National Cancer Institute

NCI Center for Strategic Scientific Initiatives

July 27, 2010

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Contents

Center for Strategic Scientific Initiatives (CSSI)

Overview

The Center for Strategic Scientific Initiatives (CSSI) is an operating entity of the Office of the Director, National Cancer Institute (NCI). The beginning of CSSI dates back to the late 1990s with the creation of the Unconventional Innovation Program (UIP), trans-divisional Integrated Molecular Analysis Technologies program (IMAT), and Cancer Genome Anatomy Program (CGAP) located in the Office of the NCI Director. The concept of an innovation center for NCI began to develop in 2003, by leveraging the success of the UIP, IMAT, and CGAP programs along with due diligence on issues that were identified as major potential barriers for all cancer researchers, specifically biospecimen resources and bioinformatics platforms.

The overall conceptual framework for the center evolved to include a continuum inclusive of foundational resources and standards development programs that would be critical to the work of nearly all cancer and biomedical research communities (e.g., biospecimens, bioinformatics, biomarkers); large-scale genomics programs; exploration and development of advanced technology programs that could support advances in cancer research; and new higher risk areas that may not be mainstream but hold promise to inform cancer research in ways that may question existing paradigms and lead to hypothesis testing.

All of CSSI's programs begin with a series of meetings with extramural scientists, generally including all sectors. From these think tanks, consensus ideas and input are generated which are captured in derivative reports – and evolve and mature into a concept that is first presented to the NCI Executive Committee (EC) for review. All of CSSI's programs receive a second level of review by the NCI's Board of Scientific Advisors (BSA) and funding approval by the National Cancer Advisory Board (NCAB). Both the NCAB and BSA also receive regular updates on selected programs with the Center. Nearly all of CSSI's programs utilize support mechanisms that create centers of excellence, emphasize team science, and, most importantly, make data publicly available.

Overall the concept of an innovation center as part of the NCI's portfolio is in keeping with similar efforts in both the government and private sectors. The Center was established to undertake programs that will enable all NCI Divisions

and Centers and the investigators they serve and concomitantly to allow the Institute to explore new ideas and fields of science to both further explore and build the science for an emerging area (e.g., nanotechnology) and anticipate future scientific opportunities and directions.

The Center currently consists of six offices with a total of 50 staff. The actual budget for the Center in 2009, inclusive of grants, contracts, and operating (RMS) funds, was $147.34M. The 2010 budget projected cost is $138.7M, again inclusive of grants, contracts, and operating (RMS) funds. In terms of operating costs only, the Center's overall RMS cost is approximately $7M (~5.1%), which compares favorably with the NCI's overall RMS costs of 384.6M (~7.7%). Selected programs in the Center received Recovery Act funding, notably The Cancer Genome Atlas (TCGA), cancer Human Biobank (caHUB), Therapeutically Applicable Research to Generate Effective Treatments (TARGET), and administrative supplements for selected existing grants.

The establishment of CSSI has allowed the NCI to leverage cross-cutting advanced technologies for cancer and overall to capitalize on the convergence of the molecular sciences with advanced technologies. The Center is designed to fulfill a fundamental need in the cancer research community by enabling synergy between individualized, investigator-driven research and team-oriented, technology-based projects. Systems-based strategies to create and analyze large volumes of data have enabled the overall research community by providing unprecedented data and information to individual researchers and clinicians. These strategies create a more comprehensive suite of tools, standards, and data that can be shared by the entire cancer research community.

Since its inception, CSSI has undertaken a number of programs and initiatives that have contributed significantly to addressing major barriers and opportunities in cancer research. All of these initiatives are also designed to enable advances that can be translated to the clinic and substantially impact patient care. The Center's programs vary in their maturity and achievement, but overall they are focused on scientific excellence and have all achieved their goals in large measure. As in any innovation center, not all ideas are accepted and developed. Some are developed to a point and then repositioned within the NCI. The intent of the Center is to maintain initiatives as long as needed to address the problem/opportunity identified, but not to become entitlement programs. However, programs such as TCGA or caHUB may have a longer period of performance than initiatives that are designed to achieve key goals within a specific timeframe. CSSI is succeeding as an innovation center, and the programs that constitute the Center are examples of how programs can both address major barriers and enrich opportunities for individual investigators.

The Offices that currently comprise the Center, and the programs within, are described and summarized in the following sections. These include:

- Office of Physical Sciences-Oncology (OPSO)
- Office of Cancer Nanotechnology Research (OCNR)
- The Cancer Genome Atlas (TCGA) Program Office
- Office of Cancer Genomics (OCG)
- Office of Clinical Cancer Proteomics Research (OCCPR)
- Office of Biorepositories and Biospecimen Research (OBBR)

Anna D. Barker, Ph.D.
Deputy Director
National Cancer Institute, NIH

Dr. Barker serves as the Deputy Director of the National Cancer Institute (NCI) and as the Deputy Director for Strategic Scientific Initiatives. In this role she has developed and implemented multi/trans-disciplinary programs in strategic areas of cancer research and advanced technologies, including the Nanotechnology Alliance for Cancer; The Cancer Genome Atlas (TCGA); and the Clinical Proteomics Technologies Initiative for Cancer. She participates actively in these programs and serves in a team leadership role for TCGA. Recently she led the development of a new initiative to develop a network of trans-disciplinary centers focused on the elucidation of the "physics" of cancer at all scales through the establishment of Physical Sciences-Oncology Centers. Dr. Barker has also led and collaborated on NCI's effort to develop contemporary resources for cancer research in the areas of biospecimens and bioinformatics (cancer Biomedical Informatics Grid) to support molecularly based personalized medicine. She serves as the co-chair of the NCI-FDA Interagency Task Force and co-chair of the Cancer Steering Committee of the FNIH Biomarker Consortium, and oversees the NCI's pilot international cancer research programs in Latin America and China.

Dr. Barker has a long history in research and the leadership and management of research and development in the academic, nonprofit, and private sectors. She served as senior scientist and subsequently a senior executive at Battelle Memorial Institute for 18 years, where she developed and led a large group of scientists working in drug discovery and development, pharmacology, and biotechnology, with a major focus in oncology and NCI-supported programs. She cofounded and served as the CEO of a public biotechnology drug development company and founded a private cancer technology-focused company. She has served in numerous volunteer capacities for cancer research and advocacy organizations including the AACR, where she led the Legislative Affairs Committee for 10 years and was a member of the Board of Directors. She has received a number of awards for her contributions to cancer research, cancer patients, professional and advocacy organizations, and the ongoing national effort to prevent and cure cancer. Her research interests include small molecule experimental therapeutics, tumor immunology, and free-radical biochemistry in cancer etiology and treatment. Dr. Barker completed her M.A. and Ph.D. degrees at The Ohio State University, where she trained in chemistry, immunology, and microbiology.

Jerry S.H. Lee, Ph.D.
Deputy Director, Center for Strategic Scientific Initiatives
Office of the Director, National Cancer Institute, NIH

Dr. Lee serves as Deputy Director for the NCI's Center for Strategic Scientific Initiatives (CSSI). He provides scientific input and expertise to the planning, coordination, development, and deployment of the innovation center's strategic scientific initiatives. He serves and leads various trans-NCI working groups and is a non-voting member of the NCI Executive Committee. Dr. Lee also represents CSSI at various NIH, HHS, and external committees and other activities to develop effective partnerships across Federal agencies and to build collaborations with key external stakeholders.

Dr. Lee is responsible for providing day-to-day administrative and programmatic management for CSSI's offices, including (1) The Cancer Genome Program Office (TCGA PO); (2) Office of Cancer Nanotechnology Research; (3) Office of Biorespositories and Biospecimen Research (OBBR); (4) Office of Cancer Genomics (OCG); (5) Office of Cancer Clinical Proteomics Research (OCCPR); and (6) Office of Physical Sciences-Oncology (OPSO). He serves as Acting Director for the Office of Physical Sciences-Oncology and is responsible for initiatives at the interface of the physical and life sciences including the NCI's Physical Sciences-Oncology Centers (PS-OCs) program. His previous experience at NIH includes serving as a program manager for the NCI's Innovative Molecular Analysis Technologies (IMAT) program and the NCI Alliance for Nanotechnology in Cancer program, where he was Program Director of fellowships to support multidisciplinary training in cancer nanotechnology. Dr. Lee's previous research experiences in coordinating collaborations among the U.S. Naval Research Laboratory, NCI-Frederick Laboratory, Johns Hopkins University Medical Oncology Division, and Institute for NanoBioTechnology also contribute to carrying out his current efforts.

Scientifically, Dr. Lee has extensive research experience in using engineering-based approaches to examine mechanisms of age-related diseases and cancer progression focused on combining cell biology, molecular biology, and engineering to understand various cellular reactions to external stimuli. Specifically, Dr. Lee's research has emphasized increasing the understanding of RhoGTPase-mediated nuclear and cellular mechanical responses to fluid flow, 3D culture, and contributions to laminopathies such as progeria. He has coauthored numerous papers, two book chapters, and one book, and has spoken at various cell biological and biomedical conferences.

Dr. Lee currently serves as adjunct assistant professor at Johns Hopkins University, where he earned his bachelor's degree in biomedical engineering and Ph.D. degree in chemical and biomolecular engineering.

Office of Physical Sciences-Oncology (OPSO)

1. Mission

The mission of the National Cancer Institute's (NCI) Office of Physical Sciences-Oncology (OPSO) is to facilitate the development of innovative ideas and new fields of study that converge perspectives and approaches of physical sciences and engineering with cancer biology and clinical oncology. By fostering a culture that encourages different perspectives and serving as a nexus for the development and implementation of physical sciences-based initiatives, we support and nurture new trans-disciplinary environments and cancer research for NCI as well as its integration across trans-NIH and inter-agency activities. Through the use of various funding mechanisms and outreach activities, we hope to join these often disparate areas of science to better understand the physical and chemical forces that shape and govern the emergence and behavior of cancer at all levels which will lead to exponential progress against cancer.

2. Physical Sciences-Oncology Centers Program

In September 2009, the Physical Sciences-Oncology Centers (PS-OC) program awarded cooperative agreements for 12 specialized Centers that comprise a virtual Network. The PS-OC Network brought together expert teams from the fields of physics, chemistry, mathematics, engineering, cancer biology, and oncology to assemble and develop capabilities and research programs that will enable the convergence of the physical sciences with cancer biology. The strategic goal of the program is to have the PS-OCs, both individually and collectively, support and nurture a new trans-disciplinary environment and research that (1) originates and tests novel, non-traditional physical sciences-based approaches to understanding and controlling cancer; (2) generates orthogonal sets of physical measurements and integrates them with existing knowledge of cancer; and (3) develops and evaluates theoretical physics approaches to provide a comprehensive and dynamic picture of cancer.

2.1 Background

Extramural Input – "Think Tanks" Meetings

The genesis for the Physical Sciences in Oncology initiative started in February 2008 with the first of three NCI-sponsored strategic "think tanks." These meetings brought together over 300 extramural thought leaders from the fields of physical sciences and engineering with leaders in the fields of cancer biology and clinical oncology to ascertain how NCI could more effectively engage the physical sciences in cancer research. The first NCI-sponsored meeting, "Integrating and Leveraging the Physical Sciences to Open a New Frontier in Oncology," was held in Washington, D.C., February 26-28, 2008, and highlighted four thematic areas that emerged from the meeting in which physical sciences approaches and principles could profoundly influence and improve our knowledge of cancer biology. These thematic areas were:

- *Physics (Physical Laws and Principles) of Cancer:* Defining the role(s) of thermodynamics and mechanics in metastasis and determining how this knowledge might be employed in new intervention strategies.
- *A New Look at Evolution and Evolutionary Theory of Cancer:* Developing a comprehensive theoretical inclusive construct that would provide a foundation for understanding and predicting cancer heterogeneity.
- *Information Coding, Decoding, Transfer, and Translation in Cancer:* Pursuing theoretical and supportive experimental approaches that define what information is and how it is decoded and managed in terms of cell signaling and contextual information translation in cancer.
- *De-convoluting Cancer's Complexity:* Applying theoretical and experimental approaches from the physical sciences to cancer complexity that will inform a new fundamental level of understanding of cancer that may facilitate prediction of viable pathways to develop novel interventions.

Subsequent think tank meetings delved more deeply into a specific thematic area. The second think tank, "A New Look at Evolution and Evolutionary Theory in Cancer," identified a number of the major research questions in the field and elaborated a number of "grand challenges" that, if met, would significantly improve our understanding of the role of evolution in cancer. The role of information and information theory in cancer, specifically those changes that confer selective advantages, emerged as an area where a great deal of knowledge is needed to elucidate the role of information flow at all scales in understanding the emergence of the malignant phenotype. This triggered the last think tank, "Physical Sciences-Based Frontiers in Oncology: The Coding, Decoding, Transfer, and Translation of Information in Cancer," which better defined this complex field relative to its potential role in understanding and controlling cancer.

2.2 Development Process and Funding History

Following the three extramural think tanks, a request for concept approval was presented to the NCI's Executive Committee and Board of Scientific Advisors in the fall of 2008 (figure 1). The concept to support the development

of PS-OCs was approved at the 41st meeting of the NCI's Board of Scientific Advisors. The Request for Applications (RFA-CA09-009) was distributed to the public in December 2008. A combined total of 35 applications were received on March 13, 2009, in response to the RFA. One of the largest study sections (over 110 reviewers, see picture) assembled by the NCI convened June 29-30, 2009. The large number of reviewers was needed due to several factors: (1) the number of applications received, (2) the multicomponent nature of the proposed Center, and (3) the diverse subject matter expertise needed to properly review all the applications.

Eight applications were funded utilizing appropriated funds ($22.5 million) when the PS-OC program launched in the fall of 2009. In addition to appropriated funds for RFA-CA-09-009, limited Recovery Act funding ($7.6 million) was applied to support four Centers at significantly reduced levels. Appropriate reductions were made in these applications to reflect elimination of specific aims/projects, and a 20% reduction was also applied to reflect the mandate that Recovery Act funds cannot be restricted. Two thematic areas were recommended for additional funding using Recovery Act dollars to balance and complete the PS-OC Network. These meritorious applications represent innovative and high-risk areas of research at the interface of physical and life sciences that are aligned with NCI's strategic vision of accelerating cancer research and advancing innovations through Recovery Act support. The funded Centers are distributed across the four themes defined by the workshops as well as length scales from DNA to the patient (figure 2).

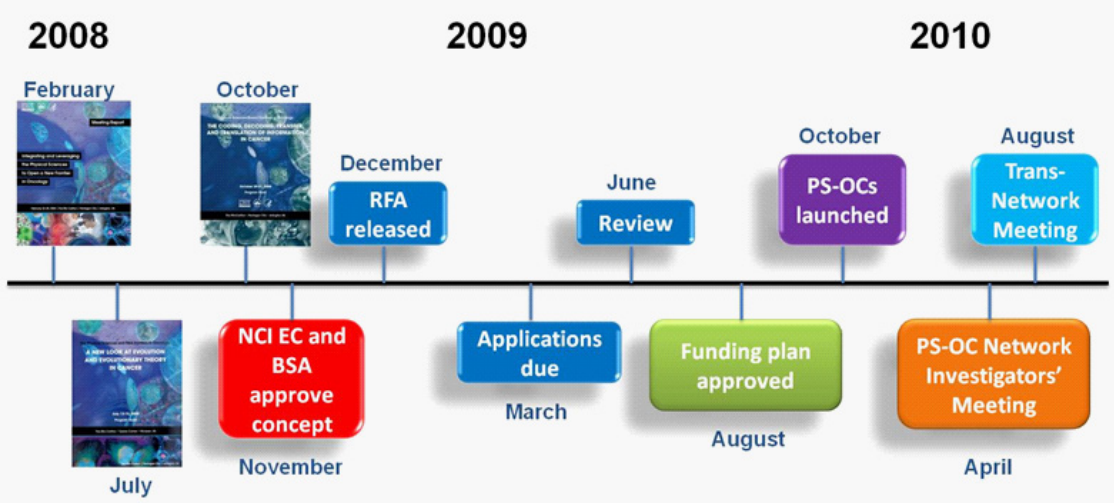


Figure 1. Timeline of Major Events in the Development of the PS-OC Program.

2.3 Strategic Approach/Plan

The three strategic think tanks were paramount for guiding the overall structure of the PS-OC program. While the distinctions between the physical sciences and life sciences disciplines have been noted (e.g., A New Biology for the 21st Century, National Academies Press [2009]; Research at the Intersection of the Physical and Life Sciences, National Academies Press [2010]), there was a consensus among the participants to establish trans-disciplinary physical sciences-oncology Centers composed of integrated physical sciences-oncology teams to overcome the traditional barriers (silos) that have existed between these two scientific communities. If we want to bring the physical science perspective (i.e., *"Bring the physics, not just the physicist, to the problem of biology"*), the Centers should be led by a physical scientist with the senior co-investigator from the oncology or cancer biology field. In addition to establishing an integrated team of physical scientists/engineers with cancer biologists/oncologists within their own Center, PS-OC investigators should also be closely integrated with other investigators from different PS-OCs, forming a comprehensive PS-OC Network.

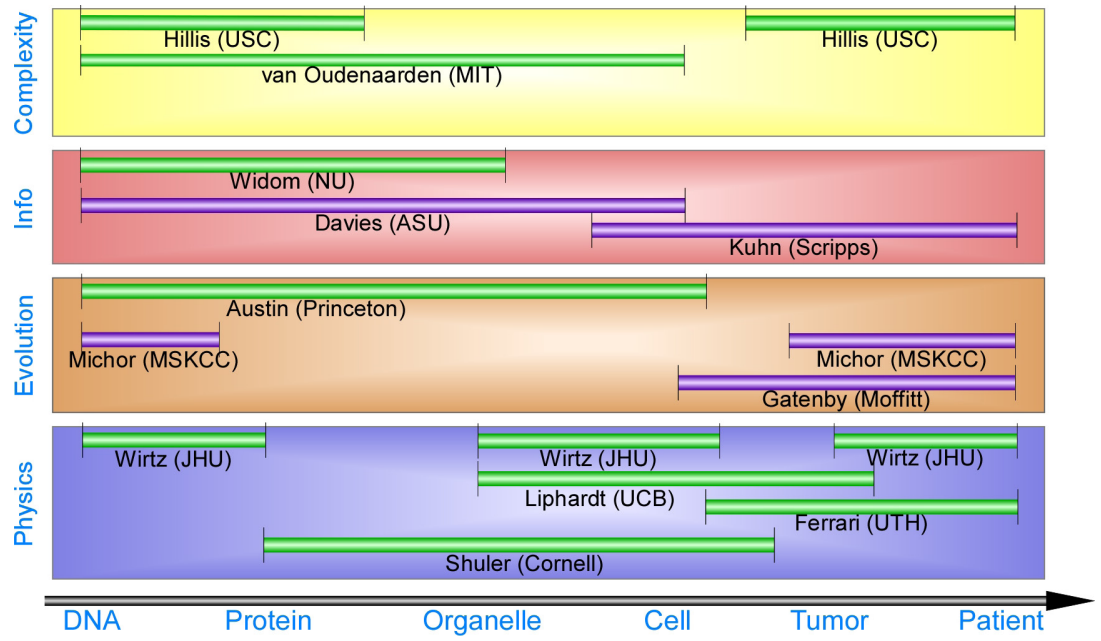


Figure 2. PS-OCs by Theme and Length Scale. The PS-OCs (by PI and institution) are arranged by thematic area (Y-axis) and length scale (X-axis). Appropriated PS-OCs are indicated in green and ARRA-funded PS-OCs are indicated in purple.

A Focus on Addressing Big Questions in Oncology

Starting with the think tank meetings and throughout the duration of the PS-OC program, asking (and revisiting) the "big questions" in cancer is a strategic component of this program at various levels. First, to help focus a Center's activities, an organizing Framework or "school of thought" is proposed that defines the overall research direction and utilizes a novel physical sciences-based perspective *to address a major barrier/question in cancer research,* vs. narrow questions pertaining only to a specific disease or model system. The organizing Framework should draw attention to research that could result in *paradigm-shifting progress against cancer* in one or more of the following thematic areas established in the first think tank meeting.

On another level, we have asked that investigators in the Network to pose "Big Questions" that the Network, as a whole, can make progress in addressing. The Network is continually refining and adapting the questions of interest. The following are examples of questions that have been posed at the think tank meetings and by the PS-OC Network investigators:

- Information Transfer in Cancer Through an Evolutionary Lens
  - Can novel therapeutic strategies be developed based on increasing the genetic load of mutations in a cancer cell population that will lead to extinction of this population?
  - What genetic and epigenetic features define a cancer stem cell?
  - Do oncogenic mutations confer self-renewal to cells?
  - What is a gene?

- Time Domain of Cancer Metastasis and Therapy
  - Is the fluid phase biopsy of solid tumors an accurate real-time representation of the disease over the course of the patient's lifetime?
  - How does the heterogeneity of a tumor impact drug response?

- The Mechanics of Cancer Metastasis
  - Is mechano-therapy of cancer possible?
  - "Follow the genes" is the dominant paradigm. Can we develop a complementary "follow the physics" approach?
  - What is the role of forces in metastasis?

- Physical Parameters of the Tumor Cells, Microenvironment, and Host
  - How does a tumor cell change its genetic, epigenomic, and metabolomic signature, as it becomes "successful," i.e., invasive, metastatic?
  - Is the transport oncophysics of the microenvironment what really matters?
  - What is the energy budget of a cancer cell?

- Understanding Physical Emergent Properties: What Is Cancer?
  - How can we change the physical microenvironment (selective pressures) to prevent cancer?
  - Is cancer curable? Can it be controlled through manipulation of the microenvironment?
  - Why do tumors ultimately make a phase transition to a metastatic phenotype?

Management Structure of the PS-OC Network

The notion that each PS-OC has a school of thought requires that these ideas/concepts be rigorously tested by other PS-OC investigators. Such transparency and openness would necessitate that a network of PS-OCs be coordinated differently, which allows for flexibility yet maintains scientific excellence. A cooperative specialized research Center (i.e., U54) mechanism was selected because this mechanism affords the ability for a high degree of integration and coordination among researchers from the disparate fields represented in the PS-OCs. Furthermore, unique and selected resources that exist in the physical sciences community could be shared among the PS-OCs; exchange of computation constructs (both dynamic and kinematic), as well as experimental verification protocols, are important to maximize the effectiveness of each of the Centers. Moreover, the active involvement and guidance of experienced program managers with relevant backgrounds in physics, engineering, and mathematics who also have training in cancer biology have been critical to guide the development and maturation of the Centers and build the Network. The interactions with program staff accelerate facilitation of multiscale, multilevel methodologies, both within and across Centers as well as provide linkages to complementary programs at the NCI.

2.4 Program Description and Goals

Overall Goals of the PS-OC Network

The primary objective of the PS-OC Program is *to unite the fields of physical science with cancer biology and oncology* to assemble *trans-disciplinary teams* and infrastructure to better understand the physical and chemical forces that shape and govern the emergence and behavior of cancer at all levels.

This program will foster the coordinated, iterative, trans-network development and testing of innovative, perhaps nontraditional, approaches to understanding cancer processes, and new fields of study based on knowledge of both biological and physical laws and principles that define normal and tumor systems at all length scales. This, in turn, will cultivate *paradigm-shifting* science leading to exponential progress against cancer.

- To generate *new knowledge* and catalyze *new fields of study* in cancer research by utilizing physical sciences/engineering principles to gain a better understanding of cancer and its behavior at all scales, thereby generating answers to some of the major questions and barriers in cancer research.
- To identify new perspectives and approaches which facilitate *paradigm-shifting* science and lead to exponential progress against cancer rather than looking for new tools to do "better" science.
- Build *trans-disciplinary teams* and infrastructure to better understand and control cancer through the convergence of physical sciences and cancer biology.

As a first step of this initiative, a program consisting of a virtual network of PS-OCs was launched in the fall of 2009. The management of the network involves a cooperative agreement collaboration between the NCI OPSO project team, the awarded Center principal investigators, and the PS-OC Steering Committee. The PS-OC Program uses the U54 mechanism to fund 12 Centers to achieve thematic balance across the PS-OC Network (8 appropriated U54s for 5 years, and four American Recovery and Reinvestment Act of 2009 (Recovery Act) U54s for 2 years) (figure 3).

