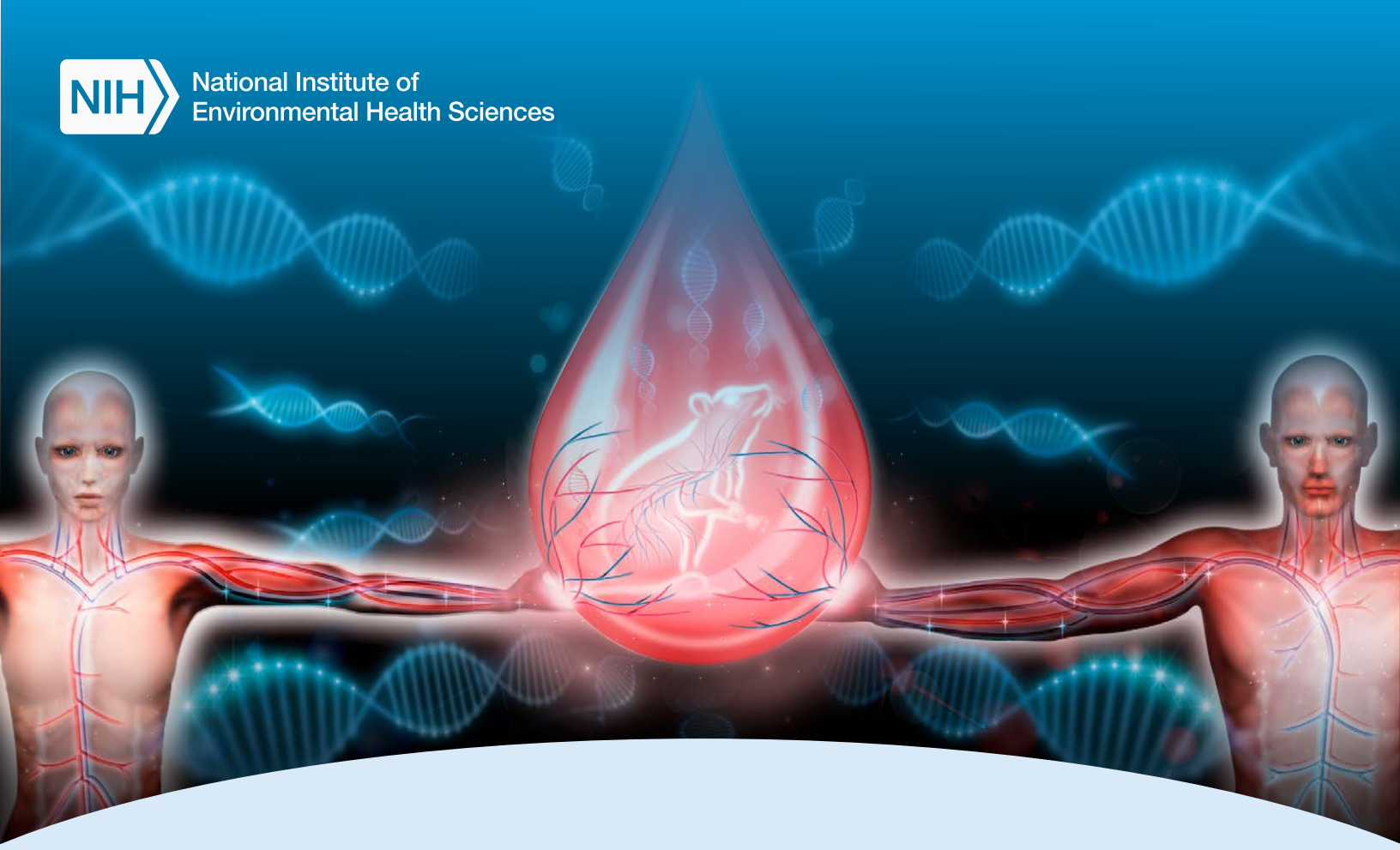
National Institute of Environmental Health Sciences



NIEHS Inflammation Faculty Workshop

Circulating Cell-Free DNA: Applications in the Clinical and Toxicology Setting

September 24-25, 2018

NIEHS Building 101, Rodbell Auditorium

Research Triangle Park, N.C.

