, Woods Hole, MA.
S. Monti	Breast Cancer: A Panel on Causes and Prevention Possibilities	Boston University
S. Monti	Life Science Seminar Series,	ShanghaiTech University, Shanghai, China.

October 2018

Dr. Stefano Monti's Lab:

With over 85,000 chemicals registered by the EPA, and thousands more coming on line every year, it is virtually impossible to screen all of them for their carcinogenicity (ability to induce cancer) using standard techniques.

Therefore, Dr. Monti's laboratory is working towards building a higher tech approach, one that will enable us to predict whether or not a chemical is carcinogenic at a price and speed that has never been seen before. Together with collaborators at the Broad Institute, the National Toxicology Program, and Dr. Sherr's laboratory, Dr. Monti has published work demonstrating the feasibility of this new platform and shown that, even in these early stages, it accurately predicts 75%-85% of the carcinogens tested. In the near future, and with funding through Find the Cause, Dr.

Monti's laboratory will work towards greater than 95% accuracy and towards gearing the prediction platform up to evaluate hundreds if not thousands of chemicals.

Dr. David Sherr's Lab:

Dr. Sherr's laboratory has been dissecting the mechanisms through which environmental chemicals cause cancer. In so doing he and his laboratory have discovered that the very mechanism that chemicals use to cause cancer can be exploited to not only block tumor cells from forming but also to activate the immune system to kill a nascent cancer. Although his studies have historically focused on triple negative breast cancers, his most recent studies demonstrate that the immune system can be roused to fight oral and brain cancers as well. In the next year he will be collaborating with computational biologists at Boston University to determine if signs of cancer can be detected prior to overt cancer formation and if the immune system can be recruited at these early stages, that is, well before cancer happens at all.

Dr. Gail Sonenshein's Lab:

In keeping with the theme of cancer prevention, Dr. Sonenshein's laboratory has been testing methods for detecting cancers well before current technologies can, including expensive imaging technologies such as MRI or even mammography, using what would be a simple blood test and very high tech molecular biology.

Furthermore, these kinds of measurements may one day be able to identify what carcinogen contributed to the formation of these early cancers, a kind of "holy grail" for cancer epidemiologists.

Dr. Charlotte Kuperwasser's Lab:

Dr. Kuperwasser has similarly been evaluating how normal cells transform into malignant cancers. Among other results, she has identified the remains of ancient virus DNA integrated into the DNA of human cells that influence how normal cells behave. Her results suggest that these snippets of DNA may help identify cells that are becoming malignant and may be exploited to enhance the immune response to newly forming cancers.

June 2018

Preventing cancer metastasis:

In May, Dr. Sherr's laboratory published an important paper that demonstrated exactly how environmental chemicals accelerate breast cancer. Using a series of cutting-edge technologies, they demonstrated that aggressive human triple-negative breast cancers and even more aggressive human inflammatory breast cancer cells migrate much more quickly in the presence of dioxin, a prototypic, carcinogenic environmental pollutant. Because these experiments revealed the molecular targets of dioxin and related environmental chemicals, the Sherr team was able to design drugs that could block their effects and slow cancer migration. Furthermore, in an intriguing model that closely replicates metastasis in humans, they demonstrated that these drugs were able to completely block metastasis of human triple-negative breast cancer, cervical cancer, and oral cancer cells in zebrafish, demonstrating that these findings impact other cancers. These results help explain how environmental chemicals exacerbate several different kinds of cancers and provide an opportunity for real prevention by blocking the pro-migratory effects of environmental chemicals.

Immuno-enhancing drugs:

The Sherr and Kuperwasser laboratories have opened a second front of investigation: Analysis of how these same environmental chemicals suppress the immune system. In so doing they were able to generate a nonmalignant version of human cancer that induce a completely protective immune response. This "vaccine" approach is akin to vaccinating people with a virus to protect against later infections.

Finally, the Sherr laboratory has generated robust data suggesting that the novel drugs that block environmental chemical activity also enhance the immune system in a fashion similar to the immunotherapeutics that have recently generated so much excitement in the oncology world. With funding from Find The Cause, the consortium hopes to test the hypothesis that these modified tumor cells, and the immuno-enhancing anti-pollutant drugs, will completely protect an individual from cancer relapse.

Liquid Biopsy:

In a significant step towards determining if someone has been exposed to environmental chemicals, the Sonenshein laboratory has developed a molecular technology that detects extremely small molecules in the blood that appear when an individual is exposed to any given environmental carcinogen. The ultimate goal of these studies is to make the so-called "liquid biopsy" a standard technique for evaluating carcinogen exposure and thereby to assessing the risk to any given

individual engendered by chemical exposure.