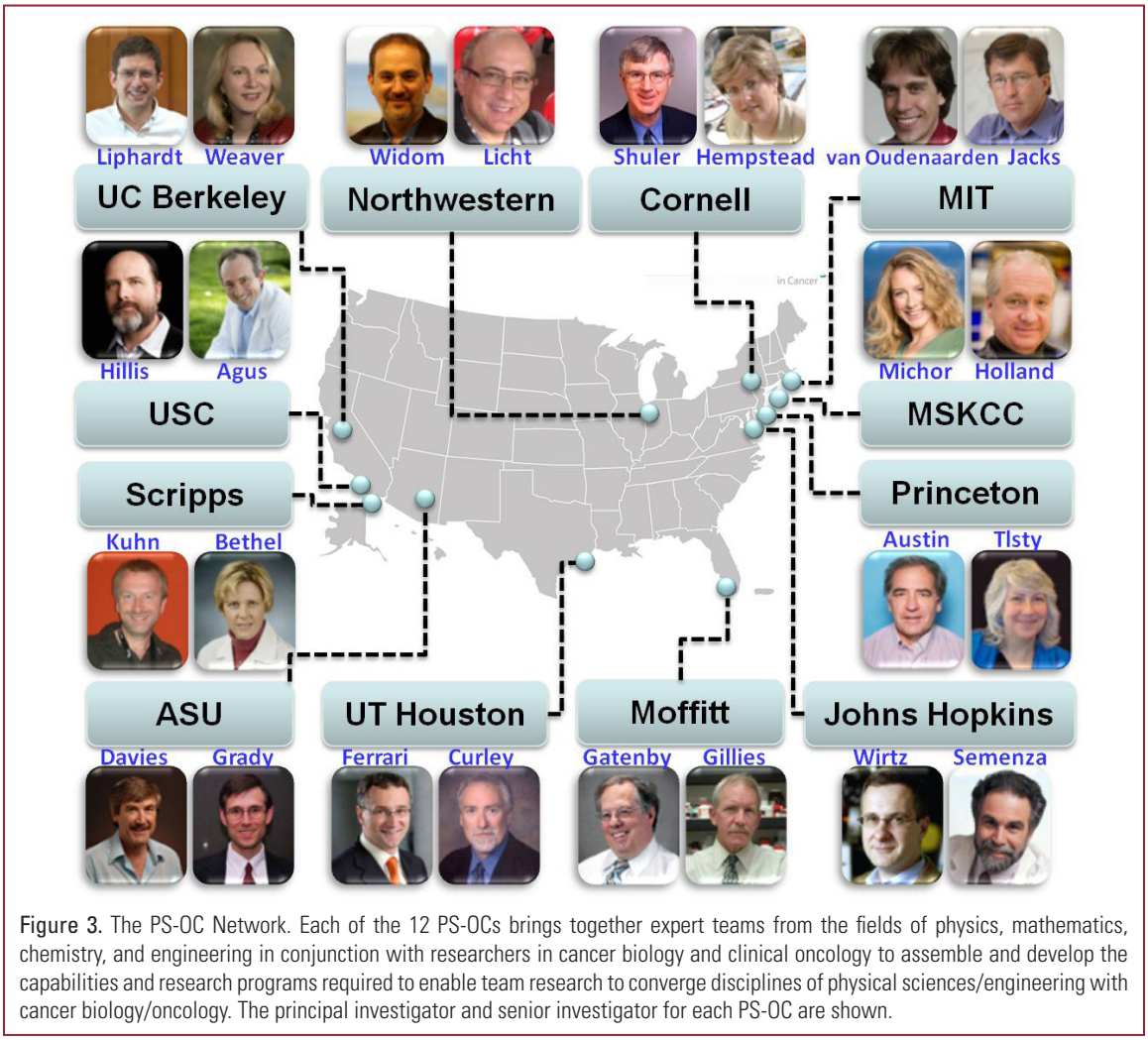


Figure 3. The PS-OC Network. Each of the 12 PS-OCs brings together expert teams from the fields of physics, mathematics, chemistry, and engineering in conjunction with researchers in cancer biology and clinical oncology to assemble and develop the capabilities and research programs required to enable team research to converge disciplines of physical sciences/engineering with cancer biology/oncology. The principal investigator and senior investigator for each PS-OC are shown.

Arizona State University Physical Sciences-Oncology Center

PI: Paul Davies, Ph.D. SI: William M. Grady, M.D.

Arizona State University Physical Sciences-Oncology Center's (ASU PS-OC) foremost aim is rigorously questioning the central tenets of cancer biology and creating innovative paradigm-shifting tactics that challenge the barriers of contemporary cancer research and treatments. This team hypothesizes that cancer progression is linked to systematic physical differences in cells. Pioneering methods (e.g., single-cell tomography) to survey these physical changes are being employed and theoretical evolutionary models are applied to establish the evolution of a metastatic cancer cell from a physical context.

Cornell University Physical Sciences-Oncology Center

PI: Michael Shuler, Ph.D. SI: Barbara L. Hempstead, M.D., Ph.D.

Cornell University Physical Sciences-Oncology Center (CU PS-OC) uses its expertise in manufacturing nano- and microfluidic devices to devise and assemble a three-dimensional tumor model to delve into the impact of physicochemical factors in tumor vascularization and cancer progression. This design imparts spatial and temporal resolution far greater than obtained by conventional two-dimensional tissue culture models. This platform facilitates the monitoring of non-linear responses to a combination of physical, chemical, genetic, and epigenetic stimuli and will lead to a better understanding of the signaling pathways that regulate the angiogenic switch.

H. Lee Moffitt Cancer Center & Research Institute Physical Sciences-Oncology Center

PI: Robert A. Gatenby, M.D. SI: Robert J. Gillies, Ph.D.

H. Lee Moffitt Cancer Center & Research Institute Physical Sciences-Oncology Center's (MCC PS-OC) mission incorporates physical science concepts into the investigation of carcinogenesis. Both genetic alterations and microenvironmental selection pressures need to be deciphered in order to impede somatic evolution. Applied mathematical modeling is being used to determine whether oncogenesis is regulated by the escape from tissue homeostasis and provide further insight into the complex problems associated with cancer.

Johns Hopkins University Physical Sciences-Oncology Center

PI: Denis Wirtz, Ph.D. SI: Gregg L. Semenza, M.D., Ph.D.

Johns Hopkins University Physical Sciences-Oncology Center (JHU PS-OC) explores the mechanical forces in cancer that bolster the tumor metastatic cascade. The team is studying and modeling cellular mobility and the assorted biophysical forces involved in the metastatic process. One such pressure includes hypoxia located within the tumor. Hence, the effects of increased levels of HIF-1 on the mechanical properties of the extracellular matrix and the impact of hypoxia on cellular signaling are being evaluated. Micropatterned extracellular matrix is also used to uncover the dynamics of cell migration.

Massachusetts Institute of Technology Physical Sciences-Oncology Center

PI: Alexander van Oudenaarden, Ph.D. SI: Tyler Jacks, Ph.D.

Massachusetts Institute of Technology Physical Sciences-Oncology Center (MIT PS-OC) employs innovative technology and analytical and computational tools to explore the process of carcinogenesis and better understand the complexity of cancer at the single-cell level. This team utilizes pioneering single-cell mRNA counting techniques to model stem cell differentiation and reprogramming signaling networks as well as to probe the connection between cell growth and the cell cycle. Gene expression of various transcripts in individual cells is being surveyed over time to measure the quantity and pattern during these processes as well as to establish computational models of neoplastic progression.

Memorial Sloan-Kettering Cancer Center Physical Sciences-Oncology Center

PI: Franziska Michor, Ph.D. SI: Eric C. Holland, M.D., Ph.D.

Memorial Sloan-Kettering Cancer Center Physical Sciences-Oncology Center (MSKCC PS-OC) intertwines the physical sciences with cancer biology and oncology by engaging evolutionary theory to address several critical issues concerning cancer research. Iterative modeling is being employed to study the evolution of brain, lung, and hematopoietic tumors. One major evolutionary focus of this Center is to resolve which cell serves at the cell of origin for brain and hematopoietic tumors. Knowledge of the cells that initiate and drive cancer progression is critical for determining treatment options against cancer.

Northwestern University Physical Sciences-Oncology Center

PI: Jonathan Widom, Ph.D. SI: Jonathan Licht, M.D.

Northwestern University Physical Sciences-Oncology Center (NU PS-OC) probes the molecular basis of information flow within a malignant cell and is providing a basic understanding of how normal gene expression is calibrated and of how the epigenome and proteosome are regulated. They are studying the diverse characteristics of gene expression and storage by exploring the 3-D organization of the genome and the higher order chromatin structure using leading-edge physical techniques. Insight into chromatin structure modifications in malignant cells has the potential to expedite the development of tools for the early diagnosis of cancer.

Princeton University Physical Sciences-Oncology Center

PI: Robert H. Austin, Ph.D. SI: Thea D. Tlsty, Ph.D.

Princeton University Physical Sciences-Oncology Center (PU PS-OC) focuses on how to control the evolution of cancer resistance to chemotherapy by understanding its origin and dynamics. Using the basics of physics to evaluate stress response mechanisms in both fundamental and clinically relevant studies, the team hypothesizes that evolution in a small, stressed microenvironment will generate the rapid emergence of resistance. Microfabricated microenvironments and single-cell genomic analysis are used to evaluate metabolic and mechanical stressors and determine whether stress can alter the types of mutations accumulated by cells.

The Scripps Research Institute Physical Sciences-Oncology Center

PI: Peter Kuhn, Ph.D. SI: Kelly J. Bethel, M.D.

The Scripps Research Institute Physical Sciences-Oncology Center (TSRI PS-OC) is pursuing the mechanisms that regulate the survival of circulating tumor cells and probing the biophysical factors implicated in the endurance of individual circulating tumor cells while in the bloodstream and in their progression to metastatic disease. Fluid phase biopsies from epithelial cancers are being employed to assess and model the physical attributes (e.g., cell size, mechanical properties, ultrastructural complexity, etc.) of these tumor cells over the course of the disease across various body compartments.

University of California, Berkeley Physical Sciences-Oncology Center

PI: Jan Liphardt, Ph.D. SI: Valerie M. Weaver, Ph.D.

University of California, Berkeley Physical Sciences-Oncology Center (UCB PS-OC) is determining how mechanobiology influences tumorigenesis in breast cancer: malignant phenotype is maintained by exchanges with its microenvironment and reversion can occur if these pressures are normalized. This Center also examines how mechanical signals trigger genetic changes that induce tumorigenesis via the integration of state-of-the-art tools in the physical, theoretical, and biological sciences that will cultivate models of various interactions of model systems with their microenvironment.

University of Southern California Physical Sciences-Oncology Center

PI: W. Daniel Hillis, Ph.D. SI: David B. Agus, M.D.

The University of Southern California Physical Sciences-Oncology Center's (USC PS-OC) overall goal is to thoroughly understand therapeutic response by establishing a predictive model of cancer that can be utilized to determine tumor steady state growth and drug response, particularly those involved in the hematological malignancies of acute myeloid leukemia and non-Hodgkin lymphoma. Furthermore, multiscale physical measurements are being conducted under unified conditions to facilitate the development of a model that can derive the tumor's traits during its growth and after any distress, such as chemotherapeutic treatment.

University of Texas Health Science Center at Houston Physical Sciences-Oncology Center

PI: Mauro Ferrari, Ph.D. SI: Steven A. Curley, M.D.

The University of Texas Health Science Center at Houston Physical Sciences-Oncology Center (UTHSCH PS-OC) integrates mathematics, innovative engineered transport probes, and state-of-the-art imaging to elucidate the transport physics of various physical and biological barriers related to tumorigenesis and drug delivery. Notably, this trans-disciplinary team is studying the physical barriers to the evolution of liver metastasis from colorectal cancer and the administration of novel carriers to surpass these barriers. Ultimately, these studies will provide a clearer grasp of the function and physics of biological barriers, and in turn accelerate basic discovery and the design for potential therapeutics.

2.5 Composition of the PS-OC Program

2.5.1 Organization and Policies of the PS-OC Network

In order to facilitate dialogue and collaboration among investigators throughout the PS-OC Network, a number of mechanisms described below were built into the program that encourage and reward collaboration.

PS-OC Steering Committee

The PS-OC Steering Committee serves as the main governing board for the PS-OC Network and is responsible for ensuring the scientific progress and oversight of the Network. The Steering Committee was jointly established by and comprises members from the awarded Centers and NCI PS-OC program staff. The PS-OC Steering Committee consists of (a) two representatives from each awarded Center (PI and SI) and (b) four NCI PS-OC Project Scientists. Each Center and each project scientist have one vote. The setup is designed to allow NCI program staff to facilitate and promote inter-Center collaboration pilot projects based on synergistic Center expertise and projects. Additional expertise will be solicited from non-voting external scientific members as needed.

The PS-OC Steering Committee meets bimonthly to discuss both critical Network policies and scientific progress. This forum serves as a critical point of interaction between members of each Center and between physical scientists and cancer biologists. The Steering Committee meetings have hosted important discussions varying from (1) the pros and cons of using model systems versus primary samples to (2) implementing systems for data sharing and data analysis. Additionally, the Steering Committee has initiated and implemented a number of key Network-wide activities including the Cell Line Pilot Study, Trans-Network Projects program, and the Network Data Sharing Agreement and Pilot Data Coordinating Center.

PS-OC Network Working Groups

Several working groups have been established for the PS-OC Network to facilitate achievement of program goals, and participation is on a voluntary basis. Each working group contains at least one NCI PS-OC program staff member. Additional working groups will be established on the basis of need and interest. Working groups have been created for the following areas:

- Physics
- Evolution of Drug Resistance
- Data Integration
- Education and Training
- Science Outreach and Dissemination
- PS-OC Steering Committee Operations Subgroup

Trans-Network Projects

Each of the appropriated PS-OCs includes a minimum of $100,000 in direct costs to be allocated specifically for Trans-Network projects. These funds provide each Center an opportunity to catalyze new perspectives and test unique research ideas through the development of robust collaborations within the PS-OC Network. These funds allow potential "outside the box" projects that originate from discussions between Network investigators an avenue to achieve funding. The goal is then to transition successful projects to independent funding when possible.

PS-OC Network Investigators' Annual Meeting

The PS-OC Network Investigators' Annual Meeting has participants from across the Network ranging from Principal Investigators to postdoctoral fellows and graduate students. The annual meetings highlight scientific efforts within the PS-OC Network, promote collaborations, and provide a venue for working group discussions that will explore the physical laws and principles that shape and govern the emergence and behavior of cancer at all scales. The Annual Meetings also host tutorial sessions and training groups to provide education and guidance to the next generation of young scientists in the many disciplines that contribute to the PS-OCs. Finally, the Trans-Network Projects and Young Investigator Trans-Network Projects programs are implemented during the PS-OC Annual Meetings.

2.5.2 Organization and Policies of Individual PS-OCs

Each PS-OC is a "virtual" Center, headed by a Principal Investigator (PI) and a Senior Investigator (SI) and is composed of research facilities from two or more collaborating institutions. In order to begin merging the perspectives of physical sciences and oncology within each Center from the top down, the PIs are trained and have significant experience in the physical sciences while the SIs are trained and have significant experience in areas of basic and/or clinical cancer research. Each PS-OC consists of a collaborative trans-disciplinary research team of investigators with complementary abilities focused by an organizing construct that addresses major questions/barriers in cancer research, which are substantiated through projects that support the overall Center Framework. In order to effectively integrate the physical sciences and cancer biology perspectives, cross-train young investigators in this emerging field, and grow the field outside the PS-OC Network, each PS-OC is composed of Projects, Resource Cores, and Education and Outreach Units and is governed by an Administrative Unit and an Overarching Framework (figure 4).

Framework: A physical science-based overarching organizing Framework is required for the PS-OC to address major questions and barriers in cancer.

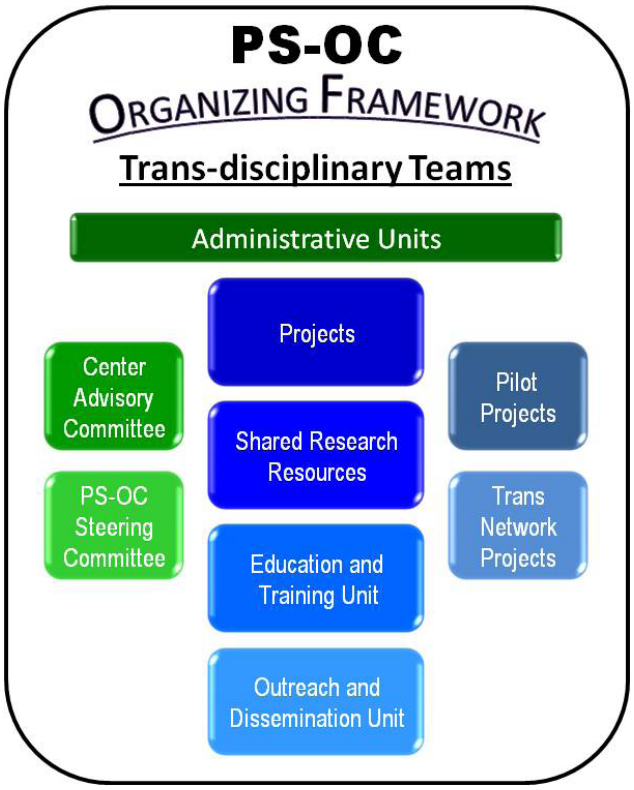


Figure 4. Center Organization. Each PS-OC is organized beginning with an overarching Framework designed to identify and address major questions in cancer research. Beneath the Framework sits an Administrative Unit that organizes and ensures progress of the Center as a whole. Within the Center there are a number of scientific Projects, Resource Cores, an Education and Training Unit, and Outreach and Dissemination Unit. Other features of the PS-OCs include both Center and Outreach Pilot Projects which allow the Centers to meet their changing needs by quickly adding additional projects.

Projects: Each PS-OC consists of between three and five major projects that combine one or more thematic areas described above and demonstrate, integrate, and support the overall PS-OC's overarching organizing Framework.

Shared Research Resources (Cores): These Cores may support and/or provide expertise to the PS-OC as either a physical or virtual infrastructure (i.e., fabrication and/or biological specimens, or computational physics modeling and/or mathematical theory development).

Administrative Units: The Administrative Unit (1) develops individual Center administration, including the Center Advisory Committee (CAC) and (2) participates in overall Network activities, including the PS-OC Steering Committee. The Unit will provide administrative support, coordinate Center activities, and assist the PI in interfacing with NCI Program Directors and Project Scientists.

- *Center Advisory Committee (CAC):* Each PS-OC is governed by a CAC consisting of four voting key Center personnel of whom one is the PI (with two investigators representing physical sciences and two representing cancer biology or clinical sciences), one voting NCI Project Scientist, and nonvoting external scientific advisors. The CAC acts to ensure Center scientific progress and to develop Pilot Project processes.

- *PS-OC Center Pilot Projects:* Each PS-OC includes approximately 5% of total Center direct costs to be allocated specifically for individual PS-OC Pilot Projects. Pilot Projects are solicited, evaluated, and awarded by the CAC.

Outreach and Dissemination Unit: The Unit will develop outreach programs (i.e., seminar series, workshops, and Web sites) to disseminate information to cancer biology and physical sciences communities about PS-OC capabilities, projects, and advances and develop mechanisms for exchanging personnel with investigators outside the PS-OC Network (min. $50,000/yr set-aside). The Unit also develops strategies to solicit Outreach Pilot Projects (min. $50,000/yr set-aside) to bring in expertise outside the individual PS-OC that will enhance specific PS-OC's efforts in its overarching Framework.

Education and Training Unit: The Unit will develop modules for integrative training of graduate students and postdocs that include programs to develop a knowledge base relevant to cancer biology and physical sciences (e.g., graduate programs, courses, seminars, and workshops) (min. $50,000/yr set-aside). The Unit will also develop and oversee mechanisms to share and exchange graduate and postdoctoral trainees and junior and senior investigators among participating PS-OCs (min. $50,000/yr set-aside).

Annual Site Visits: NCI program staff members conduct annual administrative site visits that are one full day in length. During the annual site visits all PS-OC Project/Core/Unit leaders are required to attend and present their scientific and programmatic progress (background, results, future directions, and red-flags/concerns). In addition, the site visits will provide the CAC the opportunity to meet face to face.

2.6 Scientific Accomplishments to Date

While the PS-OC Network has been in existence for less than 1 year, a considerable amount of progress has already been made. From publishing high-profile papers to establishing a Network-wide data-sharing policy, to generating dozens of proposals for Pilot and Trans-Network Projects, the Network is moving forward in integrating the approaches and techniques of the physical sciences with traditional oncology and cancer biology research.

2.6.1 PS-OC Project Scientific Advances

Despite the recent establishment of the PS-OC Program, rapid scientific progress has been made. This has included the publishing of several high-profile high-impact papers by members of the Network. Many of these papers have made significant advances in addressing the themes and questions of the PS-OC Network. To date 31 papers with an average impact factor of 10.6 have cited PS-OC Network funding. Some highlights include:

Restriction of Receptor Movement Alters Cellular Response: Physical Force Sensing by EphA2

Salaita K, Nair PM, Petit RS, Neve RM, Das D, Gray JW, Groves JT

Science 2010 Mar 12;327(5971):1380-5

A novel study was published in *Science* on March 12, 2010, by Jay Groves' laboratory, project leader in the University of California, Berkeley, PS-OC, demonstrating that the geometric arrangement and physical interactions of membrane-bound receptors can alter cell signaling processes, and that measurable changes in these biophysical variables correlate with tumor cell invasiveness. In this publication highlighting the physics theme of the PS-OC initiative, the researchers created an artificial membrane containing ephrin-A1, the natural ligand for the EphA2 receptor; this receptor is known to play a role in tumor invasiveness in many types of cancer. The investigators used this membrane to simulate the interaction of ephrin-A1 in one cell with EphA2 in an immediately adjacent cancer cell while being able to control the membrane geometric relationship between ephrin-A1 and EphA2. By observing how cell signaling changed as the geometric interactions of these two molecules were altered, the investigators showed that a correlation exists between spatial organization and the invasiveness of a given cancer cell line (figure 5).

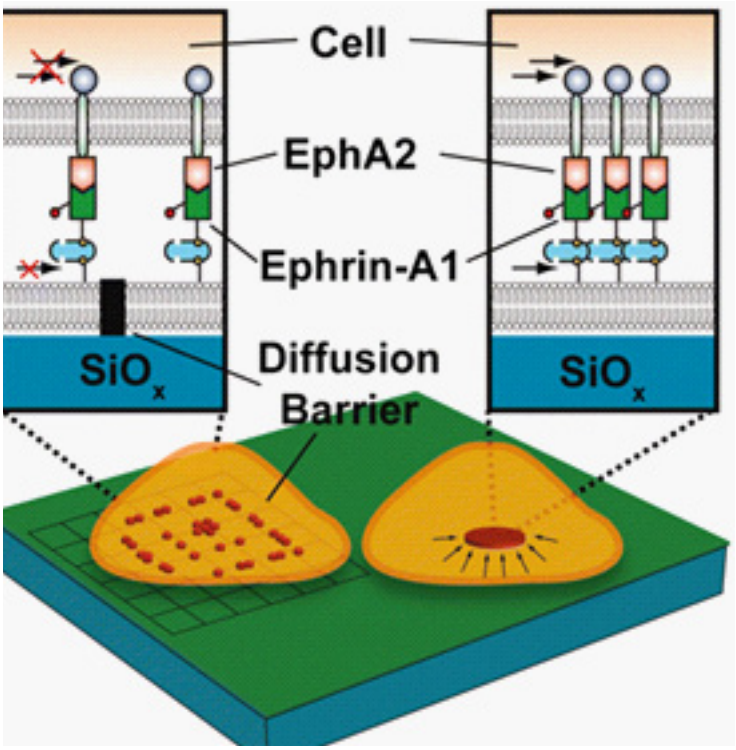


Figure 5. Scheme of the Experimental Platform Used to Trigger and Manipulate the EphA2 Receptor on the Surface of Living Cells. EphA2-expressing mammary epithelial cells are cultured onto a supported membrane displaying laterally mobile, fluorescently labeled ephrin-A1 ligand. Receptors engage ligands, form clusters that coalesce, and are transported to the center of the cell-supported membrane junction. Nanofabricated chromium metal lines 10 nm in height and 100 nm in line width (left cell) act as diffusion barriers and impede the transport of receptor-ligand complexes, leading to an accumulation of Eph-ephrin clusters at boundaries. [*Science* 327, 1380 (2010)]

A Distinctive Role for Focal Adhesion Proteins in Three-Dimensional Cell Motility

Fraley SI, Feng Y, Krishnamurthy R, Kim DH, Celedon A, Longmore GD, Wirtz D

Nat Cell Biol 2010 Jun;12(6):598-604

Also highlighting the physics theme of the PS-OC initiative, Denis Wirtz's laboratory has published its most recent findings in *Nature Cell Biology*. This publication illustrates the importance of studying cell motility in a physiologically relevant three-dimensional (3D) culture compared to traditional two-dimensional (2D) culture using human fibrosarcoma cells. Using sophisticated live-cell microscopy imaging techniques, the authors showed distinct localization patterns in 2D and 3D culture of a repertoire of focal adhesion proteins. Furthermore, they showed that focal adhesion proteins affect cell speed and persistence in distinct ways depending on the geometry of the matrix. For example, knock down of p130Cas, which results in increased cell speed in 2D, dramatically reduces cell speed in 3D. In addition, cell speed and persistence were shown to be regulated by the spatial geometry of the matrix, rather than solely the mechanical properties of the matrix. These compelling results provide insight into the molecular mechanisms of cancer cell migration and metastatsis (figure 6).

A Microfluidic Device for Continuous Cancer Cell Culture and Passage with Hydrodynamic Forces

Liu L, Loutherback K, Liao D, Yeater D, Lambert G, Estévez-Torres A, Sturm JC, Getzenberg RH, Austin RH

Lab Chip 2010 Jul 21;10(14):1807-13.