National Institutes of Health • U.S. Department of Health and Human Services

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Background

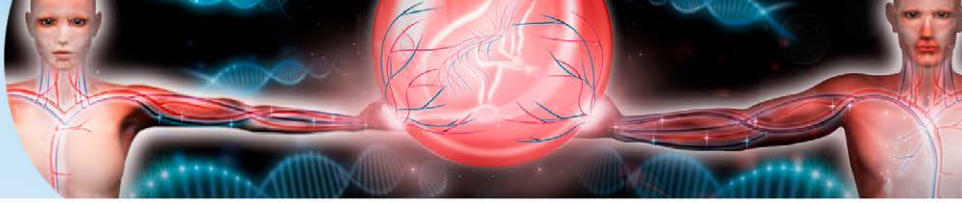
Sequence analysis of extracellular DNA circulating in blood is an exciting development in clinical and experimental medicine liquid biopsy applications. Circulating, cell-free DNA (ccfDNA), with its short half-life, is normally found in blood at low concentrations (ng/ml) when DNA of nuclear or mitochondrial origin is released after cellular breakdown.

First described in the 1940's, ccfDNA has recently been coupled with targeted or genome-wide sequencing to emerge as a novel, non-invasive tool in disease diagnosis, staging, and biomarker discovery. Liquid biopsies have gained much attention in a variety of clinical conditions, such as cancer, maternal/fetal disorders, cardiovascular disease, autoimmune disorders, and sepsis. New areas of utilization include molecular epidemiology, predictive toxicology, and precision medicine. This NIEHS workshop explores applications and utilities of ccfDNA in research and lessons learned from the clinical setting while introducing its development as a potential means to investigate and predict similar disease outcomes in exposure biology and toxicology.

Workshop Framework

The workshop, sponsored by the NIEHS Inflammation Faculty, is organized into three major sessions over 1 ½ days and connects 1) technical isolation and features of ccfDNA, 2) clinical applications using ccfDNA, and 3) experimental and environmental applications. Background with regard to ccfDNA isolation technologies and sequence characterizations are presented on day one with speakers discussing their research project and experiences in ccfDNA research in the fields of cancer, maternal/fetal, and autoimmune disorders. The final half day is dedicated to a poster session with selected poster presentations, and concludes with a panel discussion into future research directions for the ccfDNA and liquid biopsy field that have been formulated from proceedings of the workshop. The NIEHS ccfDNA workshop committee invites all attendees to submit a poster related to their work with ccfDNA. Attendees selected for a platform presentation will receive a travel award to attend the meeting.

This workshop will bring together experts investigating ccfDNA in the clinical and toxicology disciplines sharing lessons learned and providing direction for this liquid biopsy tool to be utilized in environmental health science.



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AGENDA

Day One – Monday, September 24

8:00 – 8:30 a.m.	**Registration**
8:30 – 8:40 a.m.	**Welcome and Introductions** *Mike Humble, Ph.D., NIEHS*
8:40 – 8:50 a.m.	**Opening Remarks and Workshop Background** *Linda Birnbaum, Ph.D., D.A.B.T, A.T.S., Director, NIEHS and NTP*
8:50 – 9:20 a.m.	**Biomarker Development and Application to Toxicology** *Andrew Nixon, Ph.D., Duke University Medical Center*

Session One – Technology: Collect, Quantify, Analyze

9:20 – 9:25 a.m.	**Introduction** *Kevin Gerrish, Ph.D., NIEHS*
9:25 – 9:55 a.m.	**CcfDNA: Characterization, Isolation, and Applications** *Douglas White, Promega*
9:55 – 10:15 a.m.	**CcfDNA: The NIEHS Experience** *Brian Elgart, NIEHS*
10:15 – 10:30 a.m.	**Break**

Session Two – ccfDNA Clinical Applications

10:30 – 10:35 a.m.	**Introduction** *Frederick Miller, M.D., Ph.D., NIEHS*
10:35 – 11:05 a.m.	**Utility of ccfDNA for Detecting Prostate Cancer Development and Progression: Advantages and Limitations** *Adam Sowalsky, Ph.D., National Cancer Institute, Center for Cancer Research*
11:05 – 11:35 a.m.	**Cell-Free Screening: Complexities and Challenges of Clinical Implementation** *Neeta Vora, M.D., University of North Carolina School of Medicine*