Proving chemicals innocent or guilty in the court of computational biology:
The Monti laboratory has made major strides in developing a platform for screening thousands of chemicals for potential carcinogenicity. By combining robotics that handle hundreds of chemicals at a time with machine learning or artificial intelligence Dr. Monti has shown that one can predict whether an unknown chemical is a carcinogen with approximately 83% accuracy. With additional funding, this first phase of experimentation can be expanded and, according to the algorithms, can make this platform better than 95% accurate. That would be more than enough to identify likely carcinogens and to evaluate them in greater detail in more labor-intensive studies to truly implicate the dangerous chemicals and exonerate those that aren't.

December 2017

Drugs and the immune system:

Cancer vaccines have been a long sought-after goal for cancer prevention. Our consortium believes that the drugs currently being researched that inhibit the effects of environmental carcinogens may also boost cancer- specific immune responses to fight cancers. The drugs that Dr. David Sherr's laboratory (Boston University School of Public Health) have developed and the mechanisms that Dr. Charlotte Kuperwasser's laboratory (Tufts School of Medicine) have been researching are designed to enhance the immune system. Any enhancement of the immune system can contribute to cancer prevention. That would be all cancers. This approach, if successful, will truly be a game changer.

Drugs for high risk populations:

Dr. Sherr's lab is developing drugs that could block the effects of many environmental chemicals and might be used for people who are at high risk of getting breast cancer. For example, people with high occupational exposures to carcinogens (e.g., Armed Forces personnel) and particularly susceptible populations (e.g., children).

Drugs for preventing cancer recurrence:

As an offshoot of the consortium's environmental chemical studies, Dr. Sherr and Dr. Gail Sonenshein's (Tufts School of Medicine) labs are developing drugs that could be used to prevent cancer recurrence in people that have been treated with standard chemotherapies and/or radiation therapy. This is particularly true since it is cancer stem cells that survive the initial treatment. It is those cancer stem cells that these drugs target.

Identifying toxic chemicals in our environment:

Dr. Stefano Monti's (Boston University School of Public Health) high-throughput computational biology predictive model can quickly identify which chemicals are likely to be carcinogens. With this information, we could be able to eliminate these chemicals from our environment AND prevent them from being replaced by new chemicals that may be just as carcinogenic.

September 2016 - August 2017

Dr. Sherr's lab has advanced the plausibility argument by demonstrating that common environmental chemicals accelerate conversion of relatively benign breast and oral cancer cells to highly aggressive, metastatic cancers through mechanisms that do not involve genetic mutations. As a spinoff, the Sherr lab demonstrated that a novel new drug is capable of blocking the effects of environmental chemicals and of reversing the aggression of tumors even after the environmental chemical exposure has ceased. This drug appears to have efficacy in triple negative breast cancer, oral cancer, melanoma, and brain cancer. Indeed, the most recent studies suggest that this drug works both by blocking signaling of the aryl hydrocarbon receptor (AHR), a cellular receptor that recognizes environmental chemicals and drives cancer cell metastasis and enhances the immune response to the cancer. Interestingly, in a collaboration with a neurobiologist at Boston University, Dr. Sherr's laboratory demonstrated that the same signaling pathway may be responsible for environmental chemical induced ALS (amyotrophic lateral sclerosis). This work was published in 3 manuscripts during 2017. Dr. Sherr will be presenting some of his work on cancer at the National Cancer Institute later in August, at the University of Memphis in November, and at an international (AHR) cancer conference in Paris early in 2018.

Dr. Monti's computational biology laboratory has beta tested a robotic platform to rapidly predict what new chemicals may be carcinogenic. Using human breast cells his team was able to accurately predict 3 out of 4 carcinogens. Projections indicate that another round of approximately 100 known chemicals will raise the accuracy of prediction well over 80%. More chemicals = more accuracy. Amy Li, the Find The Cause Seed-The-Scientist awardee from 2016, is working in Dr. Monti's laboratory and has generated a web portal through which other scientists can access and mine the literally million bits of information collected in the robotic platform for hints of cancer-causing signaling pathways. This work has been presented at 2 NIH-sponsored conferences and a manuscript describing the work is being prepared. The portal can be accessed at *https://carcinogenome.org*

Dr. Sonenshein's lab has made significant progress in mapping the downstream molecular effects of environmental chemicals. Specifically, she has demonstrated that a cascade of events is triggered by changes in cells resulting from environmental insults. Surprisingly, the most profound outcome observed was an increase in a molecule, "Adam 8", which enables cancer cells to migrate through the body. Proof of principle was demonstrated by the nearly complete inhibition of breast cancer migration with antibodies, generated by Dr. Sonenshein's laboratory, that bind to Adam 8 on the surface of breast cancer cells.

Dr. Kuperwasser's laboratory, has also made progress in demonstrating that environmental chemicals, many of them the same as those implicated in Dr. Sherr's work, bias development of normal breast cells to the types of cells that mature into the most aggressive form of breast cancer. Furthermore, her laboratory now has evidence that other types of environmental chemicals, known generally as endocrine disruptors, generate the same adverse outcome, i.e., skewing of the normal cell development pathway towards cells capable of becoming aggressive cancers