Robert Austin, Principal Investigator of the Princeton PS-OC, and his collaborators recently describe their new device in the journal *Lab on a Chip*. The team has developed a simple, inexpensive microfluidic device that not only provides a suitable environment for cancer cells to grow, but also can gently release specific numbers of cells on command for further study in other regions of the microfluidic chip. Moreover, a single device can continue functioning for at least a month, creating the opportunity to conduct studies on how the behavior and physical properties of cancer cells derived from the same tumor change over time. Their device on a glass slide uses the biocompatible polymer PDMS to create gas active valves that can apply a hydrodynamic shear force to the cells growing on a gelatin coating in the device's growth chamber. Unlike the microfluidic cell culturing devices developed by other investigators, this one does not require an enzymatic treatment to release the cells from the growth chamber. As a result, the device lasts longer (the enzymatic treatment destroys the gelatin layer essential for cell growth) and

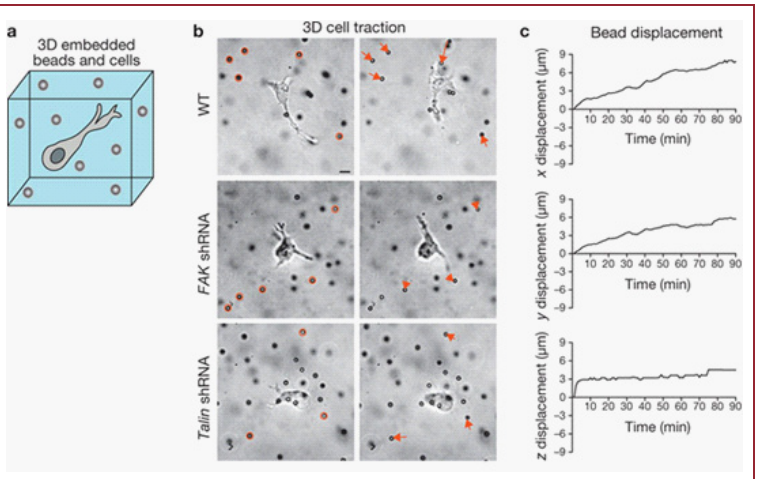


Figure 6. (a) Schematic of the method used for the measurements of local matrix traction mediated by embedded cells, whereby large fiduciary beads are tightly embedded in the matrix and are monitored by high-resolution 3D multiple-particle tracking. (b) Typical movements of fiduciary beads in the vicinity of a wild-type (WT) cell and cells depleted of talin and FAK, as indicated. Left and right micrographs show the initial and final positions, respectively, of the beads after 90 min. Arrows indicate the magnitude and direction of the displacements of the matrix-bound beads, which were magnified three times for ease of visualization. Scale bar, 10 μm. (c) Typical x, y, and z displacements of an individual matrix-bound bead in the vicinity of a WT cell in the 3D matrix. [*Nat Cell Biol* 12, 598 (2010)]

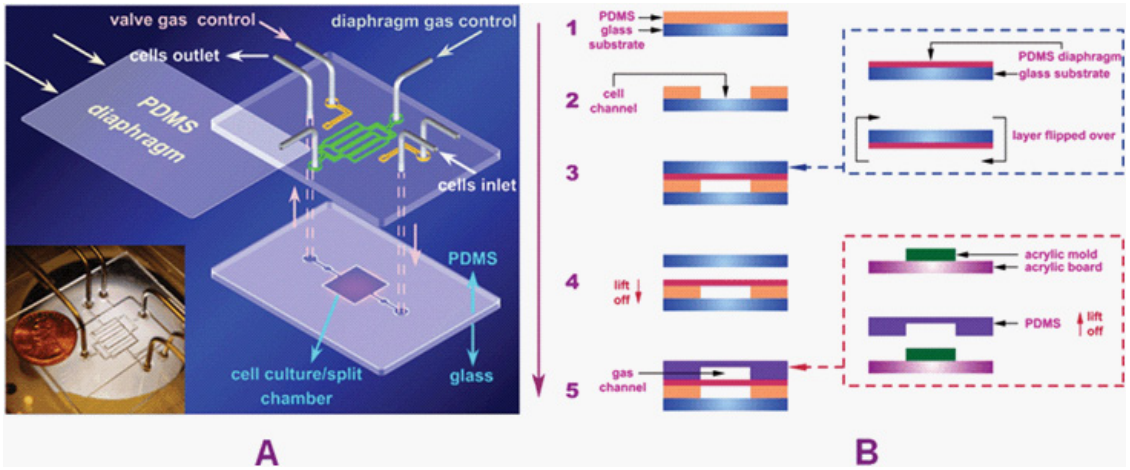


Figure 7. (A) Schematic illustrations of the 3D layered structure of the PDMS-based microfluidic chip for the continuous culture and passage of PC-3 cancer cells. The cell culture chamber with microfluidic channels in PDMS layer combined with glass substrate is located on the lower layer, while the air channels for cell passage controls are on the upper layer. A thin diaphragm is sandwiched between the upper and lower layers, by which air forces are transmitted to manipulate the passaging process. The left lower inset shows the chip with media and gas supply tubes linked, placed beside a 1-cent coin. (B) A process-flow diagram illustrates the fabrication procedure of the micro-device with multiple layers. [*Lab Chip 10*, 1807 (2010)]

requires less human intervention, reducing the likelihood that the device will become contaminated during its lifetime. A digital, programmable controller opens and closes the PDMS valves. These devices will provide the ideal setting for this PS-OC to investigate the evolutionary processes driving the development of drug resistance (figure 7).

2.6.2 Center Pilot Projects

Each PS-OC includes approximately 5% of total Center direct costs to be allocated specifically for individual Center Pilot Projects. These funds are used to develop new projects that advance the overarching Framework of the Center with the goal of transitioning successful projects to their own funding sources. While the funds are administered with input from NCI Project Scientists, each of the PS-OCs is free to implement Pilot Projects in a way that they deem to be most beneficial for their Center. This can range from (1) soliciting projects that, if successful, have the potential to replace underperforming or completed projects in a potential renewal proposal to (2) high-risk high-reward solicitations that are looking for projects that, if successful, have the potential to make major advances in cancer research. Highlights of funded Pilot Projects include:

Role of dc Electric Fields in the Motility of Cancer Cells
Johns Hopkins University PS-OC Pilot Project Solicitation
Peter C. Searson and Denis Wirtz, Johns Hopkins University

The goal of this project is to determine how dc electric fields (dcEFs) influence the mechanics of cancer cells with a long-term goal of elucidating the role of dcEFs in the metastatic process. This will be accomplished by using microfabrication techniques to quantitatively assess the global response of normal and cancer cells to an exogenous dc electric field. Immunofluorescence microscopy and real-time live cell imaging will then be used to quantitatively determine the influence of dc electric fields on key regulators of cell motility.

Microribonucleic Acids in the Physical Properties of Cancer Cells
Johns Hopkins University PS-OC Pilot Project Solicitation
Yiider Tseng, University of Florida, and Konstantinos Konstantopoulos, Johns Hopkins University

This pilot project will examine the biophysical effects of miRNA dysregulation as it pertains to tumorigenesis and metastasis. The investigators will measure the effects of exogenous miR-10b, which has been linked to metastasis, on cellular characteristics including proliferation, adhesion/detachment, migration, resistance to shear stress, and cell mechanics.

A Quantitative Description of MicroRNA-Transcriptome Interactions
Northwestern University PS-OC Pilot Project Solicitation
Richard W. Carthew, Northwestern University, and Sascha Hilgenfeldt, University of Illinois, Urbana-Champaign

This pilot project seeks to understand the rules describing microRNA interactions with the transcriptome. The investigators will first experimentally determine genome-wide maps of physical association between transcript mRNAs and the miRNAs miR-7 and miR-9 and determine expression levels of transcript mRNAs and miRNAs. These results will be used in modeling to identify miR-7 and miR-9 binding sites and then determine what parameters are important for association of the sites with miRNAs.

2.6.3 Education and Training Accomplishments

Each PS-OC includes approximately $100,000 per year specifically to develop training mechanisms and exchange trainees across the Network. Several PS-OCs have used these funds to run seminar series and journal clubs, hold workshops to introduce physical scientists to cancer biology research (and vice versa), and to fund summer students. Some highlights include:

- The ASU PS-OC in conjunction with Agilent held a 2-day hands-on workshop for 10 participants to learn techniques and applications of atomic force microscopy.

- The MIT PS-OC held a 2-day hands-on workshop for five participants to learn about application of the single-molecule FISH protocol to both cell culture and tissue samples (figure 8). Single-molecule FISH is an application of RNA FISH utilizing several short oligo probes, which allows for counting absolute numbers of transcripts on a single-cell basis (even in the context of tissues).

2.6.4 Outreach and Dissemination Accomplishments

Each PS-OC includes approximately $50,000 per year specifically for Outreach Pilot Projects. These funds are designed to allow PS-OCs to rapidly add expertise from outside the PS-OC Network to help address their Center Framework. Several PS-OCs have utilized these Pilot Project funds to recruit new investigators to their Centers and cancer research in general.

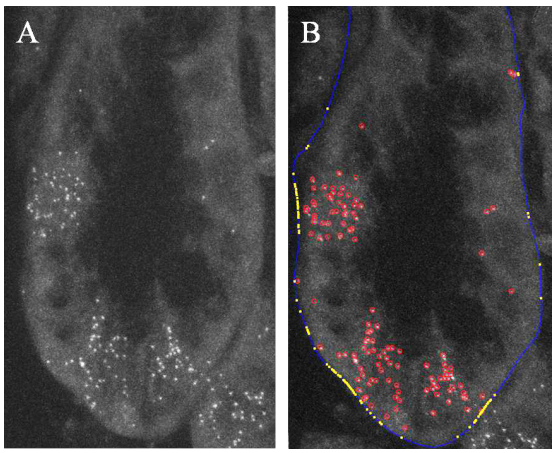


Figure 8. Lgr5 Transcript Counting in Intestinal Stem Cells by Single Molecule FISH. Raw (A) and processed (B) images for single molecule FISH to detect Lgr5 transcripts in intestinal crypts. The high signal-to-noise ratio achieved by using 40-50 small oligo FISH probes allows the detection and counting of individual transcripts in individual cells. The single molecule FISH technique is applicable to both cells and tissue samples and can detect cell-to-cell differences in transcript levels.

- The USC PS-OC has awarded Dr. Milind Tambe, a Professor of Computer Science at the University of Southern California, and Dr. Veronica Eliasson from the Department of Aerospace and Mechanical Engineering at the University of Southern California for their innovative proposals to examine cancer as a dynamic stochastic graphical game and wave propagation effects on small objects, respectively.
- Marilyn M. Bui and Mark Lloyd from the Analytic Microscopy Core at H. Lee Moffitt have provided their expertise to the MCC PS-OC via the Outreach Pilot Project mechanism.
- Terence Hwa, Professor of Physics and Biology from the University of California, San Diego, has joined the PU PS-OC to develop a quantitative model of drug resistance evolution.

2.6.5 Physical Characterization of Cell Lines Pilot Study

The Cell Line Pilot Study was initiated in November 2009 by the PS-OC Steering Committee to test the feasibility of providing a standardized "benchmark" protocol to be used by participating laboratories for showcasing the diverse physical science technologies across the PS-OC Network. Center PIs and other Network physical scientists provided input into the specific types of technologies that could be applied to cell lines and would showcase the capabilities of the Network while Center SIs, and other cancer biologists, provided insight into specific cell lines that could be used for this pilot project. The PS-OC Steering Committee reached a majority consensus that two human mammary cell lines would be used for the studies: MCF-10A and MDA-MB-231. These two cell lines could be used for comparative studies of "pre-malignant" versus "malignant" signature. While it was realized that no cell line would fill all criteria for being representative of any given cancer, the purpose of the chosen lines was not to represent the disease per se but to have commonly cultured standards for pilot experiments to showcase the various new technologies being developed or utilized by the multiple Centers.

While it may seem paradoxical that members of the PS-OC Network have been asked to blaze novel avenues of research using two cancer cell lines that have been in common use for several decades, it actually makes sense for these selected pilot experiments. The notion is that every group needs a common language in order to communicate, and applying the multitude of different technologies to one or two common cell lines in a pilot study has helped to initiate this process within the PS-OC Network. In collaboration with a lead PS-OC investigator, Dr. Thea Tlsty, a

common in vitro culture protocol was developed for the cell lines. Dr. Tlsty provided a single lot number of each cell line at the same passage to each PS-OC along with a starter package of cell culture medium and serum so that as many technical variables as possible could be removed when participants discuss and integrate their results. The cell lines were distributed to the PS-OCs in February 2010, only 4 months after the program launch. All 12 PS-OCs have participated in the Cell Line Pilot Study, generating data from an array of technologies across a range of length-scales. Investigators from nine PS-OCs presented preliminary results at the first annual Network Investigator's Meeting in April 2010, only 2 months after initial receipt of the cell lines. A followup meeting was held in June 2010 where significant progress was presented by several of the participating PS-OCs.

To facilitate future Trans-Network collaborations, a PS-OC Network Bioresource Core Facility (PBCF) is being established. The PBCF will be a centralized resource of biological specimens for program investigators, and it shall function to increase the time and cost efficiency of the transfer of biological specimens to PS-OC Network investigators. Specifically, the PBCF shall be a centralized biodistributor and biorepository that serves to provide PS-OC Network investigators with common stocks of authenticated cell lines and primary cells (non-malignant and cancerous), cell culture reagents, and related standard operating protocols upon request. In collaboration with NCI's Office of Biorepositories and Biospecimen Research (OBBR) and caHUB, the PBCF services will eventually expand to be a biorepository of human biological specimens, acquiring and authenticating tissues from PS-OC Network investigators in accordance with the NCI Best Practices for Biospecimen Resources guidelines. The PBCF shall also have the capability to prepare and distribute extracts of RNA, DNA, or protein from human cell lines, primary cells, and tissues. Moreover, any modified cell lines could be deposited by PS-OC Network investigators to the PBCF for authentication and distribution to collaborators within the PS-OC Network.

2.6.6 Trans-Network Projects

A critical and unique component for realizing the full potential of the Physical Sciences-Oncology Center (PS-OC) program is the availability of funds for Trans-Network projects. Each of the appropriated Centers includes funds in the amount of a minimum of $100,000 in direct costs to be allocated specifically for Trans-Network projects. These funds provide each Center an opportunity to catalyze new perspectives and test unique research ideas through the development of robust collaborations within the PS-OC Network. Trans-Network Projects are meant to (1) help generate answers to a major question or barrier in cancer research through *unconventional approaches* and (2) enhance the PS-OC Network interaction through establishing and developing new collaborations.

PS-OC Young Investigators Trans-Network Projects

Another important component of the Trans-Network Program is the establishment of a Network environment in which young investigators are encouraged to seek out and embrace different perspectives and execute multidisciplinary research. To this aim, a small portion of the total Trans-Network funds was utilized by the PS-OC Steering Committee to solicit proposals from young investigators (graduate students and postdocs) and to award a small number of applications at the annual PS-OC Network Investigators' Meeting.

The Young Investigators program required participants to establish a collaboration, write a proposal, and present to the Steering Committee all approximately within a 24-hour period. Eleven Young Investigator Trans-Network Proposals were received and the Steering Committee expressed enthusiasm about the level of projects planned and presented by young investigators in such a short period of time. Based on the level of proposals and the distribution of scores, the Steering Committee voted to fund six $10,000 projects, two of which are described below:

Probing Transcriptional Response as a Function of Spatial Organization of Signaling
University of California, Berkeley PS-OC and Massachusetts Institute of Technology PS-OC

This project aims to examine the downstream transcriptional effects of modulating receptor organization by physical forces. The project combines the MIT PS-OC's expertise in quantifying gene expression on a single-cell level using RNA FISH with the UCB PS-OC's expertise in modulating mechanical forces on single cells and tissues. The single-molecule

FISH approach will be used to quantify transcript counts of key downstream genes following perturbation of EphA2 organization and as a function of location in 3D tissue structures.

Identification and Characterization of Circulating Tumor Cells by Partial Wave Spectroscopy
The Scripps Research Institute PS-OC and Northwestern University PS-OC
This project is utilizing the partial wave spectroscopy (PWS) technique employed at the Northwestern PS-OC to isolate circulating tumor cells (CTCs) from patient samples. PWS is an innovative optical spectroscopy tool capable of measuring cellular disorder, a unique quantitative measure of mass density fluctuations in cells not always detectable by standard cytopathology methods. The goal is to develop an unconventional *label-free*, physical sciences-based approach to enable the reliable high-throughput detection and isolation of CTCs for further characterization.

Trans-Network Projects – Round 1, July-August 2010

Following the success of the Young Investigators Trans-Network Program, the Network has initiated the first round of large-scale Trans-Network Projects. A total of $600,000 is available to fund two to three projects that address the aims of the Trans-Network Program, focus on major problems in cancer, and enhance the developing Network of researchers at the interface of physical sciences and oncology.

Investigators were asked to develop their proposals in an open collaborative environment. Proposals were posted and edited on the PS-OC Intranet Wiki pages. Following a 3-week proposal generation period, investigators were asked to comment on the proposals submitted by other investigators. The commenting period generated 16 comments from five different investigators, with all proposals receiving at least one comment. Following the commenting period, investigators had 1 week to revise their proposal and address comments. Final proposal development and evaluation will occur at a meeting August 1-2 where investigators will receive another round of comments from their colleagues following a presentation of their proposal and then be evaluated by an external panel on the basis of the final proposal and presentation. Nine proposal teams will participate in the August meeting, two of which are described below:

Development of Models of Penetration of Resistance
University of Southern California PS-OC, Memorial Sloan-Kettering Cancer Center PS-OC, and Princeton University PS-OC
This proposal aims to examine the penetration of drug resistance by following the dynamics of drug-sensitive and drug-resistant cancer cells during growth and in response to therapeutic intervention using an integrated computational and experimental approach. The USC and Princeton teams will collaborate to examine population dynamics in cells already resistant to drugs in both 2D and 3D environments. The MSKCC team will develop a new mathematical Framework to study the dynamics of the penetrance of resistance during diverse selection pressures (e.g., targeted drug treatments, pan-target treatments, chemotherapeutics, etc.).

Role of Cellular Microrheology in the Metastatic Adhesion of Circulating Tumor Cells
University of Southern California PS-OC, Cornell University PS-OC, Arizona State University PS-OC, and Johns Hopkins University PS-OC
The investigators on this application propose to evaluate the role played by cytoskeletal mechanics in the metastatic potential of circulating tumor cells. The investigators will merge the microrheology approaches of the JHU PS-OC with the micro-scale flow approaches of the Cornell PS-OC to track cells prior to flow, in flow, upon adhesion, and following pseudopodia projection. The ASU, USC, and Cornell PS-OCs will collaborate on using the data to generate a multiscale model of circulating tumor cell convection, adhesion, and extravasation. Finally the USC and JHU PS-OCs will use intravital microscopy techniques to transition the studies to animal models.

2.6.7 Data Sharing

Scientific research depends on the free flow of information and ideas. The PS-OC Network is committed to establishing both an environment that promotes sharing of data and a mechanism to disseminate data both within the Network and to the broader scientific community.

Network Data Sharing Agreement

The PS-OC Network is unique in the manner in which it collaborates with broad cross-sections from both the cancer biology/oncology and physical sciences/engineering communities. With each PS-OC actively engaged in data generation, characterization, and analysis, the integration of these datasets will accelerate orthogonal exploration by all PS-OC investigators to help generate answers to some of the major questions and barriers in cancer research and support the development of clinical advances.

The PS-OC Steering Committee established, and all 12 of the PS-OCs have signed, a Data Sharing Policy to achieve two high-level goals:

- Facilitate collaboration between PS-OCs for purposes of achieving the goals of the program and establishing a robust PS-OC Network.
- Disseminate PS-OC results in a format that can be utilized efficiently and harmoniously by PS-OC investigators, and, after public dissemination, to the broader research community.

Pilot Data Sharing Center

The PS-OC Network will eventually establish a full-fledged Data Coordinating Center (DCC). However, the diversity of the measurements generated (and the corresponding diversity in length-scale) by the Network makes using existing DCC models problematic. In order to begin examining the DCC needs of the PS-OC Network, Carl Kesselman and the USC PS-OC have established a Pilot Data Sharing Center that is being used to house the data from the Cell Line Pilot Study previously described. The Pilot Data Sharing Center has existed for just over 1 month, but already 20 datasets have been uploaded to the database.

PS-OC Intranet Site

The development of a secure intranet site was mandated by the PS-OC Steering Committee in October 2009 and launched approximately 2 months later in late December 2009. The site launched with 40 initial users and now has more than 160 registered users. To date, the site has been used as a calendar to post dates and times of PS-OC meetings and events, announce conferences and symposia being presented by only PS-OC members, and host the agendas, slides, and minutes from the PS-OC Steering Committee meetings. Additionally, the intranet site has played a critical role in (1) the PS-OC Cell Line Pilot Study, hosting data, presentations, and manuscript drafts, and (2) the Trans-Network Projects program, where it hosts proposals in an open Wiki environment that enables collaborative proposal generation and commenting by other investigators.

2.6.8 Program Evaluation

Establishing a Baseline

In order to establish a baseline state of the Physical-Sciences Oncology field, to which we can compare the field after the existence of the PS-OC Network, we worked with the Science and Technology Policy Institute to interview individuals from the research community and program staff. Interviews will continue to be performed as a metric to monitor the progress of the program and its impact on the broader field. To date the following interviews have been conducted:

- Four extramural researchers who were members of the SEP and/or attended the think tank workshops held during the planning process for PS-OC;
- Two directors of comprehensive cancer Centers or their designated representatives;

- One NIH program staff member who has managed and evaluated Centers programs designed to support cross-disciplinary work;
- Nine PS-OC principal and senior investigator pairs shortly after the launch of the program.

Prospective Evaluation of the PS-OC Program

The PS-OC Program has submitted an application for NIH Evaluation Set-Aside funds to perform a prospective evaluation of the program. The PS-OC Process/Outcome Evaluation will assess the extent to which the PS-OC Program has been successful in reaching the goals stated above. The PS-OC Program is the only program or initiative at NCI dedicated exclusively to building trans-disciplinary teams and infrastructure to better understand and control cancer through the convergence of physical sciences and cancer biology. This is a new field, and there is no precedent on evaluations. Thus, we are putting infrastructure together for this by spending our own funds to develop a database comprising data from the semi-annual progress reports to facilitate future evaluations. We are also planning to do an assessment of the program on an ongoing basis. Determining whether and how PS-OC funding builds infrastructure and sustains trans-disciplinary science at awarded institutions will aid program managers in identifying and maintaining the most successful components of the program while adjusting or removing other components which are not effective.

The recommended evaluation component is prospective data collection on activities and key outputs/outcomes. To help facilitate the collection of data, we have developed an Extended Scientific Reporting form that Network investigators use for the NIH-annual and NCI-semi-annual progress reports. Aside from the traditional descriptions of scientific progress, investigators are asked to identify collaborations within and outside the Network, indicate red flags, and identify the Center's most novel finding for the reporting period. Collecting data on program activities and outputs prospectively serves several purposes: (1) activities and outputs can be monitored by program managers so that changes can be made as needed and (2) any errors or inadequacies that are detected in the data can be addressed sooner rather than later. These advantages must be balanced against the inefficiency of collecting and analyzing information as it becomes available relative to a single retrospective data collection effort. For this reason, prospective data collection for the PS-OC activities and outputs/outcomes for which data are most readily available is recommended.

2.7 Goals and Plans for the Remaining Funding Period

As the PS-OC Program was launched at the end of September 2009, and the goals and plans for the remaining funding period are correspondingly similarly to those outlined in the previous sections. Adjustments, if any, to these goals will be reflected in the analysis and feedback obtained through the progress reports, annual site visits, and annual PS-OC Network Investigators' Meeting, as well as from the PS-OC Steering Committee.

3. Future Vision for OPSO

Within the next 5 years, OPSO – through such activities as the PS-OC Program – brought not only "a physicist" to the organization; *OPSO will have successfully brought the physics to the problems of (cancer) biology*. By establishing trans-disciplinary teams in an integrated Network and actively facilitating strong interactions between investigators, the PS-OC program will have catalyzed new fields of studies in cancer research through physical sciences/engineering approaches and principles; nurtured the use of different perspectives to generate paradigm-shifting science; and significantly accelerated the scientific progress to better understand and control cancer at all length-scales through the convergence of physical sciences and cancer biology. Consequently, physical science and engineering approaches/principles will start to be second nature in cancer biology research.

Physical parameters (e.g., mass, density, energy dynamics, force, viscoelasticity, Young's modulus, etc.) are routinely being measured by the PS-OC Network. These physical measurements together with new and existing genomic and proteomic datasets are integrated into novel computational physics and evolutionary models to provide a more complete understanding of cancer initiation and progression. This clearer understanding of cancer is starting to answer some of the big questions in cancer with implications toward improving diagnosis, treatment, and prevention of the disease.

In addition to the PS-OC Program bringing several tens of "new" physical scientists and engineers to cancer biology, OPSO continues to reach out and invoke the physical sciences community to look at cancer with a different perspective through various mechanisms and inter-agency activities.

"NEWTON" – NEW Transdisciplinary Teams in ONcology

The Newton concept is designed to allow a venue for individual investigator-driven applications to pursue research which combines physical sciences with cancer biology. The overwhelming number of applications for the Physical Sciences in Oncology Center (RFA-CA-09-009) and Challenge Grant (Physical Sciences and Cellular Mechanics, 06-CA-116) provides a strong indication that merging physical sciences with oncology is stimulating interest in both of these communities. At the various Physical Sciences in Oncology workshops held in 2008 and several physical sciences conferences, a common theme by investigators has been the difficulties on entry into the NIH granting system. In particular, two categories of investigators ("traditional" physical scientists and junior investigators) have had the most difficulty in receiving an NCI research project grant (i.e., R01). As a way to introduce these groups to NCI, an investigator-based stepwise grants approach is proposed. There are several advantages to this approach:

- Physical scientists/junior investigators have an entry into the NCI culture while reducing risk to NCI by awarding smaller R03 awards ($50K/year direct cost).
- "NEWTON" investigators will also have a venue to seek higher level of funding through either an R21 or R01 mechanism. The two mechanisms will naturally solicit applications with stronger connectivity of physical sciences-based approaches toward oncology.
- The number of awards will be tapered in proportion to the funding amount. In other words, more smaller grants to test concepts and fewer mature concepts for R01 awards.
- All awardees (R03, R21, and R01) will have immediate entry into the PS-OC Network and can leverage the Centers Shared Research Resources to advance their program.
- In addition, "blue sky" projects can also be pursued which have the potential to bring paradigm-shifting ideas with minimal risk.

NCI/NSF "PLIER" – Physical/LIfe Sciences Early-Stage Research Awards Program

A collaborative interagency partnership between NCI and the National Science Foundation (NSF) is based on the premise by both agencies that significant advances may be expected as the result of continued investments in inter- and multidisciplinary research at the intersection of the engineering/physical sciences and the life sciences, with a focus on advancing the fundamental understanding of cancer biology to underpin translational research that promotes the prevention, detection, and treatment of cancer diseases. PLIER will serve to promote the exciting trans-disciplinary research being cultivated within the PS-OC Program by seeding new innovative, individual investigator-led pilot projects using NSF/NCI funds. Under the partnership, NSF will dedicate and match NCI funds, specifically for this purpose. Moreover, the goal of the partnership fits ideally with the NCI OPSO mission to support innovative concepts from potential "new" NCI grantees. The joint NSF/NCI plan would be to fund investigators for 3 years at $100K/year.