11:35 a.m. – 12:05 p.m.	**Cell-Free Mitochondrial DNA is Elevated in Sickle Cell Disease Patients, and Serve as a Potential Proinflammatory DAMP** *Swee Lay Thein, D.Sc., National Heart, Lung, and Blood Institute*
12:05 – 12:10 p.m.	**Session Summary** *Frederick Miller, M.D., Ph.D., NIEHS*
12:10 – 1:10 p.m.	**Lunch**

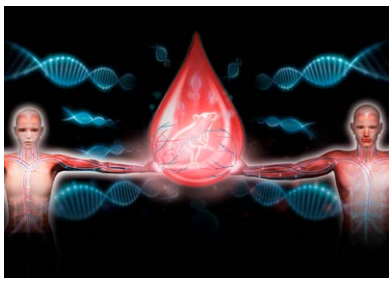
Session Three – ccfDNA Toxicology Applications

1:10 – 1:15 p.m.	**Introduction** *Julie Foley, B.S., NIEHS*
1:15 – 1:45 p.m.	**Circulating Cell-Free RNA as Biomarkers of Exposure to Toxic Substances** *Rebecca Fry, Ph.D., University of North Carolina Gillings School of Global Public Health*
1:45 – 2:15 p.m.	**Epigenetics of Cell-Free DNA in Health and Diseases** *Peng Jin, Ph.D., Emory University School of Medicine*
2:15 – 2:45 p.m.	**Detection of Stress-Induced mitoDAMPs** *Jennifer Martinez, Ph.D., NIEHS*
2:45 – 3:05 p.m.	**Break**
3:05 – 3:35 p.m.	**The Role of Cell-Free DNA in Systemic Lupus Erythematosus** *David Pisetsky, M.D., Ph.D., Duke University Medical Center*
3:35 – 4:35 p.m.	**Panel Discussion of Overarching Questions** *Moderators: Andy Nixon, Ph.D., Duke University Medical Center; Kevin Gerrish, Ph.D., NIEHS; Mike Fessler, M.D., NIEHS*
4:35 – 4:40 p.m.	**Wrapup** *Frederick Miller, M.D., Ph.D., NIEHS*
4:40 p.m.	**End of Day One**

Day Two – Tuesday, September 25

Session Four – Poster Session/Discussion Panel

8:30 – 8:40 a.m.	**Welcome and Reflections from Day One** *Alex Merrick, Ph.D., NIEHS*
8:40 – 9:00 a.m.	**Exploring Combinations of Pan-Cancer Epigenetic Markers for Cancer Screening** *Karen Funderburk, National Human Genome Research Institute*
9:00 – 9:20 a.m.	**ZNF154 Promoter Methylation Density Enhances Detection of Ovarian Cancer** *Brendan Miller, National Human Genome Research Institute*
9:20 – 9:50 a.m.	**Sequencing Plasma DNA to Classify and Monitor Cancer** *Margaret Gulley, M.D., University of North Carolina Medical Center*
9:50 – 10:50 a.m.	**Poster Session**
10:50 – 11:50 a.m.	**Panel Discussion: Technology, Clinical, and Toxicology Applications** *Moderators: Kevin Gerrish, Ph.D., NIEHS; Andy Nixon, Ph.D., Duke University Medical Center; Alex Merrick, Ph.D., NIEHS*
11:50 – 11:55 a.m.	**Summary of Findings and Next Steps** *Julie Foley, NIEHS*
11:55 a.m. – noon	**Closing Remarks** *Mike Humble, Ph.D., NIEHS*
Noon	**End of Workshop**



Biographies

Linda Birnbaum, Ph.D., D.A.B.T., A.T.S.
National Institute of Environmental Health Sciences
www.niehs.nih.gov/about/od/director/index.cfm

Linda S. Birnbaum, Ph.D., D.A.B.T., A.T.S., became the Director of the National Institute of Environmental Health Sciences (NIEHS), one of the National Institutes of Health (NIH), and the National Toxicology Program (NTP) on January 18, 2009. In these roles Birnbaum oversees federal funding for biomedical research to discover how the environment influences human health and disease. Several advisory boards and councils provide Birnbaum and NIEHS/ NTP staff with input to accomplish this large task.

Birnbaum is the first toxicologist and the first woman to lead the NIEHS/NTP. She has spent most of her career as a federal scientist.

Birnbaum has received numerous awards and recognitions, including being elected to the Institute of Medicine of the National Academies, in October 2010, one of the highest honors in the fields of medicine and health.

Birnbaum's own research and many of her publications focus on the pharmacokinetic behavior of environmental chemicals; mechanisms of actions of toxicants, including endocrine disruption; and linking of real-world exposures to health effects.

Birnbaum also finds time to mentor the next generation of environmental health scientists. For example, she serves as an adjunct professor in the Gillings School of Global Public Health, the Curriculum in Toxicology, and the Department of Environmental Sciences and Engineering at the University of North Carolina at Chapel Hill, as well as in the Integrated Toxicology Program at Duke University.

A native of New Jersey, Dr. Birnbaum received her M.S. and Ph.D. in microbiology from the University of Illinois at Urbana-Champaign.

Brian Elgart
National Institute of Environmental Health Sciences

Brian Elgart is a postbaccalaureate trainee at the National Institute of Environmental Health Sciences in the Molecular Genomics Core Laboratory. He graduated summa cum laude from East Carolina University in May 2017 with a Bachelor of Science in biology. In the Molecular Genomics Core, he works as part of a collaborative, cross-divisional project developing methodologies to study circulating cell-free DNA in clinical and toxicology applications. Elgart recently received an outstanding poster award at the National Institute of Health Postbac Poster Day for his work. He is currently applying to medical school and plans to matriculate in the fall of 2019.

Mike Fessler, M.D.
National Institute of Environmental Health Sciences
www.irp.nih.gov/pi/michael-fessler

Mike Fessler is acting chief of the Immunity, Inflammation, and Disease Laboratory. Fessler received his A.B. in philosophy from Princeton University in 1992, and his M.D. from Harvard Medical School in 1996. He subsequently completed an internal medicine residency at Massachusetts General Hospital, and a pulmonary-critical care medicine fellowship at the University of Colorado Health Sciences Center. During his research fellowship, Fessler trained in the innate immunity laboratory of G. Scott Worthen at National Jewish Health, and then joined the National Jewish faculty from 2002-2006. In 2006, Fessler started as a tenure track investigator in the Intramural Research Program of the NIEHS, in which role he heads the Clinical Investigation in Host Defense Group. Fessler has received several awards, including the NIEHS Early Career Award, NIEHS Intramural Research Award, and American Thoracic Society Carol Basbaum Award. He also serves on the editorial board of PLoS One and as a faculty member of F1000.

Julie Foley
National Institute of Environmental Health Sciences
www.niehs.nih.gov/research/atniehs/labs/bmsb/moltox/staff/foley/index.cfm

Julie Foley is a health scientist in the Molecular Toxicology and Genomics Group within the Biomolecular Screening Branch of the National Toxicology Program (NTP). In her current role, Foley is involved in working with the NTP Biorepository and Cellular and Molecular Pathology Branch to implement a sample tracking, management, and freezer inventory software program. This will enable the NTP to be better able to deposit and manage NTP samples for future molecular analysis. She is involved with the development of new technologies to assist with toxicity screening and carcinogen classification.

Prior to joining the Molecular Toxicology and Genomics Group, Foley was a member of the Cellular and Molecular Pathology Branch within the NTP where she was group leader of the Special Techniques group which provided pathology support core services in the areas of mouse embryo phenotyping, laser microdissection, image analysis, and special miscellaneous techniques involving tissue collection and handling.

Rebecca Fry, Ph.D.
University of North Carolina Gillings School of Global Public Health
www.sph.unc.edu/adv_profile/rebecca-fry-phd

Rebecca Fry, Ph.D., is director of the Institute for Environmental Health Solutions. Her lab focuses on understanding how environmental exposures are associated with human disease, with a particular focus on genomic and epigenomic perturbations. Using environmental toxicogenomics and systems biology approaches, the researchers aim to identify key molecular pathways that associate environmental exposure with diseases. A current focus in the lab is to study prenatal exposure to various types of metals including arsenic, cadmium, and lead.

Fry also aims to understand molecular mechanisms by which such early exposures are associated with long-term health effects in humans. For example, she examines DNA methylation profiles in humans exposed to metals during the prenatal period. This research will enable the identification of gene and epigenetic biomarkers of metal exposure. The identified genes can serve as targets for study to unravel potential molecular bases for metal-induced disease.