In summary, OPSO is now bringing "the physics" to the problems of cancer biology; and looking beyond the next 5 to 10-year period, *OPSO will promote a physics perspective to the problems of clinical oncology.*

Appendix 1: Current Staffing

Larry A. Nagahara, Ph.D., Program Director, OPSO

Dr. Nagahara is the Program Director for the Physical Sciences-Oncology Centers (PS-OC) Program. Previously, he served as the Nanotechnology Projects Manager for the NCI's Alliance for Nanotechnology in Cancer program. Dr. Nagahara has been actively involved implementing physical sciences approaches and nanotechnology for 20 years, most notably in novel scanning probe microscopy development, carbon nanotube applications, molecular electronics, nanoenergy, and nanosensors. Prior to joining NCI, he was a Distinguished Member of the Technical Staff at Motorola and led its nanosensor effort. Dr. Nagahara currently represents NCI on the Trans-NIH Nano Task Force, as well as NCI's Project Scientist for the NIH's Nanomedicine Development Centers and NIH's Genes and Environment Initiative (GEI), Exposure Biology Program. Dr. Nagahara also currently serves as adjunct professor at Arizona State University, where he earned his Ph.D. degree in physics. He was a postdoctorate fellow at the University of Tokyo, Japan, later joined the Faculty of Engineering as an assistant professor, and then spent 12 years at Motorola Labs before joining NCI in 2007.

Anna Maria Calcagno, Ph.D., PS-OC Project Manager, OPSO

Dr. Calcagno serves as a Project Manager for the PS-OC Program, and in this role, she assists in the oversight and scientific management of PS-OC projects by encouraging interdisciplinary collaborations of investigators and researchers within the PS-OC Network. Dr. Calcagno oversees and manages the PS-OCs at Moffitt Cancer Center, Princeton University, University of Southern California, and The University of Texas Health Science Center. Prior to joining the PS-OC program, Dr. Calcagno was a senior research fellow in the Laboratory of Cell Biology, Center for Cancer Research, NCI. Her work evaluated how treatment regimens regulate the function and expression of ABC drug transporters and how the overexpression of these drug pumps can be inhibited in tumor cells to prevent multidrug resistance. In addition, she was also awarded the NIGMS Pharmacology Research Associate (PRAT) Program Fellowship from 2005 to 2008 as well as a 2007 AACR-WICR Brigid G. Leventhal Scholar Award. She initiated and co-chairs the PS-OC Evolution of Drug Resistance Working Group, which fosters collaborations between members of the PS-OC Network working in the area of drug resistance. Dr. Calcagno received her bachelor of science degree in pharmacy from West Virginia University and a master of science degree in pharmaceutics from the University of Michigan. Her doctoral studies were completed at the University of Kansas in pharmaceutical chemistry.

Sean E. Hanlon, Ph.D., PS-OC Project Manager, OPSO

Dr. Hanlon serves as a Project Manager for the PS-OC Program, assisting in the oversight and scientific management of PS-OC projects and encouraging interdisciplinary collaborations of investigators and researchers within the Network. As a Project Manager, Dr. Hanlon is specifically responsible for oversight of the PS-OCs at Memorial Sloan-Kettering Cancer Center, Massachusetts Institute of Technology, Northwestern University, and The Scripps Research Institute. Dr. Hanlon organized and co-chairs both the Steering Committee Operations Subgroup, which acts as a liaison between the Steering Committee and individual Centers, and the Education & Training Working Group, which helps coordinate education programs across the Network. He also manages and implements both the PS-OC Trans-Network programs and the Network data-sharing program. Prior to joining the NCI as an AAAS Science & Technology Policy Fellow, Dr. Hanlon was a postdoctoral fellow at the Carolina Center for Genome Sciences at the University of North Carolina at Chapel Hill. His postdoctoral work used genomics and bioinformatics approaches to address problems in transcriptional regulation on a genome-wide scale. This work helped further the understanding of how cells and organisms ensure that each gene in the transcriptome is expressed and repressed only at the appropriate time. Dr. Hanlon received his Ph.D. degree in molecular biology and biochemistry from Rutgers University in 2003, where his work focused on understanding how chromatin structure influences transcription and cell-cycle progression.

Nastaran Zahir Kuhn, Ph.D., PS-OC Project Manager, OPSO

As a Project Manager for the PS-OC program, Dr. Kuhn oversees and manages PS-OC scientific research projects by evaluating scientific progress and encouraging interdisciplinary collaborations among PS-OC Network investigators. She is responsible for managing the Arizona State University, Cornell University, Johns Hopkins University, and University of California, Berkeley PS-OCs. Dr. Kuhn established and co-chairs the PS-OC Physics Working Group, which seeks to create standardized metrics and protocols for biophysical measurements across the PS-OC Network. Additionally, Dr. Kuhn manages the PS-OC Cell Line Pilot Study, in which two common cell lines have been utilized to showcase the physical science technologies of the PS-OCs. Dr. Kuhn's scientific expertise lies in using an engineering approach to study microenvironment regulation of breast cancer and stem cell fate. Dr. Kuhn received her Ph.D. degree in bioengineering from the University of Pennsylvania, where she studied the effects of aberrant mechanical cues from the extracellular matrix on mammary epithelial cell morphogenesis and therapeutic response. Dr. Kuhn completed her postdoctoral fellowship at the Cartilage Biology and Orthopaedics Branch, NIAMS, NIH, where she investigated the regulation of bone marrow-derived mesenchymal stem cell differentiation by the extracellular matrix microenvironment.

Nicole M. Moore, Sc.D., PS-OC Project Manager, OPSO

Beginning in September 2010, Dr. Moore will serve as a Project Manager for the PS-OC Program, where she will manage and evaluate scientific progress of individual Centers within the PS-OC Network and more broadly act to encourage interdisciplinary collaborations among PS-OC Network investigators. Prior to joining the Office of Physical Sciences-Oncology, Dr. Moore was a research chemist in the Biomaterials Group at the National Institute of Standards and Technology (NIST). Her research efforts focused on developing click chemistry gradient substrates for high-throughput measurement of cell response to functionalized materials. This work highlighted key concentrations of immobilized biomolecules that direct osteogenic differentiation and induce inflammation promoting rational design of biomaterials. While at NIST, Dr. Moore was awarded an exploratory research grant to develop new technology for measuring intracellular trafficking of nanoparticles and the Material Science and Engineering Laboratory Work-Life and Diversity Award. Dr. Moore received her doctorate in chemical engineering from Washington University in St. Louis, where she systematically explored the effect of peptides on the intracellular trafficking of nanoparticles culminating in the development of a non-toxic and efficient multifunctional polyethylene glycol vehicle for gene therapy. Upon completion of her dissertation, she was awarded a National Research Council Postdoctoral fellowship at NIST in the Biomaterials Group. Dr. Moore earned her B.S. degree in biomolecular and chemical engineering from the University of Notre Dame.

Office of Cancer Nanotechnology Research (OCNR)

1. Mission

Nanotechnology involves research and technology development at the atomic, molecular, or macromolecular levels, on the length scale of approximately 1-100 nanometer range, and allows the creation and use of functionalized structures, devices, and systems that take advantage of specific properties of matter that exist at the nanoscale. Nanoscale structures can be manipulated on the atomic scale and integrated into larger material components, systems, and architectures. Nanotechnology-based structures and devices are already enabling a large number of novel applications in various fields – including medicine.

The Office of Cancer Nanotechnology Research (OCNR) at the National Cancer Institute (NCI) develops and implements programs with and for the extramural research community. The overarching goal of these initiatives is to discover and develop innovative nanotechnologies for application(s) ranging from discovery through translation and delivery of innovative clinically relevant technologies for cancer prevention, diagnosis, and treatment. These initiatives include programmatic efforts known collectively as the NCI Alliance for Nanotechnology in Cancer (ANC).

2. Program Background and History

Novel, nanotechnology-enabled properties have been creatively utilized in biomedical devices and tools. Applications in oncology can leverage nanomaterials and nanodevices to tackle difficult problems in prevention, diagnostics, and drug delivery. The NCI recognized this opportunity early and, in the late 1990s, established the Unconventional Innovative Program (UIP) to work with university groups and small companies to evaluate potential nanotechnology applications in cancer. Building upon the solid experience of the UIP program, NCI established the ANC program in September 2004.

The ANC initiative is funded through a set of RFAs (Requests for Applications); the first set was issued in 2004 and the second set in 2009. All awards are funded for a period of 5 years. The strategic areas of scientific focus for requested proposals are established based on the input of the extramural community.

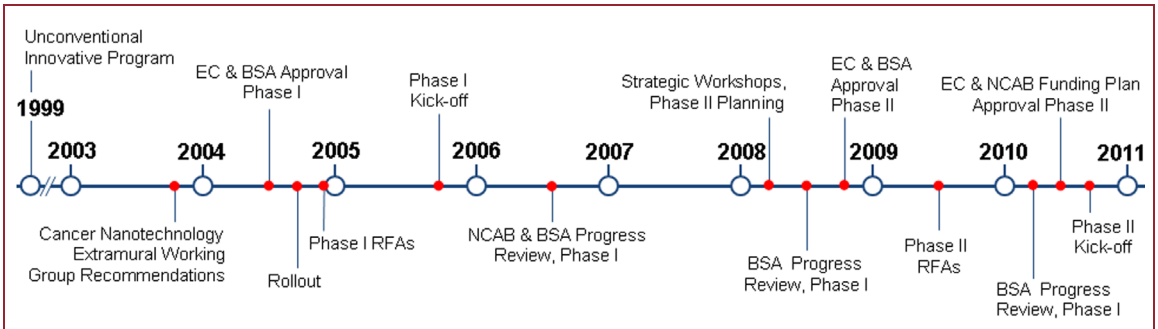


Figure 1. Timeline for the Development and Execution of the ANC Program.

The progress of the program is monitored by the NCI Program Office, independently reviewed by the Board of Scientific Advisors (BSA) and the National Cancer Advisory Board (NCAB), and evaluated by an independent contractor (Science and Technology Policy Institute). Figure 1 depicts a timeline describing approval and evaluation steps in the program. Phase I of the program is being completed this year. The reissuance of the initiative (new awards made in 2010) was initially approved by the Executive Committee (EC) of the institute in September 2008. Final approval was granted by the BSA at NCI in November 2008. The proposals in response to the RFAs of Phase II were received in October and December 2009, and reviewed over the period of February to March 2010.

3. Program

3.1 Structure – Phase I

The Phase I funding period (2005-2010) involved funding (figure 2) a constellation of 8 Centers for Cancer Nanotechnology Excellence (CCNEs) and 12 Cancer Nanotechnology Platform Partnerships (CNPPs), together with Multidisciplinary Research Training and Team Development awards (11 awardees) and the Nanotechnology Characterization Laboratory (NCL). CCNE teams are focused on developing integrated nanotechnology solutions with future potential for clinical applications. The CCNEs have evolved into research organisms having distinct area(s) of technical excellence and core resources (e.g., fabrication and materials development, diagnostic assays, toxicology, drug delivery, in vivo technology validation, informatics). The CNPPs are smaller R01 projects. The CCNEs provide infrastructure and translational support to the CNPPs where appropriate. The Multidisciplinary Research Training and Team Development program is dedicated to training graduate students and postdoctoral fellows. NCI also formed an intramural laboratory, the Nanotechnology Characterization Laboratory (NCL), to serve as a centralized facility to characterize nanomaterials. NCL is a formal collaboration with the National Institute of Standards and Technology (NIST) and U.S. Food and Drug Administration (FDA). NCL's role in the ANC is to perform standardized characterizations of nanoscale materials developed by researchers from academia, government, and industry.

The Alliance is *governed* by the Coordinating and Governance Committee (CGC). CGC membership includes at least one member from each CCNE, the NCI Program Director, and a public advocacy group representative. The CGC identifies new research opportunities, establishes priorities, and considers policy recommendations. The program office maintains close contact with its funded investigators and serves as a hub for exchanging information and establishing collaborations, by connecting researchers with synergistic capabilities. In addition, each CCNE has its own *Steering Committee* (in some cases scientists from one CCNE serve on the steering committee of another); a few of the CCNEs have also formed *Industrial Advisory Committees* that provide help in developing commercialization strategies. The *Principal Investigator* (PI) meeting, held annually in autumn, is the main venue for ANC investigators to meet in person and exchange their ideas and experiences as well as to develop collaborations.

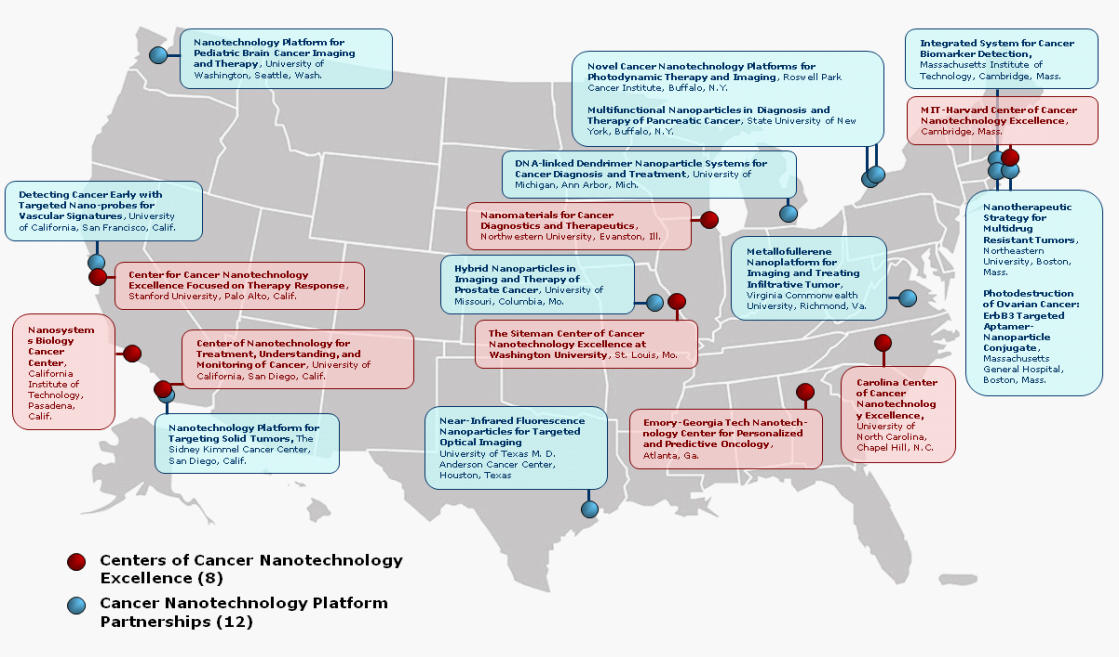


Figure 2. Centers of Cancer Nanotechnology Excellence (CCNEs) and Cancer Nanotechnology Platform (CNPPs) Awards in Phase I of the ANC Program. The detailed list of participating investigators and institutions is given in the Appendix.

The Program Office also works to develop further and support cancer nanotechnology as a research field. The office also organizes or co-organizes symposia, conference sessions, and conferences at the crossroads of nanotechnology, cancer biology, and oncology. Among others, it is currently organizing the 1st International Gordon Conference on Cancer Nanotechnology to be held in July 2011 at Colby College in Maine.

3.2 Strategy

The ANC program was designed to develop research capabilities for multidisciplinary team research, with the goal of advancing prevention, diagnostic, and/or treatment efforts from the research discovery to preclinical and early clinical development stages. The ANC's development model calls for the most promising strategies discovered and developed by ANC grantees to be handed off to for-profit partners for effective clinical translation and commercial development. In its first round, the ANC focused on basic research and developmental efforts in the following six major challenge areas:

- Molecular Imaging and Early Detection
- In Vivo Nanotechnology Imaging Systems
- Reporters of Efficacy
- Multifunctional Therapeutics
- Prevention and Control
- Research Enablers

The details of these challenge areas were outlined in the Cancer Nanotechnology Plan, published in 2004. The ANC program continues to work on strategy updates through consultation with the extramural community. Recently (2008), three strategic workshops were held (see discussion in section 4.1.), and currently, there is work on a new Cancer Nanotechnology Plan for Phase II.

3.3. Program Accomplishments

3.3.1. Extramural Scientific and Clinical Achievement

Chemotherapies. Polymeric nanoparticles for targeted delivery and controlled drug release are expected to improve the efficacy of cancer drugs. Such improvement is particularly important when administering chemotherapeutics that have toxicities that often limit their dose, resulting in suboptimal efficacy. Drs. Langer and Farokhzad from the MIT-Harvard CCNE formulated drug-encapsulating targeted nanoparticles using self-assembly of a triblock copolymer composed of poly(lactic-co-glycolic-acid) (PLGA), which is a controlled-release polymer; polyethyleneglycol (PEG), which protects against systemic clearance as well as improves pharmacokinetics; and aptamers which bind specifically to an antigen expressed on the surface of cancer cells.

This formulation enables drug release and cell-specific targeting. The construct was used to encapsulate docetaxel and deliver it to prostate cancer cells by using an aptamer that recognizes the extracellular domain of the prostate-specific membrane antigen (PSMA). This construct binds to the PSMA proteins, which are expressed on the surface of LNCaP prostate epithelial cells and are taken up by these cells, resulting in significantly enhanced cellular toxicity as compared with nontargeted nanoparticles. This construct exhibited improved efficacy and reduced toxicity in vivo (figure 3A). Moreover, after a single intratumoral injection of this construct containing docetaxel, 100% of mice used in the experiment survived the 109-day study (figure 3B). In contrast, docetaxel alone had a survivability of only 14% (figure 3B).

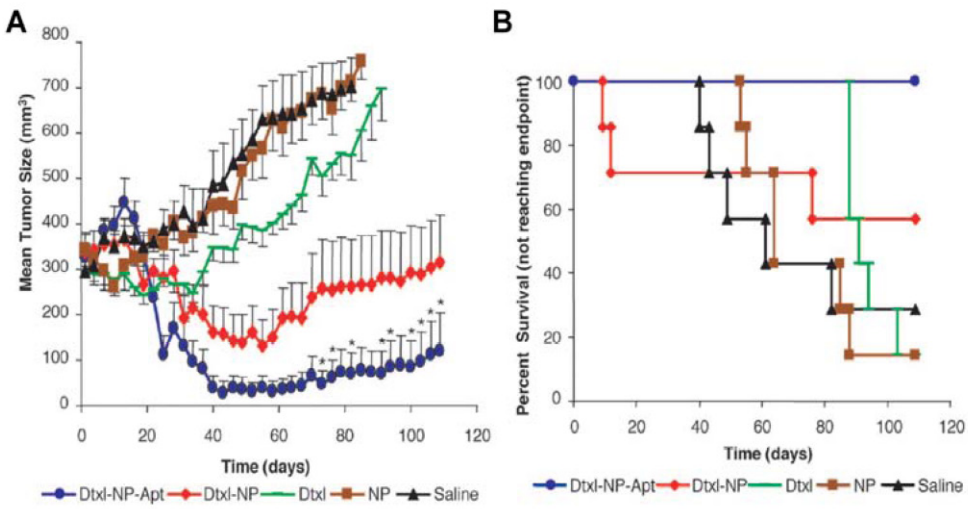


Figure 3. Comparative Efficacy Study in LNCaP s.c. Xenograft Nude Mouse Model of Prostate Cancer (PCa). (A) PCa was induced in mice by implanting LNCaP prostate epithelial cells s.c. in the flanks of nude mice and allowing the tumors to develop to appreciable size over 21 days (≈300 mm^3). The comparative efficacy study of single intratumoral injection (day 0) of (i) saline (black); (ii) pegylated PLGA NP without drug (NP, brown); (iii) emulsified Dtxl (Dtxl, green), 40 mg/kg; (iv) Dtxl-encapsulated NPs (Dtxl-NP, red), 40 mg/kg; or (v) Dtxl-encapsulated NP-Apt bioconjugates (Dtxl-NP-Apt, blue), 40 mg/kg was evaluated over 109 days.

- Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, Kantoff PW, Richie JP, Langer R. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. *Proc Natl Acad Sci U S A* (2006) 103:6315-20.
- Gu F, Zhang L, Teply BA, Mann N, Wang A, Radovic-Moreno AF, Langer R, Farokhzad OC, Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. *Proc Natl Acad Sci U S A* (2008) 105:2586-91.

Genetic therapies. Therapeutics relying on siRNA mediated RNA interference (RNAi) to silence oncogenes have the potential to form a new class of cancer drugs. The major challenge to using these therapies in mammals is the difficulty of delivering siRNA to cells that express the target gene. In order to overcome this difficulty, Dr. Mark Davis' group from the Caltech-UCLA CCNE has developed a targeted nanoparticle delivery system that contains (1) a linear, cyclodextrin-based polymer (CDP), (2) a human transferrin protein (TF) targeting ligand displayed on the exterior of the nanoparticle to engage TF receptors (TFR) on the surface of the cancer cells, (3) a hydrophilic polymer [polyethylene glycol (PEG)] used to promote nanoparticle stability in biological fluids, and (4) siRNA designed to reduce the expression of the RRM2 gene (figure 4A). In collaboration with Dr. Antoni Ribas from UCLA, Dr. Davis is testing these nanoparticles in a Phase I clinical trial in patients with solid tumors. Clinical data so far have provided the first proof that a nanoparticle can reach a tumor and silence a target gene using RNAi. Tumor biopsies from melanoma patients obtained after treatment show the presence of intracellularly localized nanoparticles in amounts that correlate with dose levels of the nanoparticles administered. The levels of specific messenger RNA and protein were reduced when compared to pre-dosing tissue (figures 4B,C).

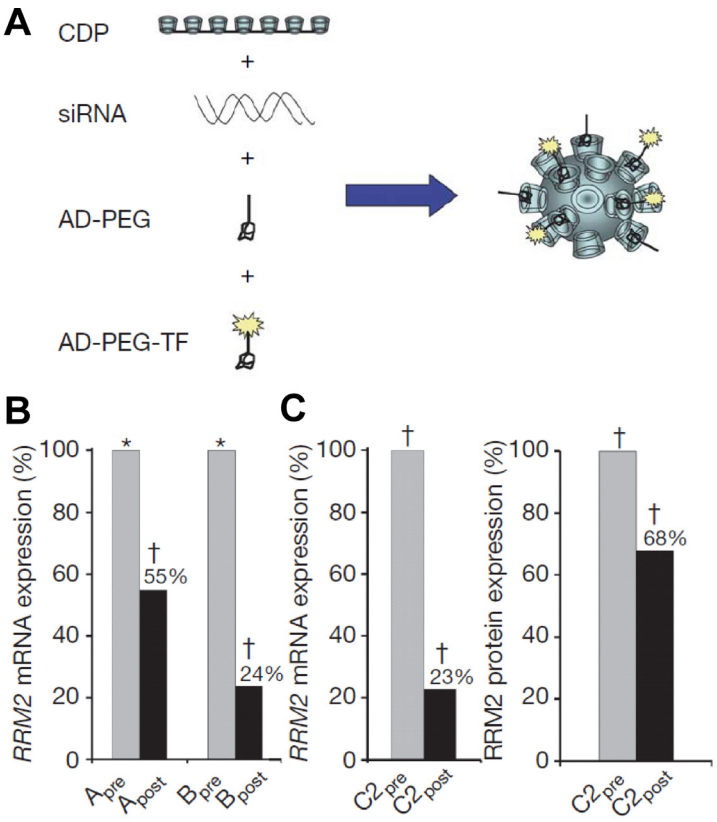


Figure 4. (A) Schematic representation of targeted nanoparticles. (B-C) RRM2 mRNA and protein expression in tumor tissue. (B) qRT–PCR analysis of RRM2 mRNA levels in samples from patients A and B before and after dosing. RRM2 mRNA levels are normalized to TATA box binding protein (TBP) mRNA levels. Results are presented as the percentage of the pre-dosing RRM2/TBP mRNA levels for each patient. (C) qRT–PCR and Western blot analysis of RRM2 protein expression from patient samples $C2_{pre}$ and $C2_{post}$.

- Davis ME, Zuckerman JE, Choi CH, Seligson D, Tolcher A, Alabi CA, Yen Y, Heidel JD, Ribas A. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* (2010) 464:1067-70.

In vitro diagnosis. Dr. James Heath's group from the Caltech-UCLA CCNE has engineered an integrated microfluidic system – the integrated blood barcode chip (IBBC) – that can sensitively sample a large panel of protein biomarkers from whole blood within 10 minutes of sample collection. A microfluidic network on the IBBC enables ~15% of the plasma to be skimmed from whole blood for detection of plasma proteins without pre-processing (figure 5A). The proteins are then detected using DNA Encoded Antibody Library (DEAL) technology also developed at the Caltech-UCLA CCNE. The DEAL barcodes in the plasma channels consist of spots of single-stranded DNA bound to protein-specific antibodies that are labeled with complementary ssDNA oligomers (figure 5B). The DNA, unlike antibodies, is stable to the processing used to create the elastomeric microfluidics chips and resists biofouling. Working with Dr. Paul Mischel of UCLA, Dr. Heath is currently using the IBBC for molecular and functional analysis of clinical glioblastoma tumor samples, to identify patients with the greatest potential for positive response to Avastin® therapy.

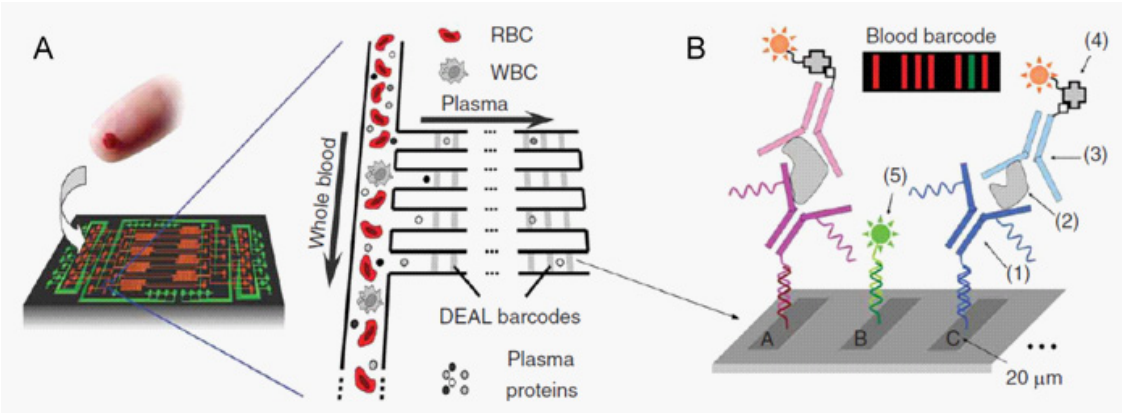


Figure 5. (A) Scheme depicting plasma separation from a finger prick of blood by harnessing the Zweifach-Fung effect. Multiple DNA-encoded antibody barcode arrays are patterned within the plasma-skimming channels for in situ protein measurements. (B) DEAL barcode arrays patterned in plasma channels for in situ protein measurement. A, B, C indicate different DNA codes. (1)–(5) denote DNA-antibody conjugate, plasma protein, biotin-labeled detection antibody, streptavidin-Cy5 fluorescence probe, and complementary DNA-Cy3 reference probe, respectively. The inset represents a barcode of protein biomarkers, which is read out using fluorescence detection. The green bar represents an alignment marker.

- Fan R, Vermesh O, Srivastava A, Yen BK, Qin L, Ahmad H, Kwong GA, Liu CC, Gould J, Hood L, Heath JR. Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. *Nat Biotechnol* (2008) 26:1373-8.

In vitro and in vivo diagnosis. Dr. Chad Mirkin's team from the Northwestern University CCNE developed the gold nanoparticles-based bio-barcode assay, which is an emerging diagnostic tool, used for the ultrasensitive detection of various protein and nucleic acid targets. In the case of proteins, the bio-barcode assay can be 2-3 orders of magnitude more sensitive than conventional ELISA-based assays. Recently, this assay has been applied to the detection of prostate-specific antigen (PSA) in the serum of male subjects who have undergone radical prostatectomy. The clinical data demonstrated that this new bio-barcode PSA assay is 300 times more sensitive than commercial immunoassays. This assay has the potential for broad applications in the detection of other cancer biomarkers (ovarian cancer will be next model studied). Dr. Mirkin has also used the gold nanoparticles to engineer nano-flares, which consist of a gold nanoparticle core functionalized with a dense monolayer of nucleic acid aptamers (figure 6). These nanoconjugates are readily taken up by the cells where their signal intensity could be used to quantify intracellular analyte concentration.

- Thaxton CS, Elghanian R, Thomas AD, Stoeva SI, Lee JS, Smith ND, Schaeffer AJ, Klocker H, Horninger W, Bartsch G, Mirkin CA. Nanoparticle-based bio-barcode assay redefines "undetectable" PSA and biochemical recurrence after radical prostatectomy. *Proc Natl Acad Sci U S A* (2009) 106:18437-42.

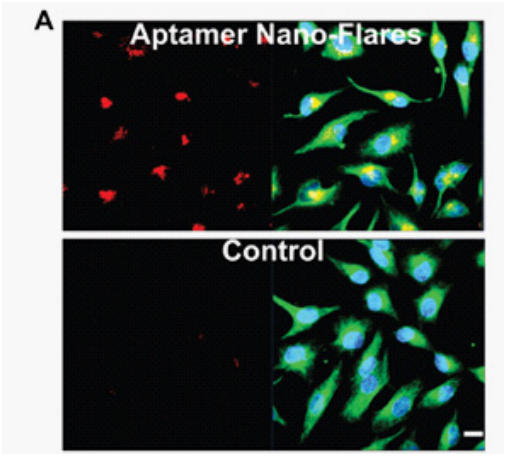


Figure 6. (A) Fluorescence microscopy images of HeLa cells incubated with aptamer nano-flares and control particles for 2 h. The red channel is Cy5 fluorescence associated with activated aptamer nano-flares, the green channel is fluorescence associated taxol-Alexa 488 and specific for tubulin, and the blue channel is fluorescence associated with Hoechst 3342 and specific for the nucleus. Scale bar is 20 μm. (B) Cell-associated fluorescence (Cy5) of populations treated with aptamer nano-flares and control particles as determined by flow cytometry. Inset numbers indicate the mean fluorescence value of the population.

- Zheng D, Seferos DS, Giljohann DA, Patel PC, Mirkin CA. Aptamer nano-flares for molecular detection in living cells. *Nano Lett* (2009) 9:3258-61.

Targeted nanosystems for therapy and imaging. A new cooperative nanosystem, developed by a multidisciplinary collaboration of Alliance researchers Drs. Michael Sailor and Erkki Ruoslahti from the UCSD CCNE and Dr. Sangeeta Bhatia from the MIT-Harvard CCNE uses the unique photothermal properties of gold nanorods to improve the tumor specificity of targeted nanoparticles for therapy and imaging. The system consists of several components which can be selected to determine final mode of action. The first component of the system is gold nanorod "activators" that populate the porous tumor vessels and act as photothermal antennas by absorbing and transducing near infrared radiation (NIR) into heat (~43 °C) (figure 7A).

The second element consists of either doxorubicin-loaded liposomes or magnetic "nanoworms." Targeted doxorubicin-loaded liposomes preferentially accumulate in tumors following photothermal treatment, resulting in a three-fold increase in doxorubicin deposition (figure 7B). Therefore, this approach could reduce the required dose of anticancer drugs and mitigate toxic side effects. Another potential secondary component of the system is magnetic nanoworms. These comprise spherical iron oxide nanoparticles linked together to form worm-like chains. The structure is composed of a chain of 5 to 10 magnetic grains, each 5 nm in diameter, with a global hydrodynamic diameter of 65 nm (figure 7A). This particular geometry was found to bind to tumor cells more efficiently in vitro because of multivalent interactions between the nanoworms and the cellular receptors, compared with spherical nanoparticle controls, and therefore could be used as an improved contrast agent for MRI.

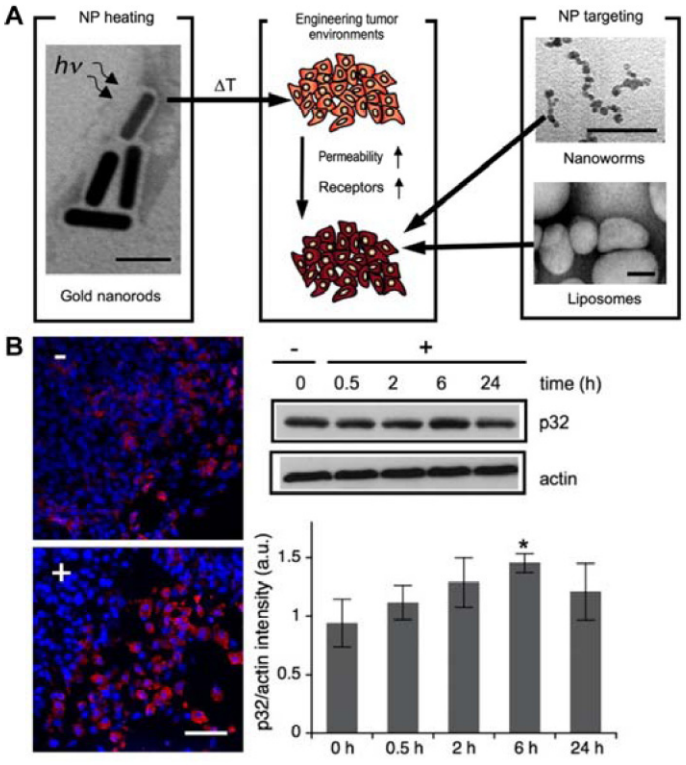


Figure 7. Characterization of the Components of Cooperative Nanosystems. (A) Schematic showing the components of the two cooperative nanomaterials systems: the first - gold nanorods (NR), and second - magnetic nanoworms (NW), or doxorubicin-loaded liposomes (LP). Irradiation of the NR with an NIR laser induces localized heating that stimulates changes in the tumor environments. The NW or LP components decorated with tumor targeting peptides bind to the heat-modified tumor environments more efficiently than to the normal tumor environments. Transmission electron microscope images of all three components are shown. Scale bars indicate 50 nm. (B) Effect of heating time on p32 expression in MDA-MB-435 xenograft tumor. Tumor in an athymic (nu/nu) mouse was heated at 45 °C for 30 min in a water bath. Images at left show cell surface p32 immunostaining of tumor sections 6 h post-treatment. Symbols + and - indicate with and without heating, respectively. Scale bar indicates 50 μm. At right are Western blot results for p32 relative to b-actin control. *indicates P<.05 for 0 h and 6 h intensity ratio (n = 3 ~ 4).

- Park JH, von Maltzahn G, Xu MJ, Fogal V, Kotamraju VR, Ruoslahti E, Bhatia SN, Sailor MJ. Cooperative nanomaterial system to sensitize, target, and treat tumors. *Proc Natl Acad Sci U S A* (2010) 107:981-6.

New material platforms. An accurate control over size, shape, chemical composition, amount of targeting moiety, and therapeutic cargo in the nanoparticle is needed to develop reproducible nano-delivery systems. A particle fabrication technology, called PRINT® (Particle Replication In Non-wetting Templates), developed by Dr. Joseph DeSimone of the University of North Carolina CCNE, takes advantage of the unique properties of elastomeric molds to produce monodisperse, shape-specific particles from an extensive array of organic precursors (figure 8A1-5). PRINT® nanoparticles allow for the elucidation of mechanisms by which organic particles of controlled size, shape, site-specific surface chemistry, tunable particle matrix composition, and tunable modulus undergo endocytosis. Obtaining knowledge about the endocytic pathway used from PRINT® particles should lead to crucial information required for not only enhancing specific cellular internalization, but also manipulating the intracellular location of particles, and minimizing cytotoxic effects (figures 8B,C). Once the mechanisms of internalization are established, it is then possible to use these findings to better engineer the intracellular release of specific cargos. This information, in combination with ongoing efforts to understand the biodistribution of shape-controlled particles, will help to establish rules toward the rational design of nanocarriers for effective in vivo delivery of various cargos, especially those cargos that need to be internalized into cells such as siRNA and antisense oligonucleotides.

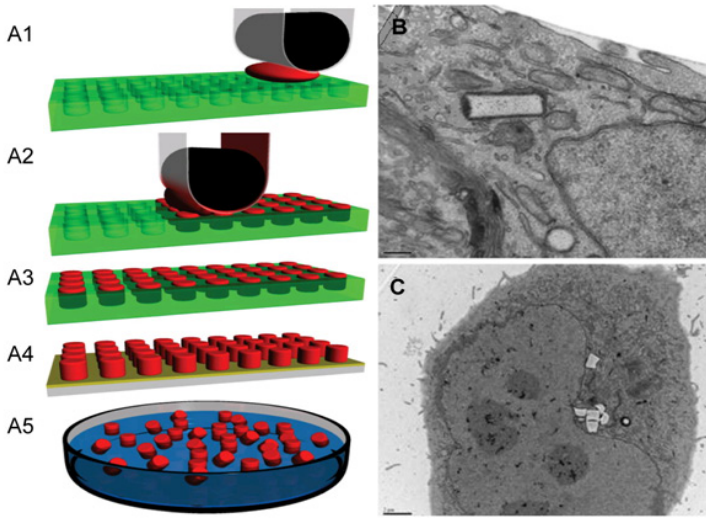


Figure 8. (A1-5) Schematic representation of the PRINT process. (A1) Empty mold (green), high surface energy polymer sheet (clear), roller (black) is brought into contact with the particle precursor solution (red) and the mold; (A2) Roller evenly distributes particle precursor solution into the cavities of mold. Excess particle precursor solution is wicked away by the high surface energy polymer sheet; (A3) Particles are cured in the mold; (A4) Particles are removed from the mold; (A5) Particles are collected or harvested using a number of different film-based techniques and ultimately are dispersed in solution. (B-C) TEM images of HeLa cells at 37 °C. Illustration depicts cellular internalization used by PRINT particles.

- Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, DeSimone JM. The effect of particle design on cellular internalization pathways. *Proc Natl Acad Sci U S A* (2008) 105:11613-8.

Cell migration studies. The groups of Drs. Milan Mrksich and Bartosz Grzybowski from the Northwestern University CCNE have played a leading role in developing methods to understand directed cell motility. They have created discrete tracks on which cells can move, using microetched glass coverslips with Au/Ti and protecting unetched portions either with oligo (ethylene glycol) alkane thiols which inhibits cell adhesion or with fibronection (or laminin) to promote cell adhesion. They have created distinct patterned surface areas on which to study directional migration and have found that B16F1 metastatic murine melanoma cells migrate in one direction whereas non-tumor cells such as Rat2 migrate in the exact opposite direction on the same ratchet pattern. B16F1 cells project their lamellipodium into the "open" vertices of the short base of the trapezoid whereas Rat2 cells extend long protrusions into the "open" vertex of the long base and anchor at the nearby spike (figure 9A). Since this directionality of migration persists even in a mixed cell population (figure 9B), the authors propose the possibility of implanted "cancer traps" whereby only metastatic cells would migrate into the trapping device. This device could also contain chemokines that specifically attract tumor cells as well as chemotherapeutic agents. Additionally, in vitro this type of bi-directional ratchet could be useful to partially purify populations of cells.

- Mahmud G, Campbell CJ, Bishop KJM, Komarova YA, Chaga O, Soh S, Huda S, Kandere-Grzybowska K, Grzybowski BA. Directing cell motions on micropatterned ratchets. *Nat Physics* (2009) 5:606-12.

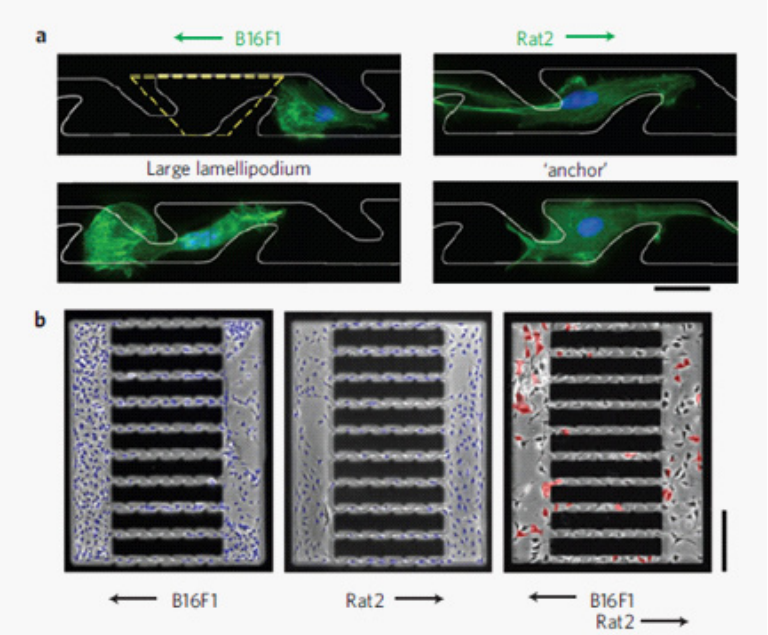


Figure 9. Cells on Bi-directional "Lines-With-Spikes" Ratchets. (A) B16F1 and Rat2 cells move in opposite directions on linear ratchets with spikes inclined at 45 degrees (staining: phalloidin, green; Hoechst 33342, blue). B16F1 cells (left column) and Rat2 (right). The trapezoid (delineated by a yellow dotted line) adhesive area is 1,300 μm^2. The borders between adhesive and nonadhesive areas are outlined by white dotted lines. The scale bar corresponds to 30 μm. (B) Distribution of B16F1 (left), Rat2 (middle), and a mixture of B16F1 and Rat2 (right) cells over a pattern of two reservoirs connected by 10 spiked ratchets (after 48 h from cell plating, serum concentration 10%; laminin). Superimposed images of phase contrast and nuclei (Hoechst 33342, blue) illustrate 54.8% bias for B16F1 cells (189 cells in the left reservoir and 156 in the right one) and 56.0% bias for Rat2 (80 and 102 cells). For the mixture of B16F1 cells and Rat2 cells, the former are stained with a red DiI dye; the image is a superposition of phase contrast and DiI. The cells of two types partly sort out and the biases from reservoir counts are 57.7% bias for B16F1 cells (15 cells in the left reservoir and 11 in the right one) and 56.5% bias for Rat2 cells (27 and 35 cells). The scale bar represents 250 μm.

New instrumentation. X-rays are indispensable in many medical applications including cancer detection, characterization, and treatment. The basic design of the x-ray tube, however, has not changed significantly in the past 100 years: a thermionic cathode is used to produce electrons, which strike on a metal target to generate x-rays. This design has several intrinsic drawbacks which include a high cathode operating temperature (~1,000 °C), which prevents miniaturization and novel source configurations that can increase imaging speed and accuracy; high imaging dose, which causes radiation damage; and low temporal and spatial resolution, which affects the size and accuracy of the features that can be detected. Carbon nanotube (CNT)-based field emission x-ray sources have the potential to not only overcome these limitations but also enable new novel imaging modalities. Dr. Otto Zhou's team at the University of North Carolina CCNE has demonstrated that the CNT-based x-ray technology can miniaturize x-ray source while allowing novel source configurations such as scanning multibeam x-ray sources for dynamic and high-speed imaging. Dr. Zhou's team used CNT x-ray technologies for improving cancer imaging methods and radiotherapy and has recently employed tomosynthesis to perform in vivo imaging of breast cancer in a quicker manner with less patient radiation exposure. The system is composed of a 25-pixel x-ray source array, a flat panel detector for full-field mammography, a control unit for x-ray sources, and a computer work station. It can acquire 25 projection images in 11 seconds at 0.2-mm resolution. By contrast, the Siemens system at the same dose requires 20 seconds to take 25 images with 0.3-mm focal spot size. The imaging system can increase the imaging speed, reduce the size and cost of the equipment, and enable experimentations on new imaging configurations, which can give better quality images not feasible with the conventional step-and-shoot method.

- Maltz JS, Sprenger F, Fuerst J, Paidi A, Fadler F, Bani-Hashemi AR. Fixed gantry tomosynthesis system for radiation therapy image guidance based on a multiple source x-ray tube with carbon nanotube cathodes. *Med Phys* (2009) 36:1624-36.

- Qian X, Rajaram R, Calderon-Colon X, Yang G, Phan T, Lalush DS, Lu J, Zhou O. Design and characterization of a spatially distributed multibeam field emission x-ray source for stationary digital breast tomosynthesis. *Med Phys* (2009) 36:4389-99.

3.3.2. Accomplishments of Nanotechnology Characterization Laboratory (NCL)

The NCL (http://ncl.cancer.gov/) performs preclinical characterization of nanomaterials intended as cancer diagnostics or therapeutics. NCL selects nanomaterials for characterization based on an application process (applications are evaluated based on published criteria, with a focus on demonstrated proof-of-concept anticancer efficacy and potential for clinical translation). Successful applicants submit nanomaterials to the NCL for characterization, which is provided at no cost to the submitting investigator.

- The NCL has developed a three-tiered Assay Cascade of tests, including physicochemical characterization, in vitro assessment, and in vivo evaluation for safety and efficacy, as a standard tool for the preclinical characterization of biomedical nanomaterials. Over 200 different nanoparticle formulations have been evaluated by the NCL, and nearly 90% of the NCL's efforts are in support of extramural nanomaterial submitters from academia, industry, and government.

- The NCL recently initiated a collaboration with FDA's National Center for Toxicological Research (NCTR) in Jefferson, Arkansas. The NCTR collaboration will give eligible NCL collaborators the opportunity to expand their animal study data to include GLP-quality pharmacokinetic studies in non-human primates. In return, NCL provides NCTR with physicochemical resources and expertise to characterize nanoparticles of interest to the FDA.

- Several NCL assays have been adopted as standards by the American Society for Testing and Materials (ASTM) and the International Standards Organization (ISO). In addition, in collaboration with NIST and ASTM, NCL coordinated an interlaboratory study involving more than 60 laboratories, which helped to expose sources of data variability in experiments on nanomaterials.

3.3.3. Data Sharing

In collaboration with the NCI Center for Biomedical Informatics and Information Technology (CBIIT), the NCI has established the caNanoLab (http://cananolab.nci.nih.gov/caNanoLab/welcome.do) database to house the results from NCL and ANC extramural researcher studies and make them accessible to the research community. caNanoLab provides access to information on nanomaterials composition, physicochemical characterizations, in vitro characterization (cytotoxicity, blood contact properties, oxidative stress, immune cell functions), nanomaterials pharmacokinetics and toxicity, protocols supporting nanotechnology characterizations, nanomaterials synthesis and preparation, radiolabeling, and safety. The caNanoLab team is working with the nanotechnology biomedical community to develop standards for capturing information on nanomaterials and their characterization in a structured fashion, in support of cross-particle analysis and advanced visualization of structure-activity relationships. These standards will assist in engineering of nanomaterials for optimal biodistribution and identifying the impact of particle physicochemical structure on biological activity.

3.4. Summary – Phase I

The first phase of the ANC was successful in meeting the initial program goals of establishing a cancer nanotechnology community that produces both important scientific discoveries and the necessary preclinical development of existing biomedical nanotechnologies. Basic discoveries to come out of the ANC program include, among others, the cell migration studies by Drs. Mrksich and Grzybowski, the cell and tumor penetrating peptides developed by Drs. Tsien and Ruoslahti, and novel quantum dots synthesized by Drs. Nie, Belcher, and Bawendi. These works are important and show the diversity of opportunities presented by nanotechnology materials design. Important development work within the first phase of the ANC, including the clinical validation of in vitro diagnostic technologies developed in the

Caltech and Northwestern CCNEs (Drs. Heath and Mirkin, respectively), the promising clinical trial results for siRNA delivery at Caltech (Dr. Davis) , the preclinical development of chemotherapeutic nano-formulations in the MIT-Harvard CCNE (Drs. Farokhzad and Langer), and the intracellular delivery studies using PRINT nanoparticles in the UNC CCNE (Dr. DeSimone), have established the feasibility of using nanotechnology tools and materials in the clinical oncology setting. The assays and protocols for nanomaterial characterization developed by the NCL are crucial foundations for the safe translation of these technologies into the clinic and provide necessary infrastructure for the uniform validation of nanomaterials for medical use.

In some aspects the ANC exceeded its early goals, as evidenced by the number and preliminary success of clinical trials launched by ANC researchers. Some examples include Dr. Davis' siRNA-nanoparticle delivery trial (Caltech/UCLA), the development of polymer nanoparticles with very long circulation time by BIND Pharmaceuticals (spinoff from Dr. Langer's lab at MIT), and a new diagnostic technique for colon cancer by Dr. Gambhir (Stanford). The last two efforts currently are approaching IND approval.

Program efforts to foster a collaborative spirit in the first phase of the ANC resulted not only in research projects and publications, but also in exchanges of personnel and materials. This personnel exchange was particularly important for the program's training components, as numerous ANC graduate students and postdoctoral researchers were able to use network connections formed at PI meetings to establish their next positions.

4. ANC Phase II – Program Structure

Phase II of the ANC, taking the lessons learned in Phase I, will use the diversity of nanotechnology platforms and will cover the range of different technology developmental stages. It will continue basic discovery and innovation, but it will also take great care in the evaluation of clinical utility of the technology and put strong emphasis on the translation. Phase II of the program will enhance the training component. Training has become increasingly critical to developing the multi- and trans-disciplinary scientists necessary to the future implementation of nano-enabled interventions in the practice of clinical oncology.

Phase II will still rely on the network of U54 CCNEs and U01 CNPPs. We will expand training efforts through the formation of R25 Cancer Nanotechnology Training Centers (CNCTs) and Path to Independence Awards (K99/R00). Both will broaden the pool of potential cancer nanotechnology researchers to institutions and regions not currently heavily involved in ANC. The ANC used an open competition to award Phase II grants, with existing grants competing equally with first-time applicants, to encourage the introduction of new researchers, institutions, and ideas to the ANC. The structure of the Phase II program is shown in figure 10.The geographical distribution of awards is shown in the map in figure 11; detailed information on all awards is included in the Appendix. One important goal of Phase II of the ANC will

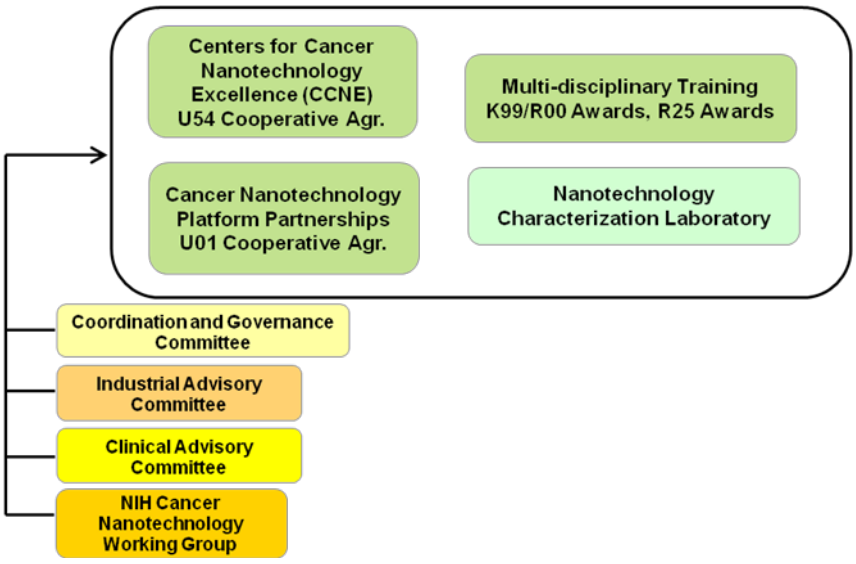


Figure 10. Proposed Structure for Phase II ANC Program.