Ultimately, her lab aims to identify mechanisms of metal-induced disease and the basis for inter-individual disease susceptibility.

Kevin Gerrish, Ph.D.
National Institute of Environmental Health Sciences
www.niehs.nih.gov/research/atniehs/facilities/molgencore/index.cfm

Kevin Gerrish is the Acting Director of the Molecular Genomics Core Laboratory. The Molecular Genomics Core Laboratory provides intramural researchers with access to cutting edge genotyping, microarray, Nanostring, nucleic acid isolation, and sequencing services. The core strives to support this work with technical and bioinformatics components for every project.

Margaret Gulley, M.D.
University of North Carolina School of Medicine
www.med.unc.edu/pathology/faculty-2/biosketch-of-dr-margaret-gulley

Margaret Gulley, M.D., is professor and director of molecular pathology in the Department of Pathology and Laboratory Medicine at the University of North Carolina at Chapel Hill. She provides clinical laboratory services to patients with cancer, heritable disease, infectious disease, transplant, or immunologic disorders. Research services involve development and validation of genomic assays supporting translational research in oncology, virology, and other chronic infections. Emphasis is on generating an evidence base to justify implementing novel gene-based assays that add value for disease diagnosis, classification, and monitoring.

Mike Humble, Ph.D.
National Institute of Environmental Health Sciences
www.niehs.nih.gov/research/supported/dert/geh/humble/index.cfm

Michael Humble, Ph.D., received his doctorate in toxicology from the University of North Carolina at Chapel Hill, performing his dissertation research in the intramural laboratories at NIEHS on a transgenic mouse model for skin cancer. Following a short postdoctoral training period, Humble began working in the Division of Extramural Research and Training at NIEHS, first as a program analyst, and currently as a program administrator. Humble oversees the extramural fellowship program, the Short Term Educational Experiences for Research (STEER) in the Environmental Health Sciences program, the R15 AREA grants program, as well as the research portfolios in skin disease, immunotoxicology, and autoimmune disease. Humble was a high school chemistry teacher prior to returning to graduate school and receiving his Ph.D.

Pen Jin, Ph.D.
Emory University School of Medicine
www.genetics.emory.edu/faculty/primary/jin-peng.html

Peng Jin received his doctorate degree in molecular and developmental biology from Cincinnati Children's Hospital/University of Cincinnati, and postdoctoral training at Emory University. At Emory, Jin is interested in the roles of non-coding RNAs and epigenetic modulation in neural development and brain disorders. Jin is the recipient of the Beckman Young Investigator Award, Basil O'Connor Scholar Research Award, Alfred P. Sloan Research Fellow in Neuroscience, and National Alliance for the Research of Schizophrenia and Depression Independent Investigator Award.

Jennifer Martinez, Ph.D.
National Institute of Environmental Health Sciences
www.niehs.nih.gov/research/atniehs/labs/iidl/pi/inflammation/index.cfm

Jennifer Martinez, Ph.D., heads the Inflammation and Autoimmunity Group, and holds a secondary appointment in the NIEHS Signal Transduction Laboratory. The Inflammation and Autoimmunity Group investigates the mechanisms by which cargo from the extracellular environment, including pathogens, allergens, and dying cells, is processed by immune cells, and how these events influence their pursuant immune response.

Alex Merrick, Ph.D.
National Institute of Environmental Health Sciences
www.niehs.nih.gov/research/atniehs/labs/bmsb/moltox/index.cfm

Alex Merrick, Ph.D., is a molecular toxicologist who joined the NTP Biomolecular Screening Branch in 2010. Merrick leads the Branch's Molecular Toxicology and Genomics Group. His responsibilities include identifying key signaling pathways and transcripts altered by environmental toxicants, and participating in the Tox21 collaboration between the National Toxicology Program (NTP), the National Institutes of Health Chemical Genomics Center (NCGC), the Environmental Protection Agency's National Center for Computational Toxicology (NCCT), and the Food and Drug Administration. He is especially interested in performing molecular analysis in NTP archival tissues to discover gene expression and epigenetic signatures from chemical toxicology studies. Collaborative work with the Cellular and Molecular Pathology Branch aims to use NTP archival research to further pathological insight, better understand mechanisms of chemical toxicity, and contribute to predictive models of toxicity and chemical prioritization as a complementary effort to the Tox21 high-throughput screening program. In addition, NextGen sequencing projects will help NTP better evaluate chemically exposed tissues for differential transcript profiles that include splice variants, low copy transcripts, and non-coding mRNA's.