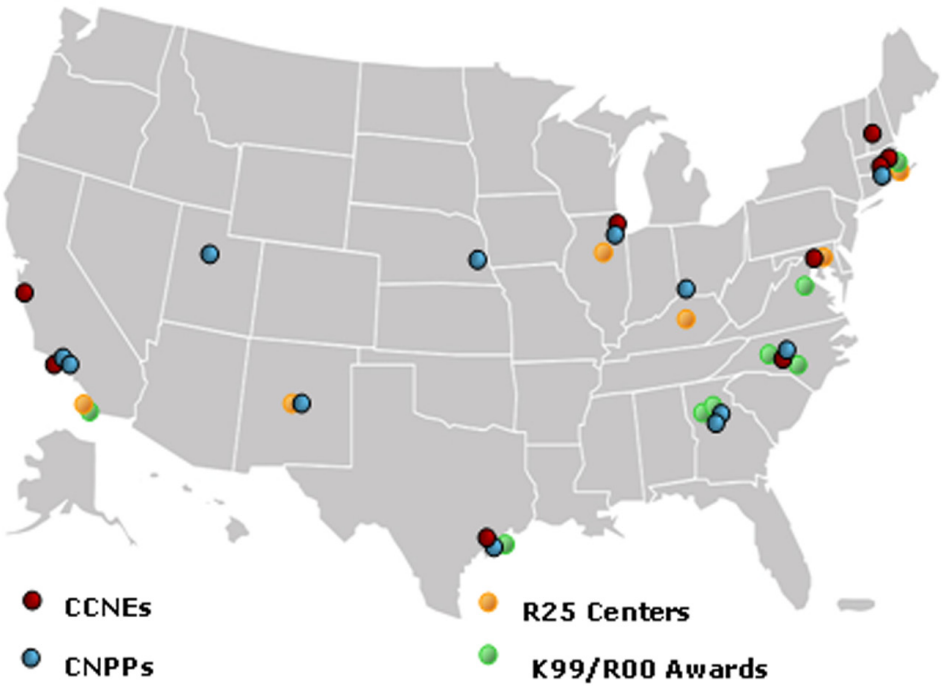


Figure 11. Centers of Cancer Nanotechnology Excellence (CCNEs), Cancer Nanotechnology Platform (CNPPs) Awards, Training Center (CNTCs) Awards, and Path to Independence Awards in Phase II of the ANC Program.

be to increase collaborative activities within the network. As such, the 2009 RFA defined funding for Trans-Alliance Challenge projects to help researchers in the program establish and foster collaborations.

The NCL will continue to act as a national resource for cancer nanotechnology researchers in preclinical characterization of nanomaterials.

4.1. Alliance Phase II – Program Strategy

Scientific strategy for the Phase II was developed based on information gathered from several sources: the lessons learned of Phase I, the evolving strategy of the National Nanotechnology Initiative (NNI), and, most importantly, the input of the extramural community. In spring 2008, we conducted three strategic workshops, which brought together clinicians and leading researchers from the extramural community. The workshops were focused on cancer nanotechnology accomplishments to date and the likely future impact of nanotechnology in clinical oncology. There was a clear consensus among participants that cancer nanotechnology had made significant advancements in both discovery and preclinical development, and that the field was poised to become a core component of cancer research and an important part of comprehensive cancer care. Workshop participants believed that early diagnosis and better monitoring of therapeutic efficacy could be achieved using emerging multiplex in vitro diagnostic techniques and novel imaging technologies such as multiplexed, multimodal molecular contrast agents. Participants stressed the importance of correlating outcomes from both approaches. On the therapeutic front, improved tumor targeting via cell surface targeting ligands, enhanced formulations for chemotherapeutics that reduce systemic toxicity and improve therapeutic index, and cooperative treatment regimes in which drug delivery is combined with tumor microenviroment engineering to improve treatment response were predicted.

- Nagahara LA, Lee JS, Molnar LK, Panaro NJ, Farrell D, Ptak K, Alper J, Grodzinski P. Strategic workshops on cancer nanotechnology. *Cancer Research* (2010) 70:4265-8. (see Appendix)

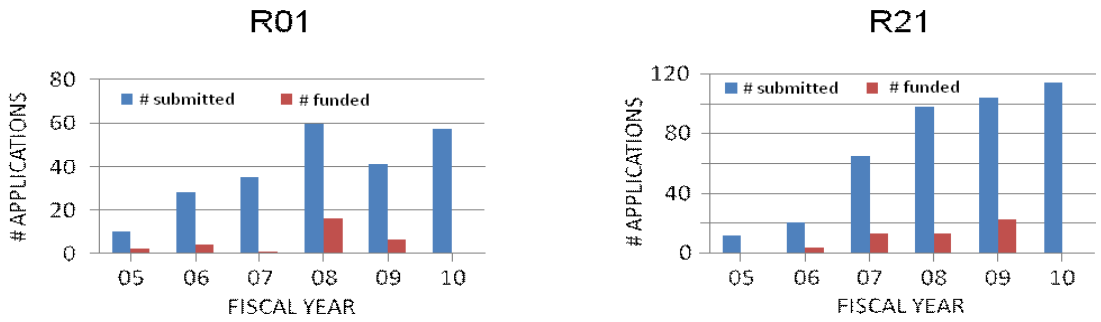


Figure 12. NCI Proposal Submissions to Nanoscience and Nanotechnology in Biology and Medicine PA 08-052 (R01) and PA 08-053 (R21)

5. ANC Impact on Other NCI and NIH Programs

The ANC program collaborates with and supports the development of other nanotechnology initiatives within NCI and NIH. There are several standing Program Announcements (PAs) soliciting R01 and R21 proposals, for example, the Nanoscience and Nanotechnology in Biology and Medicine (PA-08-052/53) and Image-Guided Drug Delivery in Cancer (PA-09-253). There has been a large growth of incoming applications, through these PAs as well as unsolicited applications, indicating a growing interest in nanotechnology applications within the cancer research community. Figure 12 shows a number of incoming applications in response to Nanoscience and Nanotechnology PA, increasing from ~10 to 60 and 10 to 120 for R01s and R21s, respectively, over the 5-year period (2005-2010).

In addition to these grant opportunities, ANC also established a small contract program to support studies in nanomaterials biodistribution and toxicity in larger animals and reformulation efforts which attempt to resurrect drugs that failed in free systemic delivery due to high toxicity, by opening their therapeutic window and delivering them using nanotechnology-based carriers.

Other NIH institutes have also formed nanotechnology programs. NIH Roadmap established a center program on nanomedicine, while NHLBI has established a nanotechnology center initiative called Programs of Excellence in Nanotechnology (PEN). NHGRI has a growing portfolio of grants dedicated to novel methods of sequencing based on nanotechnology devices. NIBIB has funded new nanoengineering concepts to support imaging. Finally, NIGMS has funded efforts on basic understanding of fundamental cellular and physiological principles using nanotechnology tools. NIH formed the Trans-NIH Nanotechnology Task Force in 2006 to coordinate efforts in this area across the agency.

6. Future Vision

The ANC has demonstrated that a multidisciplinary approach to research can catalyze scientific developments and enable clinical translation. ANC investigators have advanced diagnostic technology, using both in vitro assays and novel imaging methods, and offered improved therapies and therapeutic efficacy measures. Many of the technologies developed and clinically translated have applied novel engineering to existing cancer biology strategies. The next stage of cancer nanotechnology research should introduce new models of cancer care, where progress in cancer biology and understanding of the disease is enabled by new nanotechnologies.

Future advances in nanotherapy will be based on distinctive nanomaterial properties capabilities, such as nanoparticle-mediated hyperthermia or recognition and alteration of the tumor microenvironment. Nanoparticles will also enable resurrecting drugs which failed in free systemic delivery due to high toxicity by opening their therapeutic window by delivering them using nanotechnology-based carriers. Drugs and devices will converge in multifunctional systems

that release therapeutics in response to biochemical signals detected in the tumor or blood. Low-cost genomic and proteomic profiling will enable more detailed identification of tumor types and effective patient-therapy matching. Monitoring of patient response via molecular imaging of tumors and in vitro measurements of different biomarkers has already begun, and will advance further.

In vivo molecular imaging capabilities will enable optical biopsies, with tumors being typed and staged at the time of detection. More complete molecular characterization of lesions will also allow clinicians to recognize and prevent chemoresistance. The combination of advanced imaging with traditional surgical techniques for intraoperative guidance will enable more successful resection of cancerous growths, which is still the most effective cure available for many cancers.

Many of the advances envisioned in therapeutics and imaging will depend on advances in in vitro assay technology, particularly the identification and validation of additional cancer biomarkers. Microfluidics will be a backbone technology for many of these advances. Work on the collection and analysis of circulating tumor cells has begun, but will increase in complexity and utility in the coming years.

More research is needed in the effective use of nanotechnology tools in disease prevention. Nanoparticle formulations of chemopreventives are one avenue for investigation, but the hope is that other innovative systems for cancer prevention will also emerge. Early diagnostic techniques operating in a multiplexed manner with high sensitivity and specificity will have impact as well.

Phase II of the ANC begins in September 2010, and will consist of a newly selected group of CCNEs and CNPPs chosen in an open competition. The CCNEs of this new program edition will have a greater focus on clinically worthy technologies as compared to Phase I. The new program will emphasize more heavily cancers having particularly poor outcomes, including brain, lung, pancreatic, and ovarian cancers. The CCNEs and CNPPs will be joined by several Cancer Nanotechnology Training Centers (CNTCs) and Path to Independence Awards. The NCL will continue to act as a national resource for cancer nanotechnology researchers. Having begun the process of standardizing bio-nanomaterials, the challenge facing the ANC is to promote widespread acceptance of NCL established protocols within the research and development community. In addition to preclinical characterization and regulatory obstacles, good manufacturing procedures (GMPs) such as scale-up process, purity, and batch-to-batch consistency have to be established for nanomaterials. This is one of the major challenges facing the next stage of the ANC.

Appendix 1: Current Staffing

Piotr Grodzinski, Ph.D. Director, OCNR

Dr. Grodzinski oversees the operation of the office and works closely with the extramural community to develop strategies for the use of nanotechnology in cancer. He is a materials scientist by training, but found bio- and nanotechnology fascinating. In the mid-1990s, he left the world of semiconductor research and built a large microfluidics program at Motorola Corporate Research & Development in Arizona. After his tenure at Motorola, Dr. Grodzinski joined the Bioscience Division of Los Alamos National Laboratory, where he served as a Group Leader and an interim Chief Scientist for the Department of Energy Center for Integrated Nanotechnologies (CINT). Dr. Grodzinski received his Ph.D. degree in materials science from the University of Southern California, Los Angeles in 1992. He is an inventor on 15 patents and has authored 52 peer-reviewed publications and 7 book chapters, and delivered over 100 conference presentations.

Dorothy F. Farrell, Ph.D., Nanotechnology Program Manager, OCNR

Dr. Farrell oversees and manages training programs within the ANC, coordinates grant review and award processing with the NCI Division of Extramural Activities, and was instrumental in developing new RFAs and funding plans for Phase II of the Alliance program. She also evaluates nanomaterials research and development within the ANC. Dr. Farrell received her doctorate in physics from Carnegie Mellon University, where her thesis project focused on the synthesis and characterization of self-assembled arrays of magnetic nanoparticles. She then spent 2 years at University College London on a Royal Society USA Research Fellowship and then joined the Naval Research Laboratory for 2 years as a National Research Council Research Associate.

Krzysztof Ptak, Ph.D., M.B.A., Nanotechnology Projects Manager, OCNR

Dr. Ptak acts as liaison between the program office and the bioinformatics community. He also coordinates communication between the program office and grantees, including chairing the ANC Communications and Integration Working Group, and is responsible for ANC outreach activities, including maintenance of the ANC Web site. He also manages projects focused on imaging and therapy of different cancers. Prior to joining OCNR, he held research positions at Northwestern University and then at the National Institute of Neurological Disorders and Stroke, NIH. During Dr. Ptak's more than 10 years of research in experimental science, his focus was on the neurobiology of respiration and related specifically to the pathology of sudden infant death syndrome. Dr. Ptak earned his Ph.D. degrees in neuroscience from the Paul Cezanne University in Marseilles and the Jagiellonian University in Krakow. His dissertation was honored as the best doctoral thesis of the year by the Prime Minister of the Republic of Poland.

Nicholas Panaro, Ph.D., Senior Scientist, Nanotechnology Characterization Laboratory, OCNR

Dr. Panaro manages contracts for SAIC-Frederick and provides technical and scientific oversight of NCI programs, including developing requests for proposals and administering Small Business Innovation Research (SBIR) grants. He also serves as the liaison between the Nanotechnology Characterization Laboratory (NCL) and the ANC. Prior to joining NCL, Dr. Panaro conducted postdoctoral research at the University of Pennsylvania, where he focused on the design and fabrication of micro-electromechanical systems for genetic analysis and at NCI, where his research focused on tumor angiogenesis. He holds a Ph.D. degree in chemical engineering from the Rice University Biomedical Engineering Laboratory.

Sara Hook, Ph.D., Nanotechnology Projects Manager, OCNR

Dr. Hook oversees projects that use nanotechnology to enhance understanding of cancer biology and use nanotechnologies for the delivery of genetic therapies; she participates in NCI-wide activities in research development for specific cancer types. She is also helping to develop the cananoPLAN. She has extensive research experience in the field of molecular cancer biology focusing on regulation of the histone deacetylases and pathways that maintain genomic stability. Prior to joining the OCNR, she did postdoctoral work at the Fred Hutchinson Cancer Research Center with Dr. Robert N. Eisenman and at the University of Virginia with Dr. Anindya Dutta. She holds a Ph.D. degree in pharmacology from the Duke University Program in Cellular and Molecular Biology, where she elucidated the activation mechanism of

the Calcium/Calmodulin-dependent kinases with Dr. Anthony R. Means. Dr. Hook has published numerous papers, has earned 10 scientific and academic awards, and has enjoyed mentoring elementary, high school, undergraduate, and graduate-level students.

George Hinkel, Ph.D., AAAS Fellow, OCNR

Dr. Hinkel is joining OCNR from his recent postdoctoral position at Centre Léon Bérard in France, where he studied the mechanisms of cancer metastasis. He holds a Ph.D. degree in biomedical sciences from Baylor College of Medicine.

Appendix 2: Program Collaborations

1. The ANC entered into an Interagency Agreement with the National Science Foundation in 2005 to co-fund four Integrative Graduate Education and Research Traineeships (IGERTs) for 5 years.

2. The ANC established an Interagency Agreement in 2004 with the U.S. Food and Drug Administration and the National Institute of Standards and Technology to form the Nanotechnology Characterization Laboratory (NCL). The NCL has successfully developed assays and protocols for materials characterization and characterized over 200 materials since its inception.

3. The NCL has established numerous collaborations with universities, for-profit institutions, international organizations, and other Federal agencies to develop protocols for materials characterization and to characterize materials for clinical applications. These collaborations include:

    - Work with NIST scientists to determine the best measurement tools, protocols, and analysis algorithms for physical characterization of nanoparticles.

    - Participation in an International Alliance for NanoEHS Harmonization (IANH) (coordinated from Ireland) study to develop protocols for physicochemical testing of materials.

    - In collaboration with NIST and ASTM International, NCL coordinated an interlaboratory study (ILS) involving more than 60 participating laboratories in 2008, which helped to expose sources of data variability in experiments on nanomaterials. The ILS used NIST reference material gold nanoparticles and dendrimers.

    - A 2-year cooperative research and development agreement (CRADA) GE Global Research, the technology development arm of General Electric Company, with the goal of accelerating the development of nanoparticle-based imaging agents. In 2010, the NCL-GE CRADA is being extended for an additional 2 years.

    - NCL developed a quality assurance assay to monitor the stability of AuroShells® PEG coatings, which is important for biocompatibility and may affect their shelf life for Nanospectra Biosciences, Inc., a Houston, Texas-based company. These AuroShell® particles emit heat upon absorption of near-infrared wavelengths of light and can be delivered intravenously to tumors.

    - Work with CytImmune Sciences, Inc., to develop data supporting regulatory review of AurImune®, a PEGylated colloidal gold nanoparticle with attached tumor necrosis factor (TNF). AurImune® will enter Phase II clinical trials in 2010.

    - Work with BIND Biosciences to examine the extent to which BIND's targeted particles for drug delivery evade the immune system, bind to target sites, accumulate in target tissues, and provide the desired drug release profile.

4. The ANC established a partnership with the National Center for Nanoscience and Technology in Beijing, China, to promote collaborations between Chinese and American researchers. There was one joint meeting in Beijing in 2008 and another scheduled for September 2010 in Washington, D.C. Collaborations so far include:

    - Dr. Alexander Kabanov of the University of Nebraska Medical Center and Prof. Dr. Xi Zhang, Chair, Department of Chemistry, Tsinghua University.

    - Dr. Shuming Nie of Emory University and Prof. Yuliang Zhao of the Chinese Academy of Sciences.

    - The ANC is investigating funding exchange programs through administrative supplements to its CCNE grants or its CNTC training program.

5. The ANC Program Director, Piotr Grodzinski, holds the NCI seat on the National Nanotechnology Initiative's subcommittee on Nanoscale Science, Engineering and Technology.

6. The ANC maintains close consultation with the FDA on regulatory review of nanotechnology-enabled devices and nanomaterials for biomedical application. To support his consultation, Dr. Subhas Malghan, Deputy Director for Program Policy and Evaluation in the FDA's Office of Science and Engineering Laboratories, is on detail to the OCNR one day per week.

Meeting Report

Cancer Research

Strategic Workshops on Cancer Nanotechnology

Larry A. Nagahara[1], Jerry S.H. Lee[1], Linda K. Molnar[2], Nicholas J. Panaro[3], Dorothy Farrell[1], Krzysztof Ptak[1], Joseph Alper[4], and Piotr Grodzinski[1]

Abstract

Nanotechnology offers the potential for new approaches to detecting, treating, and preventing cancer. To determine the current status of the cancer nanotechnology field and the optimal path forward, the National Cancer Institute's Alliance for Nanotechnology in Cancer held three strategic workshops, covering the areas of *in vitro* diagnostics and prevention, therapy and post-treatment, and *in vivo* diagnosis and imaging. At each of these meetings, a wide range of experts from academia, industry, the nonprofit sector, and the U.S. government discussed opportunities in the field of cancer nanotechnology and barriers to its implementation.

Cancer Res; 70(11); 4265–8. ©2010 AACR.

Introduction

Cancer is one of the most pressing public health concerns of the 21st century. The statistics are daunting; it was projected that 550,000 people would die of cancer and that another 1.4 million would be diagnosed with the disease in 2009 in the United States alone. Five years ago, the National Cancer Institute (NCI) initiated the NCI Alliance for Nanotechnology (1), in hopes of fostering revolutionary new ways to approach cancer research and care. Nanomaterials have the potential to deliver drugs directly to cancerous tissues and to open up entirely new modalities of cancer therapy. Nanotechnology-enhanced microfluidic devices can increase sensitivity and multiplexing capability for cancer-marker identification and detection.

The first nanotechnology-based constructs for cancer care are already on the market, including liposomal doxorubicin (DOXIL, Centocor Ortho Biotech Products L.P.) and albumin-bound paclitaxel (Abraxane, Abraxis Bioscience). Similarly, diagnostic and therapeutic monitoring techniques are benefiting from nanotechnology. New assays using microfluidic-based microarrays are being used for genomic and proteomic analysis of cancerous samples, whereas novel nanoparticle-based contrast agents and molecular imaging approaches are entering clinical trials. Most nanotechnology tools currently in development aim to improve diagnostic sensitivity and specificity, or to increase the therapeutic index for established chemotherapeutic drugs via selective delivery to cancerous tissue. These tools are advancing cancer research and gradually moving toward the clinic, but are still more evolutionary than revolutionary. The identification of new areas of impact and development of novel nanotherapeutics could make the field of cancer nanotechnology more significant and powerful.

To assess the status of the field and provide guidance for future development, the NCI convened three one-day strategic workshops around the following topics:

Workshop I: *In vitro* Diagnostics and Prevention, February 20, 2008

Workshop II: Therapy and Post-Treatment, March 6, 2008

Workshop III: *In vivo* Diagnosis and Imaging, March 28, 2008

Participants were asked to consider what the most important goals for cancer research should be for the next 5 or 10 years, how nanotechnology can address these goals, and what barriers exist to the integration of nanotechnology and oncology. A summary of discussions from the workshops is presented here.

In vitro Diagnostics and Prevention Workshop

This workshop hosted presentations by Steven Rosen, MD (Northwestern University, Evanston, IL) and Gregg Shipp, MD, PhD (Nanosphere, Inc., Northbrook, IL) on clinical needs in oncology and David Walt, PhD (Yale University, New Haven, CT) and Paul Yager, PhD (University of Washington, Seattle, WA) on technological opportunities and challenges. The speakers discussed the limitations of current cancer-screening technologies: insufficient sensitivity and specificity to detect precancerous conditions or early-stage cancer with a low rate of false positives; inability to determine tumor stage or type; and high cost. They suggested alternative cancer indicators, including constitutive or stimulated proteins, peptides, anomalous cells in fluids or tissues, cell surface markers, genomic or proteomic signatures, and inflammation markers. Ideally, these indicators would distinguish between cancer types and stages and characterize immune

Authors' Affiliations: [1]Center for Strategic Scientific Initiatives, National Cancer Institute, Bethesda, Maryland; [2]Independent Consultant, [3]Nanotechnology Characterization Laboratory, Advanced Technology Program, SAIC-Frederick Inc., NCI-Frederick, Frederick, Maryland; and [4]Parrotfish Consulting, Louisville, Colorado

Corresponding Author: Piotr Grodzinski, National Cancer Institute, 31 Center Drive MSC2580, Building 31 Room 10A52, Bethesda, MD 20892. Phone: 301-496-1550; Fax: 301-496-7807; E-mail: grodzinp@mail.nih.gov.

doi: 10.1158/0008-5472.CAN-09-3716

response. Technical challenges to the development of these markers include protein heterogeneity, nonspecific binding, lack of good capture agents for cells and molecules, and the expense and difficulty of genomic sequencing.

It was agreed that single molecule or cell measurements are stochastic and potentially unrepresentative, making measurement of multiple markers necessary for reliable diagnosis at early stages. Nanotechnology-based sensors using quantum dots of multiple colors, Raman probes with distinct spectra, nanoparticle arrays, and nanoscale cantilevers are capable of the high-throughput, multiplexed screening these measurements would require. Improvements in genomic sequencing due to nanotechnology were also predicted (2), enabling recognition of cancer-specific genes, rapid sequencing of large and heterogeneous samples, and in depth profiling of single cells. Enhanced biomarker detection should also result in the discovery and validation of new cancer signatures.

A lively discussion resulted from a suggestion by Dr. Walt that the detection of very early-stage disease may be undesirable and lead to unnecessary treatment of lesions that would otherwise be destroyed by the innate immune system. The participants reached a clear consensus in favor of detection at the earliest possible stage.

The speakers also expressed a need for technologies that monitor tumor progression and recurrence, as well as delivery and bioavailability of administered chemotherapeutics and therapeutic efficacy. Dr. Shipp discussed early results from trials of a biobarcode assay that uses oligonucleotide-labeled gold nanoparticles to monitor PSA levels in patients following radical prostatectomy; the nanoparticle labeling results in inherent signal amplification. The new assay allows recognition of rising PSA levels as much as 2 years earlier than standard enzyme-linked immunosorbent assay (3), so that postsurgical disease management can be guided by knowledge of a patient's low or high risk of recurrence.

During working group discussions, participants proposed that highly sensitive and specific multiplexed nano-enabled detection technology would enable speedier marker validation; development of new marker types (e.g., cellular metabolic signatures, anoxia, and necrosis); and better characterization of tumor heterogeneity (e.g., tumor cell subset and immune cell and cancer stem cell recognition). The working groups also made recommendations for technology development, including the following:

- Devices for single-cell analysis of circulating tumor cells
- Microfluidics or nanopore-based technologies to produce a $1,000 genome
- New diagnostics using unprocessed bodily fluids, (e.g., blood, serum)
- Synthetic antibodies with superior affinity, specificity, and stability (4)

Participants also raised several technical issues affecting the development of reliable *in vitro* diagnostics: variability in biospecimen collection and preparation procedures, which complicate marker and device validation; poor understanding of molecular recognition processes; and uncertainty about how to best characterize disease states using biomarkers, such as static measurement versus dynamic tracking of markers, marker concentration versus absolute number versus binding affinity measurement, and marker multivalency. Binding to inert surfaces and nonspecific binding were considered of particular concern with nanoscale materials, which have large, high energy surfaces.

Therapy and Post-Treatment Workshop

In this workshop, David Parkinson, MD (Nodality, Inc., San Francisco, CA); James J. Baker, MD (University of Michigan, Ann Arbor, MI) presented clinical needs in oncology; Naomi Halas, PhD (Rice University, Houston, TX) and Joseph DeSimone, PhD (University of North Carolina, Chapel Hill, NC) presented clinical applications of nanotechnology.

Currently, most new anticancer drugs fail in clinical trials, or offer only marginal improvements to the standard of care. Dr. Parkinson suggested that in many of these trials, some patients experienced a strong benefit from the tested drugs, but the trials failed because most patients experienced no effect. This result is due to our poor comprehension of the complexity of cancer and interpatient heterogeneity, including variations in cell-signaling pathways, tumor microenvironment, and patient metabolism. The key question to be answered is what level of biological characterization is sufficient to handle this heterogeneity and match therapy to patient. There also needs to be a better understanding of the time progression of cancer, if it is to be managed as a chronic disease. Many other drugs fail in trials owing to unacceptable toxicity; nanoformulations that enable targeted tumor delivery with a corresponding decrease in side effects could rehabilitate these drugs.

Dr. Halas spoke of the intrinsic therapeutic potential of nanomaterials. Gold nanoshells can convert infrared light into heat at tumor sites, killing cancerous cells, as demonstrated by preclinical studies by Lal and colleagues (5). The FDA has approved clinical trials of this system in head and neck cancers. Plasmonic nanoparticles can also function as contrast agents, raising the possibility of a multifunctional nanoparticle platform combining therapy and monitoring.

However, issues of poor biodistribution and unknown toxicity must be addressed before nanomaterials can be clinically translated. Studies using highly uniform particle replication in nonwetting templates (PRINT) nanoparticles invented at the University of North Carolina indicate that biodistribution and cellular uptake of nanoparticles depend on the nanoparticle size, shape, deformability, and surface chemistry, but the reasons are poorly understood (6). There is also little understanding of how nanoparticles access the cell interior, complicating efforts to target drugs to intracellular compartments. The role and importance of targeting agents, (e.g., peptides, oligonucleotides, and antibodies) in delivering nanoparticles to cells and tissue must also be understood and compared with mechanistic effects, (e.g., enhanced retention and permeability of nanomaterials in leaky tumor vasculature).

In discussions following the presentations, participants identified several additional areas in which nanotechnology could affect clinical cancer practices in the next 5 to 10 years. Successful development of the rapid, multiplexed biomarker detection systems discussed in the previous section would lead to more rational and effective tumor stratification and therefore treatment choice. The ability to inexpensively collect large data sets of cancer markers across individuals and disease stages should also promote a better understanding of basic cancer biology and heterogeneity, resulting in more predictive models of cancer growth and patient response, including dormancy and metastasis. Personalized treatment regimes could then be constructed by combining the enhanced data collection and cancer models garnered through these detection systems. Highly tumor-specific targeting ligands could enhance diagnostic imaging, drug delivery, and *in vivo* monitoring of treatment response. Specific areas recommended for development included the following:

- Cell surface-targeting ligands
- *In situ* drug release from nanocarriers, triggered externally (e.g., plasmonic heating) or chemically (e.g., proteolytic peptide cleavage)
- Combination therapies, (e.g., nanoparticle hyperthermia and drug delivery)
- Multifunctional nanoplatforms, (e.g., liposomes encapsulating imaging agents and drugs or small interfering RNA [siRNA])

Another serious barrier to clinical development discussed is the lack of animal models that simulate human cancers. Most nanotherapeutic delivery exploits tumor structure, making appropriate models indispensible to evaluation of therapeutic efficacy. Unfavorable host immune and cellular response to nanomaterials, the mechanisms of which are poorly understood, must also be resolved before nanomaterials can be clinically useful.

In vivo Diagnosis and Imaging Workshop

In this workshop, James Olson, MD, PhD (Fred Hutchinson Cancer Research Center, Seattle, WA), Shimon Weiss, PhD (University of California, Los Angeles, CA), and Renata Pasqualini, PhD (University of Texas M.D. Anderson Cancer Center, Houston, TX) presented wish lists of desired cancer-imaging capabilities, surveyed promising nanotechnologies for imaging applications, and outlined the gaps between technology development and clinical application. Oncologists wish they had sufficient spatial resolution and molecular recognition abilities to detect very early-stage tumors, diagnose tumor type, and define metastases without surgery or pathology. This detailed imaging information could then be used to guide tumor and lymph node removal using intra-operative imaging or to track drug delivery and response in disease sites. This information could also be used to identify emerging resistance to chemotherapy. Dr. Olson suggested that amplification of signal from rare events, such as mitosis and anaplasia, could be used to differentiate diseased from healthy tissue, and that recognition of cellular differences, such as open versus closed chromatin, could be used to distinguish tumor types. Other diagnostically useful measures suggested were differences in the ratio of the nucleus to the cytoplasm in suspected tumors, differences in oxygen tension, pH differences, heterogeneity, and specific tumor markers.

Participants recognized the opportunity afforded by nanotechnology to do rapid, multiplexed molecular imaging in multiple modalities. If nanoprobes targeted to multiple markers could accumulate at the tumor and be detected using MRI, PET, and/or near-infrared imaging, the information could be combined to noninvasively diagnose tumor type and stage. Additional targeting to recognize multiple cell types (e.g., healthy, tumor, stem) or activities could give a complex picture of tumor microenvironment and metabolism and track tumor growth and therapeutic response. Nanoparticles coated with enzymatically or pH-sensitive peptides that experience aggregation, fluorescent quenching, or some other measureable signal change in response to peptide activation by tumor cells are already being explored for this type of imaging. Dr. Weiss suggested combining these probes with implantable devices, themselves recognizable by MRI, that conduct detailed molecular analyses of tumor tissue before, during, and after therapy (7). Deep-tissue imaging can also be achieved using fiber optics to access and illuminate targets that have previously been targeted with optically active nanomaterials, (e.g., quantum dots or Raman spectroscopy tags).

One promising avenue of research discussed by Dr. Pasqualini is the development of organ-specific and angiogenesis-related vascular ZIP codes (8) that enable region- or activity-based targeting strategies. This technique can be used to map molecular diversity and target accessible tumor receptors that can internalize and accumulate nanoparticles, increasing image signal. As an example, Dr. Pasqualini described a bioinorganic nanoparticle that binds to a lung vascular endothelial receptor and that may provide a predictive tool for drug response on the basis of imaging data (9).

To attain these ambitious goals, workshop participants identified several necessary developments. Existing imaging strategies must be quantified so that findings across centers can be compared and the effects of nanotechnology on imaging capabilities measured. A reasonable number of targets for imaging must be determined for development, as well as measures of therapeutic efficacy in addition to apoptosis, and automated image analysis software will be necessary to make meaningful sense of the data collected. There is also a pressing need for a battery of *in vitro* and *in vivo* tests to develop "go–no go" criteria for nanoparticles for *in vivo* use. Toxicity and targeting efficacy standards must be established. During discussions, participants also identified several specific technologies as being ready for development, including the following:

- Automated, microfluidics-based imaging probe synthesis (10)
- Carbon nanotube-based X-ray imaging CT scanners

- Nanomaterials with increased relaxivities for magnetic resonance–based imaging
- Nanoparticle contrast agents with external activation of therapeutic effect
- Substitution of a PET-suitable isotope into an approved nanoparticle-based therapeutic for biodistribution studies
- A national facility representing good manufacturing practice for scaling up nanoparticle production

These applications require development of suitable cancer biomarkers and targeting ligands, as well as tools to monitor and evaluate nanomaterial pharmacokinetics and cellular interactions *in vivo*. Studies are also required to determine the lower limits of tumor size that are detectable using *in vivo* imaging.

Summary

Workshop participants believed that nanotechnology applied to clinical oncology practice has the potential to better monitor therapeutic efficacy, provide novel methods for detecting and profiling early-stage cancers, and enable surgeons to delineate tumor margins and sentinel lymph nodes. The field is well positioned to provide improved methods for imaging and staging cancers and to more effectively deliver therapeutics in a targeted manner to tumors. However, nanotechnology-based cancer therapies and diagnostics need to pass several critical tests before the future of the field is assured. These tests include successful *in vivo* delivery of a targeted therapeutic, establishing viability of both targeting chemistry and nanomaterial pharmacokinetics, and deployment of a multiplexed *in vitro* diagnostic for cancer, establishing biomarker and capture agent validity and device design integrity.

Ultimately, if nanotechnology researchers can establish methods to detect tumors at a very early stage, prior to vascularization and metastasis, cancer will become a disease amenable to complete cure via surgical resection. The impact on the disease survival rates and disease management expenditures could be exceedingly high.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Capital Consulting Corporation, especially Amy S. Rabin and Jennifer Kostiuk, for organizing these strategic workshops.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Grant Support

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E.

Received 10/09/2009; revised 03/23/2010; accepted 03/25/2010; published OnlineFirst 05/11/2010.

References

1. NCI Alliance for Nanotechnology. Bethesda (MD): National Cancer Institute. Available from: http://nano.cancer.gov.
2. Wanunu M, Morrison W, Rabin Y, Grosberg AY, Meller A. Electrostatic focusing of unlabelled DNA into nanoscale pores using a salt gradient. Nat Nanotechnol 2010;5:160–5.
3. Thaxton CS, Elghanian R, Thomas AD, et al. Nanoparticle-based bio-barcode assay redefines "undetectable" PSA and biochemical recurrence after radical prostatectomy. Proc Natl Acad Sci U S A 2009; 106:18437–42.
4. Agnew HD, Rohde RD, Willward SW, et al. Iterative *in situ* click chemistry creates antibody-like protein-capture agents. Angew Chem Int Ed Engl 2009;48:4944–8.
5. Lal S, Clare SE, Halas NJ. Nanoshell-enabled photothermal cancer therapy: impending clinical impact. Acc Chem Res 2008;41:1842–51.
6. Gratton SE, Pohlhaus PD, Lee J, Guo J, Cho MJ, DeSimone JM. Nanofabricated particles for engineered drug therapies: a preliminary biodistribution study of PRINT nanoparticles. J Control Release 2007;121:10–8.
7. Daniel KD, Kim GY, Vassiliou CC, et al. Implantable diagnostic device for cancer monitoring. Biosens Bioelectron 2009;24:3252–7.
8. Arap W, Kolonin MG, Trepel M, et al. Steps toward mapping the human vasculature by phage display. Nat Med 2002;8:121–7.
9. Giordano RJ, Edwards JK, Tudor RM, Arap W, Pasqualini R. Combinatorial ligand-directed lung targeting. Proc Am Thorac Soc 2009;6: 411–5.
10. Lee CC, Sui G, Elizarov A, et al. Multistep synthesis of a radiolabeled imaging probe using integrated microfluidics. Science 2005;310: 1793–6.

Opinion

The NCI Alliance for Nanotechnology in Cancer: achievement and path forward

Krzysztof Ptak,[1] Dorothy Farrell,[1] Nicholas J. Panaro,[2] Piotr Grodzinski[1]* and Anna D. Barker[1,3]

Nanotechnology is a 'disruptive technology', which can lead to a generation of new diagnostic and therapeutic products, resulting in dramatically improved cancer outcomes. The National Cancer Institute (NCI) of National Institutes of Health explores innovative approaches to multidisciplinary research allowing for a convergence of molecular biology, oncology, physics, chemistry, and engineering and leading to the development of clinically worthy technological approaches. These initiatives include programmatic efforts to enable nanotechnology as a driver of advances in clinical oncology and cancer research, known collectively as the NCI Alliance for Nanotechnology in Cancer (ANC). Over the last 5 years, ANC has demonstrated that multidisciplinary approach catalyzes scientific developments and advances clinical translation in cancer nanotechnology. The research conducted by ANC members has improved diagnostic assays and imaging agents, leading to the development of point-of-care diagnostics, identification and validation of numerous biomarkers for novel diagnostic assays, and the development of multifunctional agents for imaging and therapy. Numerous nanotechnology-based technologies developed by ANC researchers are entering clinical trials. NCI has re-issued ANC program for next 5 years signaling that it continues to have high expectations for cancer nanotechnology's impact on clinical practice. The goals of the next phase will be to broaden access to cancer nanotechnology research through greater clinical translation and outreach to the patient and clinical communities and to support development of entirely new models of cancer care. *WIREs Nanomed Nanobiotechnol*

Cancer is arguably the most complex human disease. The poor understanding of its root-cause and its unnerving ability to spread metastatically to other organs make diagnosis and subsequent treatment of the disease very difficult. Cancer nanotechnology, a discipline at the intersection of engineering and the physical sciences with cancer biology and clinical practice, has the potential to radically alter disease outcomes. The unique and diverse properties of nanomaterials benefit oncology applications by enabling selective drug delivery to tumors, increasing therapeutic index of drugs by decreasing the toxicity associated with an effective dose, and enhancing imaging sensitivity, enabling early tumor detection, intraoperative guidance of tumor resection, and real-time monitoring of therapeutic response. Moreover, nanomaterial properties (size, charge, biocompatibility, solubility) can be manipulated to encapsulate therapeutic agents to prevent their degradation, increase half-life circulation, and facilitate tumor penetration. Similarly, nanotechnology devices are capable of simultaneously recognizing and monitoring minute amounts of several biomarkers in the *in vitro* or *in vivo* environments, enabling highly sensitive and specific diagnosis and therapeutic monitoring. [1–5]

*Correspondence to: grodzinp@mail.nih.gov

[1]Office of Cancer Nanotechnology Research, Center for Strategic Scientific Initiatives, National Cancer Institute, NIH, 31 Center Dr, Bethesda, MD 20892, USA

[2]Nanotechnology Characterization Laboratory, Advanced Technology Program, SAIC-Frederick Inc., NCI-Frederick, Frederick, 31 Center Dr, MD 20892, USA

[3]Office of Director, National Cancer Institute, NIH, 31 Center Dr, Bethesda, MD 20892, USA

DOI: 10.1002/wnan.98

The National Cancer Institute (NCI) at the National Institutes of Health (NIH) recognized the value of nanotechnology in oncology applications early. In the late nineties, it established the Unconventional Innovative Program (UIP) to work with university groups and small companies to evaluate potential nanotechnology applications in cancer. Building upon the solid experience of the UIP program, NCI established the Alliance for Nanotechnology in Cancer (ANC) in September 2004 and pledged $144 million to the 5 year initiative. NCI appreciated the unique benefits of combining the efforts of physical scientists, engineers, and technologists working at the nanoscale with cancer biologists and oncologists and funded large multidisciplinary Centers for Cancer Nanotechnology Excellence (CCNEs) as pillars of the Alliance, as shown in Figure 1. CCNE teams are focused on integrated technology solutions with practical clinical applications and pursue aggressive development of these solutions to the preclinical stage and provide a path to clinical translation. Twelve smaller collaborative Cancer Nanotechnology Platform Partnerships (CNPPs) pursue circumscribed nanotechnology projects with transformative potential for basic and/or preclinical development.

Realizing the need for a centralized facility to characterize nanomaterials, NCI also formed the Nanotechnology Characterization Laboratory (NCL) as part of the ANC. NCL's role is to perform standardized characterizations of nanoscale materials developed by researchers from academia, government and industry. NCL has worked with National Institute of Standards and Technology (NIST) and the U.S. Food and Drug Administration (FDA) scientists to develop an assay cascade that serves as the standard protocol for physicochemical, preclinical toxicological, pharmacological, and efficacy testing of nanoscale materials and devices. The information acquired from material and device studies at NCL and Alliance institutions is uploaded to the Cancer Nanotechnology Laboratory (caNanoLab), a comprehensive database accessible to the scientific community and the public.

PROGRAM ACCOMPLISHMENTS

The beginning of the program in 2005 was a trying experience. Each CCNE consisted of ~40 researchers: senior academics, young faculty, postdocs and students, representing a variety of disciplines. They had to learn a common language and establish a common set of goals. They have accomplished this and demonstrated that research and development performed in such multidisciplinary environments can produce highly creative results at a high productivity rate. A steady flow of innovation has opened up new opportunities to deepen understanding in cancer biology and to enable novel clinical techniques. Several principal investigators (PIs) within the program originate from disciplines that are non-traditional for NIH sponsored research, such as physics, materials science, and information technology. These researchers have begun to understand the needs of contemporary oncology through their partnerships with biologists and clinicians and subsequently directed their research toward the most relevant oncology problems. They have also introduced new research approaches, with a focus on platform modularity and rapid research returns. The centers have evolved into research organisms having distinct area(s) of technical excellence and core resources (e.g.,

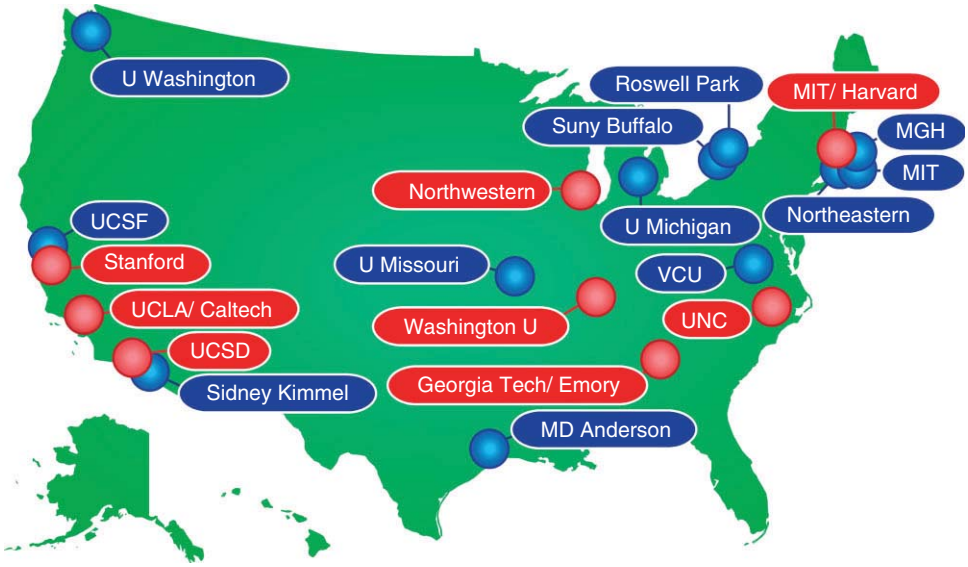


FIGURE 1 | NCI Alliance for Nanotechnology in Cancer awarded institutions (2005–2010): Centers of Cancer Nanotechnology Excellence (in red) and Cancer Nanotechnology Platform Partnerships (in blue).

fabrication and materials development, diagnostic assays, toxicology, *in vivo* technology validation, informatics). Over time, synergistic collaborations across the ANC have emerged, and several joint projects have been initiated.

To date, the ANC has generated very strong scientific output, including over 1000 peer-reviewed publications (average impact factor 7.4) and more than 250 patent disclosures and applications. This prolific output helped to establish the field of 'cancer nanotechnology'. PubMed searches for publications with the keywords 'cancer' and 'nanotechnology' indicate a steady growth in number of publications in this area over the last 5 years, shown in Figure 2.

Moreover, funding support from the ANC created a foundation of experience and accomplishment that allowed program participants to secure significant additional research and developmental funds from the federal government, philanthropic sources, industry, and foreign governments. The investigators took upon themselves the additional daunting task of commercializing the technology developed within the program. They have been prolific entrepreneurs, forming over 30 start-up companies dedicated to translational and commercial efforts. In addition, there are several mature companies (from mid-size to multinational) associated with the program; the total number of commercial entities is close to 50.

The NCI Alliance has implemented numerous training and career development mechanisms toward building an interdisciplinary field of cancer nanotechnology. The Multidisciplinary Fellowships in Cancer Nanotechnology Research were established as part of the NCI ANC program. NIH F32 and F33 National Research Service Award (NRSA) award mechanisms are used to provide postdoctoral and senior fellow trainees with interdisciplinary training specifically in the field of cancer nanotechnology. The goal of this fellowship program is to provide research scientists with an opportunity to train outside their current fields of expertise and develop multidisciplinary skill sets that can be applied in the development and testing of nanomaterials and nanodevices in cancer-related applications of diagnosis and treatment. Since the Alliance launched this program, the number of both submitted and awarded applications has steadily increased.

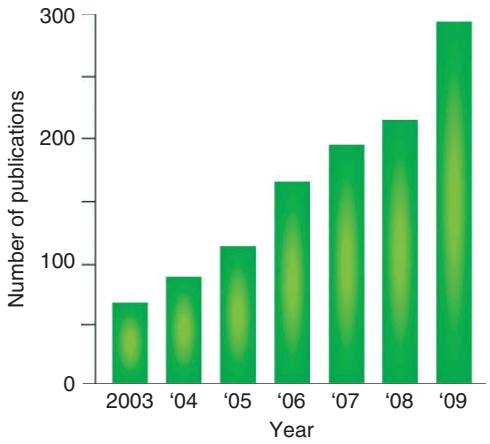


FIGURE 2 | Research Articles in Cancer Nanotechnology from 2002 to 2009. The information was retrieved from MEDLINE/PubMED indexed articles using the U.S. National Library of Medicine's Medical Subject Headings (MESH) terminology related to '*cancer*' and '*nanotechnology*'.

ANC research has focused on two complementary efforts—the development of nanomaterials and devices for cancer applications, and clinical translation of cancer nanotechnology strategies. Significant progress has been made in the development of new materials of increasing complexity and devices of superior sensitivity, speed and multiplexing capability. Input from clinicians has guided researchers in the design of technologies to address specific needs in the areas of therapy and therapeutic monitoring, *in vivo* imaging, and *in vitro* diagnostics. In what follows we will introduce some of the most exciting platforms to come out of the ANC and some of the strategies that have begun clinical translation (www.clinicaltrials.gov).

Platform Development

Dr. Joseph DeSimone, PI of the University of North Carolina (UNC), Chapel Hill CCNE (UNC CCNE), has developed a top-down manufacturing technique capable of mass production of good manufacturing practices (GMPs) quality particles with fully controllable and reproducible size, shape, matrix composition and flexibility and surface chemistry, as shown in Figure 3. His PRINT (particle replication in non-wetting templates) technology,[6] is a soft lithographic imprint technique to produce particles from diverse biologically and pharmaceutically relevant precursors.[7] PRINT is a versatile, flexible platform for preparing therapeutic materials and imaging agents. However, one of its greatest utilities so far has been preparation of model systems for the study of the *in vitro* and *in vivo* behavior of nanoparticles as a function of shape, size, stiffness, and surface charge. Studies performed on PRINT particles indicate that the effects of shape and stiffness on biodistribution and cell uptake have been insufficiently recognized and offered insight into improved design of materials for *in vivo* applications.[8] Dr. DeSimone's work is introducing a more efficient, systems engineering

approach to the preparation of nanomaterials for biomedical use, in which materials are created with precisely defined parameters optimized for specific applications.

Magnetic nanoworms are chains of iron oxide nanoparticles which greatly improved magnetic resonance imaging (MRI) contrast compared to spherical iron oxide particles.[9] They were developed through a multidisciplinary collaboration of ANC researchers that brought together the nanomaterials capabilities of Dr. Michael Sailor's group at the University of California, San Diego, the vasculature mapping and tumor microenvironment expertise of Dr. Erkki Ruoslahti's group at the Burnham Institute for Medical Research, and the medical engineering experience of Dr. Sangeeta Bhatia's group at Massachusetts Institute of Technology (MIT). Nanoworms exploit the advantageous biodistribution and reduced phagocytosis of elongated nanostructures, and coating with cell penetrating peptides[10] resulting in further enhancement of cancer cell attachment and tumor penetration in a mouse model.[11] The researchers have also developed a two stage cancer nanotechnology strategy in which gold nanorod mediated heating alters the tumor microenvironment and sensitizes the tumor to the actions of imaging (magnetic nanoworms) and treatment (liposomal doxorubicin) agents.[12] The use of nanotechnology to modify the properties of cancerous tissue for diagnostic and therapeutic effect is one of the most exciting recent trends in cancer research.