Brendan Miller
National Human Genome Research Institute

Brendan Miller is a Ph.D. student under the guidance of Laura Elnitski, Ph.D., in the National Human Genome Research Institute at the National Institutes of Health (NIH). He is also part of a graduate partnership program between NIH and Johns Hopkins University. His goal is to develop a minimally invasive cancer detection assay using methylated DNA loci as biomarkers. A second aim is to seek a better understanding of how these genomic loci become targeted for methylation during tumorigenesis. Overall, he is honored to have the opportunity to be trained by Elnitski, who has extensive experience as a graduate student mentor, as well as work on a project that allows him to bridge the gap between computational and molecular biology. He believes he will be well equipped to pursue a research career in genomics and molecular biology, whether it be in industry or academia.

Frederick Miller, M.D., Ph.D.
National Institute of Environmental Health Sciences
www.irp.nih.gov/pi/frederick-miller

Frederick Miller is chief of the Environmental Autoimmunity Group at the National Institute of Environmental Health Sciences at the National Institutes of Health (NIH), Bethesda, Maryland. He oversees investigators in his group, as well as others in national and international consortia that evaluate and conduct a wide range of basic and clinical studies on adult and juvenile autoimmune diseases. He obtained his M.D. and Ph.D. from Case Western Reserve University, went on to medical residencies at both Emory University and Stanford University, and then did rheumatology and immunology training at NIH. His work in the field of autoimmune diseases spans three decades, and involves many aspects of the environmental risk factors, epidemiology, immunology, genetics, pathogenesis, evaluation, and treatment of immune-mediated diseases. He has focused much of his work on autoimmune muscle diseases. He is leading a number of studies to identify environmental and genetic risk factors for autoimmunity and systemic autoimmune diseases.

Andrew Nixon, Ph.D.
Duke University Medical Center
www.medicine.duke.edu/faculty/andrew-benjamin-nixon-phd

Andrew Nixon, Ph.D., is associate professor of medicine at Duke University, and Director of the Duke Phase I Biomarker Laboratory, a credentialed Molecular Reference Laboratory for the evaluation of blood-based biomarkers within the Alliance oncology cooperative group. He is a nationally recognized expert regarding the development of biomarkers, serving on the American Society of Clinical Oncology (ASCO) Program Committee (chair, tumor biology), chair-elect of the ASCO Taxonomy Governance Committee, and an ad hoc reviewer of the newly created National Cancer Institute National Clinical Trials Network (NCI NCTN) Core Correlative Sciences Committee. Additionally, he serves as an associate member of the NCI's Group Banking Committee (GBC), helping to harmonize the activities of multiple cooperative group banks, and developing more consistent standardization of practices and approaches to banking activities. Within the Alliance Oncology Cooperative Group, he serves as an executive member of the Translational Research Program, vice-chair for GI correlative research, and has recently been appointed to co-chair the newly established Immuno-Oncology Working Group. His research focuses on the interrogation of circulating markers found in the blood, referred to as the 'liquid biopsy,' pursuing the development of novel biomarkers for immuno-oncology and anti-angiogenic agents. Overall, Nixon brings extensive experience in coordinating multi-investigator and multicenter analyses, having operational experience ranging from biomarker kit development to assay development, including all aspects of sample handling, processing, inventory management, data cleaning, and statistical analyses.

David Pisetsky, M.D. , Ph.D.
Duke University Medical Center
https://immunology.duke.edu/people/david-stephen-pisetsky-phd-md

David Pisetsky received his B.A. from Harvard College, and his M.D. and Ph.D. from the Albert Einstein College of Medicine. Following house staff training at the Yale New Haven Hospital, he was a clinical associate at the National Cancer Institute. He joined the faculty of Duke University Medical Center in 1978 as chief of rheumatology at the Durham VA Hospital where he has remained since. He served as chief of rheumatology and immunology at Duke from 1996-2007.

Pisetsky has conducted basic and translational research on the pathogenesis of systemic lupus erythematosus (SLE). These studies have concerned the mechanisms of production of antinuclear antibodies (ANAs) and the immunological properties of nuclear molecules. Topics investigated include the immune activity of DNA of bacterial and mammalian origin, the antigenicity of DNA, and the influence of base sequence and backbone structure on the antigenic and immunogenic properties of oligonucleotides. More recently, he has investigated the role of microparticles as an important source of extracellular DNA.

In 2001, Pisetsky was awarded the Howley Prize from the Arthritis Foundation for his work on the immune properties of DNA. In 2016, he received the Presidential Gold Medal from the American College of Rheumatology. From 2000-2005, Pisetsky served as editor of Arthritis and Rheumatism, and from 2006-2011, he was the first physician editor of The Rheumatologist.

Adam Sowalsky, Ph.D.
National Cancer Institute, Center for Cancer Research
https://ccr.cancer.gov/Laboratory-of-Genitourinary-Cancer-Pathogenesis/adam-g-sowalsky

Adam Sowalsky received his Ph.D. from Tufts University's Sackler School of Graduate Biomedical Sciences, performing his dissertation research in the lab of Larry Feig. He conducted postdoctoral training at Harvard Medical School/Beth Israel Deaconess Medical Center with Steven Balk. The central theme of Sowalsky's research is understanding the biology of the molecular events associated with prostate cancer development, progression, and resistance to therapy.

Swee Lay Thein, D.Sc.
National Heart, Lung, and Blood Institute

Swee Lay Thein, D.Sc., was educated in both Malaysia and the United Kingdom. She completed her specialist training in hematology at London's Royal Postgraduate Medical School, Hammersmith, and the Royal Free Hospital, London. In 1982, she joined the U.K. Medical Research Council Molecular Hematology Unit in Oxford where she held various positions, including clinical training fellow, Wellcome Senior Fellow in Clinical Science, senior clinical scientist, and honorary consultant hematologist.

Thein was appointed in 2000 to the position of professor of molecular hematology and consultant hematologist at King's College London, and served as clinical director of the Red Cell Centre in King's College Hospital. At the hospital, she treated adult patients with sickle cell disease, and also provided consultation to clinicians and researchers throughout the world on patients with unusual forms of thalassemias, inherited blood disorders that disrupt the normal production of hemoglobin, resulting in anemia.

She joined the National Heart, Lung, and Blood Institute in spring 2015 as senior investigator and chief of the institute's newly formed Sickle Cell Branch.

Thein is author or co-author of more than 300 peer-reviewed research publications, invited review articles, and book chapters. She has been honored for her research with awards from the U.K. Academy of Medical Sciences and the Academy of Life Sciences for Chinese in the U.K. She was also awarded a visiting professorship from Kuwait University, and an honorary professorship in pathology from the University of Hong Kong. She serves on the editorial boards of the research journals Blood, Pathology, Annals of Haematology, Hemoglobin, and the American Journal of Hematology, and is feature editor of the Blood Journal's Sickle Cell Disease hub, a micro-website that complements research published in the journal with links to articles, images and slideshows, and other multimedia.

She was chair of the European Hematology Association's scientific working group for red blood cells and iron disorders from 2011-2014, and has organized annual international conferences on sickle cell disease since 2006. She also has been instrumental in organizing scientific and educational conferences on red blood cell disorders for the European Hematology Association and European School of Hematology.

Neeta Vora, M.D.
University of North Carolina School of Medicine
https://www.med.unc.edu/obgyn/department-directory/maternal-fetal-medicine/neeta-vora

Neeta Vora, M.D., is triple boarded in obstetrics and gynecology, maternal fetal medicine, and clinical genetics. She completed her residency and fellowship at Tufts Medical Center in Boston, and moved to UNC-Chapel Hill in 2012. She is now an associate professor and director of reproductive genetics at UNC. She has a K23 from the National Institute of Child Health and Human Development to study use of new genomic technologies in obstetrics. She has authored more than 30 articles on prenatal genetics, ranging from cell-free DNA to whole exome and genome sequencing.

Douglas White, M.S.
Promega

Douglas White received his bachelor's degree in chemistry from the Illinois Institute of Technology, and his master's degree in biochemistry from the University of Wisconsin-Madison where he studied with Dave Nelson. In 1990, he joined Promega's R&D department where he worked on projects related to protein kinases and DNA purification from myriad sources. For the past five years, he has focused on the purification and analysis of circulating cell-free DNA.

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