One of the ANC's primary goals is to provide patients and clinicians with tools to make informed choices regarding the most effective and least disruptive treatment for their cancer. Dr. Ralph Weissleder, co-PI of the Harvard/MIT CCNE, has been pursuing the use of lymphotropic magnetic nanoparticle MRI contrast agents to assess lymph node metastasis and to determine patient eligibility

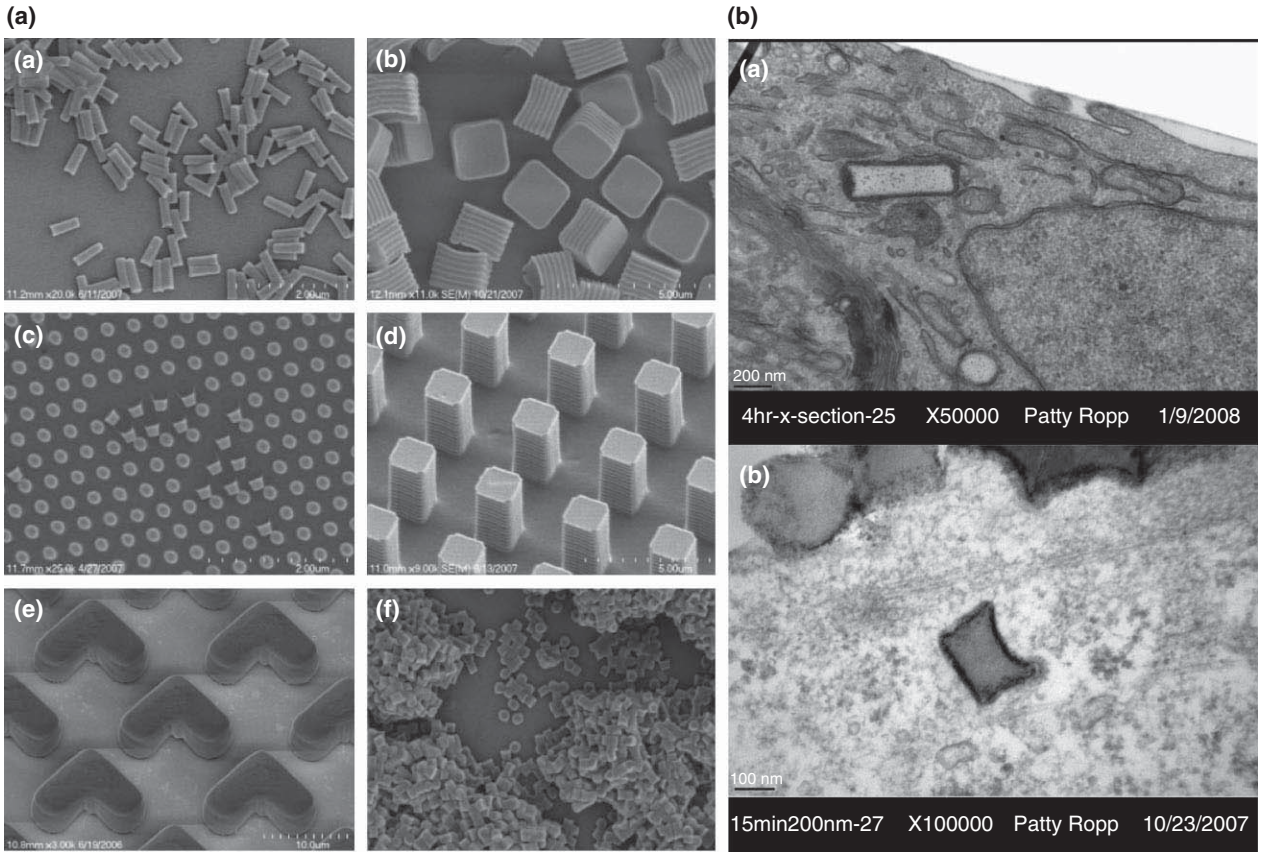


FIGURE 3 | Particle Replication In Non-wetting Templates (PRINT) and their uptakes by HeLa cells. (A) Scanning electron microscope (SEM) images of particles of various sizes, shapes, and compositions prepared via the PRINT process: (a) hydrogel rods containing antisense oligonucleotide; (b) crosslinked degradable matrix cubes containing doxorubicin HCl; (c) abraxane harvested onto medical adhesive; (d) insulin particles harvested onto a medical adhesive; (e) hydrogel 'boomerangs' containing 15 wt% iron oxide; (f) hydrogel cylinders containing 10 wt% Omniscan.
(B) Transmission electron microscopy (TEM) image showing HeLa cell internalization of 150 × 450 nm (top) or 200 × 200 nm (bottom) cylindrical particles fabricated via the PRINT process. (Panels A and B reprinted with permission from Ref 6. Copyright © 2009 John Wiley & Sons, Inc.).

for salvage radiation therapy.[13–16] Dr. Chad Mirkin, PI of the Northwestern University CCNE, is using a gold nanoparticle-based bio-barcode assay, shown in Figure 4, to monitor Prostate Specific Antigen (PSA) levels in patients following radical prostatectomy and assess response to adjuvant and salvage therapy.[17,18] This assay has protein detection sensitivity as much as six orders of magnitude higher than standard ELISA assays and the potential for broad application in the detection of other cancer biomarkers, such as peptides and nucleic acids.

Significant advances in *in vitro* diagnostic assay technology have been made in the laboratory of Dr. James R. Heath, PI of the NanoSystems Biology Cancer Center (NSBCC), a CalTech/UCLA-based CCNE. Dr. Heath's work has ranged from the development of synthetic antibodies, cheaper and more stable than the natural versions[19], to clinical testing of the Integrated Blood-Barcode Chip (IBBC),[20] capable of multiplexed detection of proteins in whole blood samples. Working with Dr. Paul Mischel of UCLA, Dr. Heath is using his IBBC for the molecular and functional analysis of glioblastoma tumors, to identify patients with the greatest potential for positive response to Avastin therapy. This is an early step toward personalized cancer care.

The ANC has also supported development of both materials and instrumentation to enable early disease detection and non-invasive typing and staging. Researchers at the Stanford University CCNE, led by PI Dr. Sanjiv Sam Gambhir, have been developing multimodal, multiplexed molecular imaging probes,

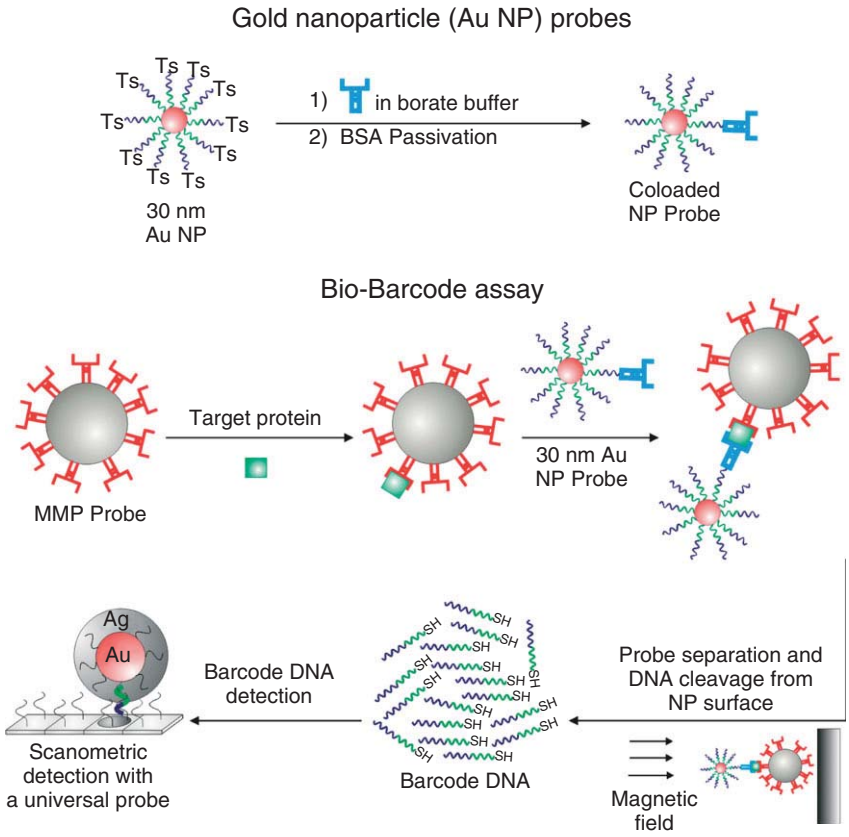


FIGURE 4 | Schematic representation of the PSA Au-NP probes (Upper) and the PSA bio-barcode assay (Lower). (Upper) Barcode DNA-functionalized Au-NPs (30 nm) are conjugated to PSA-specific antibodies through barcode terminal tosyl (Ts) modification to generate the coloaded PSA Au-NP probes. In a second step, the PSA Au-NP probes are passivated with BSA. (Lower) The bio-barcode assay is a sandwich immunoassay. First, MMPs surfacefunctionalized with monoclonal antibodies to PSA are mixed with the PSA target protein. The MMP-PSA hybrid structures are washed free of excess serum components and resuspended in buffer. Next PSA Au-NP probes are added to sandwich the MMP-bound PSA. Again after magnetic separation and wash steps, the PSA-specific DNA barcodes are released into solution and detected using the scanometric assay, which takes advantage of Au-NP catalyzed silver enhancement. Approximately 1/2 of the barcode DNA sequence (green) is complementary to the 'universal' scanometric Au-NP probe DNA, and the other 1/2 (purple) is complementary to a chip-surface immobilized DNA sequence that is responsible for sorting and binding barcodes complementary to the PSA barcode sequence. (Reprinted with permission from Ref 18. Copyright © 2009 National Academy of Science USA).

including Raman spectroscopy probes[21] and carbon nanotubes for photoacoustic imaging.[22] They are establishing the predictive power of tumor biochemical characteristics with respect to tumor progression and treatment response. Dr. Otto Zhou of the UNC CCNE has invented a carbon nanotube-based, low temperature, high density X-ray cathode,[23] which has great clinical potential for use in a new generation of high speed, low power stationary X-ray tomography machines.[24]

The ANC has also made a progress in development of biocompatible nanoparticles for molecular imaging and targeted therapy. Efforts of Dr. Nie's team from GeorgiaTech/Emory CCNE have led to the development of luminescent quantum dots (QDs) for multiplexed molecular diagnosis and *in vivo* imaging. In comparison with organic dyes and fluorescent proteins, QDs have unique optical and electronic properties, with size-tunable light emission, superior signal brightness, resistance to photobleaching, and broad absorption spectra for simultaneous excitation of multiple fluorescence colors. QDs also provide a versatile nanoscale scaffold for designing multifunctional nanoparticles with both imaging and therapeutic functions.[25] Although QDs are clinical translation ready for *in vitro* applications, most current QD compositions contain toxic heavy metal elements, limiting their use *in vivo*. In addition, the QDs currently used for most biomedical research resist internalization by cells and are prone to aggregation in the cytoplasm. Dr. Nie's lab has recently produced QDs functionalized with two polymers that show increased cellular uptake and decreased aggregation. [26]

The field of oncology may also benefit by employing dendritic polymer or dendrimers developed by Dr. James R. Baker Jr. at University of Michigan. Dendrimers can be applied to a variety of cancer therapies (such as photodynamic and gene therapies) to improve safety and efficacy and can also improve performance of nanoparticle image contrast enhancement agents.[27] Work by Dr. Baker's group on iron oxide nanoparticles functionalized with a dendrimer shell containing a folate targeting group enabled imaging of early stage tumors that overexpress folic acid receptors.[28] Dendrimer labeling was also found to improve performance by activatable cell penetrating peptides developed by Dr. Roger Tsien of the University of California, San Diego, possibly by increasing size, thereby decreasing renal filtration.[29]

Clinical Translation

One of several advantages that nanotechnology offers is targeted delivery of previously discarded therapeutic agents that have poor pharmacological properties or deleterious side effects. Dr. Mark Davis of the NSBCC has developed Cyclosert, a rationally designed delivery system based on cyclic repeating molecules of cyclodextrin, polyethylene-glycol and L-cysteine. Cyclosert is being used to deliver camptothecin, a potent naturally occurring anticancer compound with significant pharmacological shortcomings, and siRNA, which will otherwise rapidly degrade *in vivo*. Cyclosert particles are typically between 30 and 80 nm in diameter, hydrophilic with neutral surface charge and have extended blood circulation times. Camptothecin conjugated Cyclosert, IT-101, is currently in an open-label, dose-escalation clinical phase study in patients with solid tumor malignancies. A Cyclosert-siRNA formulation, CALAA-01, is being used for the targeted delivery of siRNA using human transferrin as a cancer cell targeting ligand. Its efficacy relies on the enhanced permeability and retention (EPR) effect for tumor access, uptake into cancer cells via transferrin receptor-mediated endocytosis and subsequent pH mediated siRNA release into the cytoplasm, as shown in Figure 5. CALAA-01 was used for the first treatment of a human patient with targeted siRNA delivery in a phase I clinical trial in May 2008.[30–32]

Nanotechnology is also having an impact on molecular imaging applications for cancer. Drs. Caius Radu, Owen Witte and Michael Phelps at the NSBCC have developed a new positron emission tomography (PET) imaging agent, [^{18}F]FAC (1-(2′-deoxy−2′-[^{18}F] fluoroarabinofuranosyl) cytosine), using a microfluidic circuit for rapid radiochemical synthesis.[33] This new PET probe allows visualization of immune organs and is sensitive to alternations in lymphoid mass and immune status, as shown in Figure 6, and can be used to monitor immunosuppressive therapy. Pre-therapy imaging with the [^{18}F]-FAC family of PET probes is currently undergoing clinical testing as a method to assign patients to chemotherapy drugs regimes, e.g., gemcitabine, cytarabine, fludarabine, in a variety of cancers.[34,35]

Numerous other materials developed by ANC researchers are also entering clinical trials. Drs. Greg Lanza and Samuel Wickline of the Siteman CCNE at Washington University in St. Louis have developed a nanoparticle MRI contrast agent that binds to the $\alpha_v\beta_3$-integrin found on the surface of the newly developing blood vessels associated with early tumor development.[36–38] This agent is currently undergoing Phase I clinical trials to assess its safety for human use. Drs. Robert Langer and Omid Farokhzad of the MIT/Harvard CCNE have developed a polymeric matrix for encapsulating

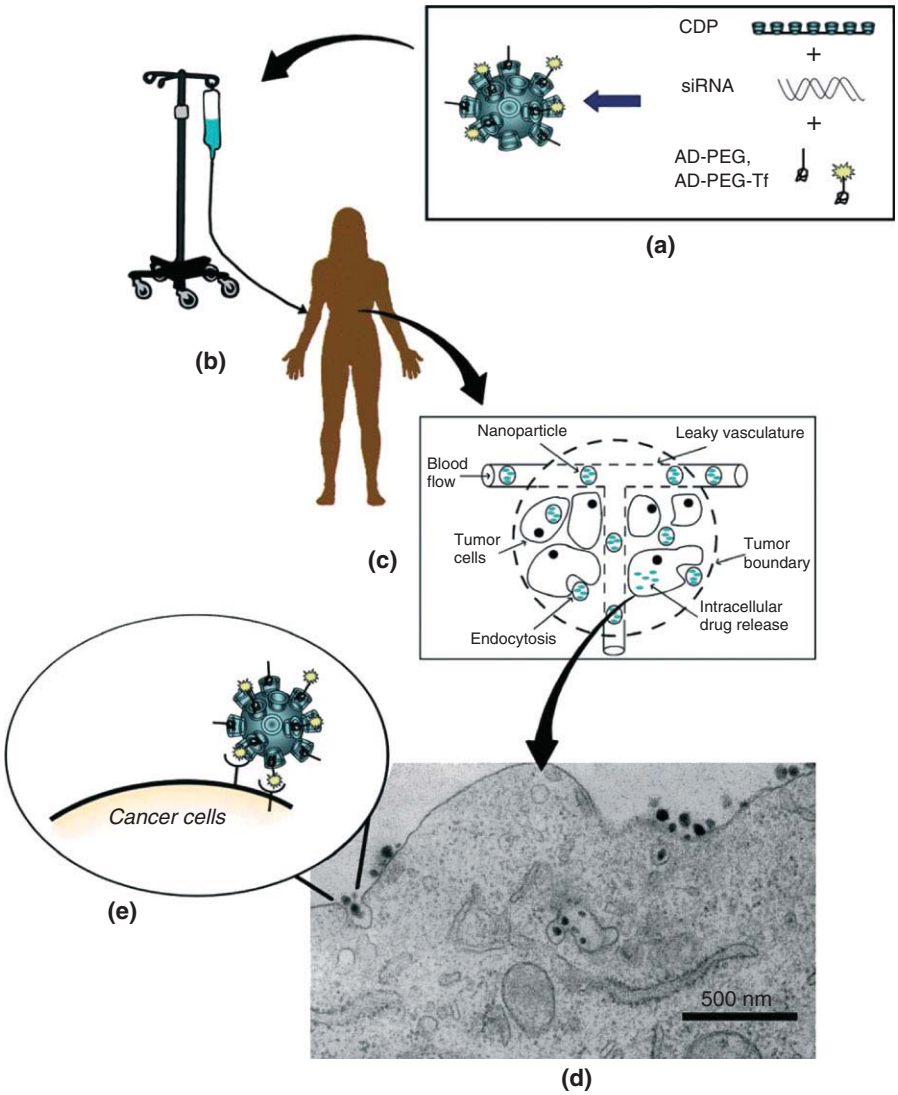


FIGURE 5 | Schematic of how the targeted nanoparticles function. (a) Nanoparticles are assembled from the four components (see Figure 2). (b) Aqueous solutions of nanoparticles are infused into patients. (c) The nanoparticles circulate in the blood of the patient and escape via the 'leaking' blood vessels in tumors. (d) Nanoparticles penetrate though the tumor and enter into cells by receptor-mediated endocytosis (transmission electron micrograph of 50 nm nanoparticles entering a cancer cell). Note that the nanoparticles enter and are initially located in vesicles within the cell and must escape and disassemble to deliver their payload. (e) Targeted nanoparticles can have numerous interactions (e.g., Tf with its receptor) on the surface of the cancer cell that then stimulate entrance into the cell. (Reprinted with permission from Ref 30. Copyright © 2009 American Chemical Society).

therapeutic payloads that also features functional surface moieties and targeting ligands which allow for particle design optimization (accumulation in target tissue, immune system avoidance, and desired drug release profile) independent of therapeutic payload. The matrix is expected to enter clinical trials this year.[39] Clinical trials are also anticipated for a combination MRI contrast agent/drug delivery system developed by Dr. Miqin Zhang of the University of Washington CNPP for Pediatric Brain Cancer Imaging and Therapy for the treatment of glioblastoma. [40–43]

GOALS FOR THE FUTURE

The ANC has demonstrated that a multidisciplinary approach to research can catalyze scientific developments and achieve clinical translation. ANC investigators have advanced diagnostic technology, using both *in vitro* assays and novel imaging methods, and offered improved therapies and therapeutic efficacy measures. Many of the technologies developed and clinically translated have applied novel engineering to existing cancer biology strategies. The next stage of cancer nanotechnology research should introduce entirely new models of cancer care, where progress in cancer biology and understanding of the disease is enabled by new nanotechnologies.

Future advances in nanotherapy will be based on distinctive nanomaterial properties capabilities, such as nanoparticle mediated hyperthermia or recognition and alteration of the tumor microenvironment. Drugs and devices will converge in multifunctional systems that release therapeutics in response to biochemical signals detected in the tumor or blood.[18] The era of personalized therapeutics will emerge, as low cost genomic and proteomic profiling will enable more

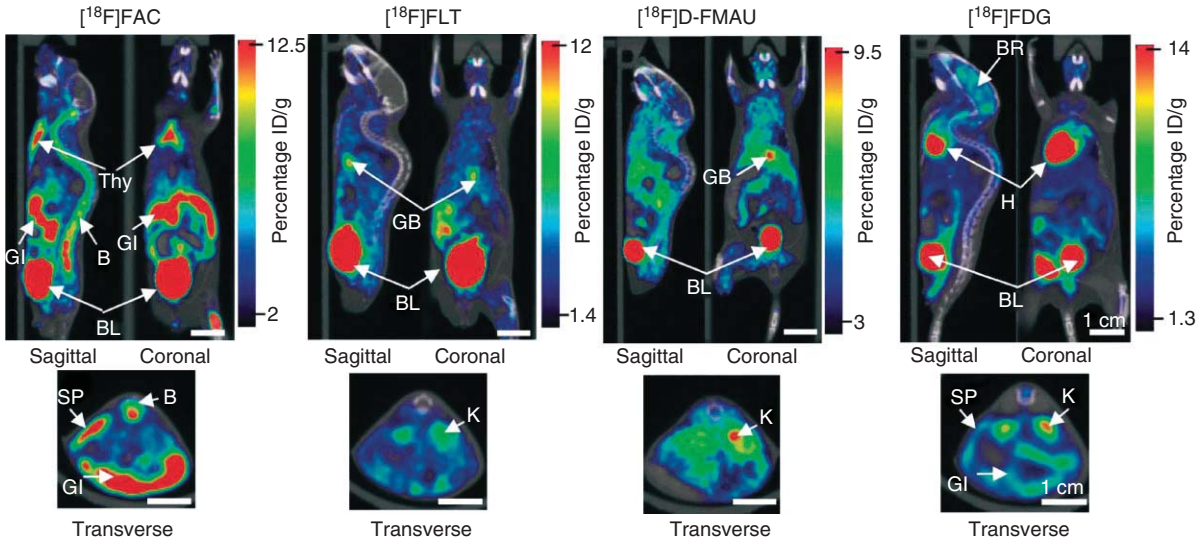


FIGURE 6 | [18F]FAC has better selectivity for lymphoid organs compared with other PET probes for nucleoside metabolism and glycolysis. C57/BL6 mice were scanned by microPET-CT using [18F]FAC, [18F]FLT, [18F]DFMAU and [18F]FDG. Mice were imaged 60 min after intravenous injection of probes. B: bone; BL: bladder; BR: brain; GB: gall bladder; GI: gastrointestinal tract; H: heart; K: kidney; L: liver; LU: lung; SP: spleen; Thy: thymus; BM: bone marrow; ST: stomach. (Adopted by permission from Ref 33. Copyright © 2008 Nature Publishing Group).

detailed identification of tumor types and effective patient-therapy matching. Smarter clinical trials will more accurately respond to the heterogeneity of cancer lesions and patient metabolisms and identify drugs effective for patient subpopulations. Monitoring of patient response via molecular imaging of tumors and *in vitro* measurement of immune response markers has already begun, but will rapidly advance.

In vivo molecular imaging capabilities will enable optical biopsies, with tumors being typed and staged at the time of detection. More complete molecular characterization of lesions will also allow clinicians to recognize and prevent chemoresistance. The combination of advanced imaging with traditional surgical techniques for intraoperative guidance will enable more successful resection of cancerous growths, which is still the most effective cure available for many cancers.

Many of the advances envisioned in therapeutics and imaging will depend on advances in *in vitro* assay technology, particularly the identification and validation of additional cancer biomarkers. These will include markers of tumor metabolism, growth and dormancy as well as type and stage. The development of biomarkers other than proteins will be essential. Microfluidics will be a backbone technology for many of these advances. Work on the collection and analysis of circulating tumor cells has begun but will increase in complexity and utility in the coming years.

The NCI would like to see more research in cancer nanotechnology prevention, currently an underdeveloped area. Nanoparticle formulations of chemopreventives are one avenue for investigation, but the hope is that other innovative systems for cancer prevention will also emerge.

The new edition of the ANC begins in September 2010, and will consist of a newly selected batch of CCNEs and CNPPs chosen in an open competition. The CCNEs of this new program edition will have a greater focus on clinical translation; it is expected that by the end of the next phase of the ANC, each Center will have at least one nanotechnology strategy in clinical trials. The new program will emphasize more heavily cancers having particularly poor outcomes, including brain, lung, pancreatic, and ovarian cancers.

The CCNEs and CNPPs will be joined by several Cancer Nanotechnology Training Centers (CNTCs), intended to prepare a cadre of researchers with multidisciplinary training who are skilled in applying the tools of nanotechnology to critical problems in cancer research and clinical oncology. Although the NCI recognizes the progress made during the first phase of the ANC in establishing the field of cancer nanotechnology, the program's next phase is intended to have greater cross-pollination of research ideas and pursuits between ANC sites. It is also an ANC goal to broaden access to cancer nanotechnology research through greater outreach to the patient and clinical communities, both by NCI program staff and extramural ANC researchers.

The NCL will continue to act as a national resource for cancer nanotechnology researchers.

Having begun the process of standardizing bionanomaterials, the challenge facing the ANC is to promote widespread acceptance of NCL established protocols within the research and development community. In addition to preclinical characterization and regulatory obstacles, GMPs such as scale-up process, purity and batch-to-batch consistency have to be established for nanomaterials. This is one of the major challenges facing the next stage of the ANC.

CONCLUSION

The mission of NCI and the ANC is to relieve the burden of suffering due to cancer; this means that the measure of success of the ANC is the creation of innovative solutions to disease prevention, diagnosis, and treatment and translation of these research findings into improved clinical practices. Reflecting a proactive approach to moving the innovative technologies developed within the ANC into the clinic, the program witnessed a substantial amount of work with animal models, experimentation with human clinical samples, and emerging human clinical trials. In re-issuing the ANC, NCI has signaled that it continues to have high expectations for cancer nanotechnology's impact on clinical practice. The ANC and the research community expect to see truly transformative technologies emerge from the next phase of the program.

ACKNOWLEDGEMENTS

This project has been funded in whole or in part with federal funds from the NCI, NIH, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

REFERENCES

1. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer* 2005, 5:161–71.
2. Nie S, Xing Y, Kim GJ, Simons JW. Nanotechnology applications in cancer. *Annu Rev Biomed Eng* 2007, 9:257–288.
3. Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat Rev Drug Discov* 2008, 7:771–82.
4. Heath JR, Davis ME. Nanotechnology and cancer. *Annu Rev Med* 2008, 59:251–65.
5. Singhal S, Nie S, Wang MD. Nanotechnology applications in surgical oncolog. *Annu Rev Med* 2010, 61:359–73.
6. Canelas DA, Herlihy KP, DeSimone JM. Top-down particle fabrication: control of size and shape for diagnostic imaging and drug delivery. *Wiley Interdiscip Rev: Nanomed Nanobiotechnol* 2009, 1:391–404.
7. Kelly JY, DeSimone JM. Shape-specific, monodisperse nano-molding of protein particles. *J Am Chem Soc* 2008, 130:5438–9.
8. Gratton SE, Pohlhaus PD, Lee J, Guo J, Cho MJ, Desimone JM. Nanofabricated particles for engineered drug therapies: a preliminary biodistribution study of print nanoparticles. *J Control Release* 2007, 121:10–8.
9. Park JH, von Maltzahn G, Zhang L, Schwartz MP, Ruoslahti E, Bhatia SN, Sailor MJ. Magnetic iron oxide nanoworms for tumor targeting and imaging. *Adv Mater* 2008, 20:1589.
10. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, Hanahan D, Mattrey RF, Ruoslahti E. Tissue-penetrating delivery of compounds and nanoparticles into tumors. *Cancer Cell* 2009, 16:510–520.
11. Park JH, von Maltzahn G, Zhang L, Derfus AM, Simberg D, Harris TJ, Ruoslahti E, Bhatia SN, Sailor MJ. Systematic surface engineering of magnetic nanoworms for *in vivo* tumor targeting. *Small* 2009, 5:694–700.
12. Park J-H, von Maltzahn G, Xu MJ, Fogal V, Kotamraju VR, Ruoslahti E, Bhatia SN, Sailor MJ. Cooperative nanomaterial system to sensitize, target, and treat tumors. *Proc Nat Acad Sci U S A* 2009, 107:981–986.
13. Fulci G, Breymann L, Gianni D, Kurozomi K, Rhee SS, Yu J, Kaur B, Louis DN, Weissleder R, Caligiuri MA, et al. Cyclophosphamide enhances glioma virotherapy by inhibiting innate immune responses. *Proc Nat Acad Sci U S A* 2006, 103:12873–12878.
14. Kelly KA, Bardeesy N, Anbazhagan R, Gurumurthy S, Berger J, Alencar H, Depinho RA, Mahmood U, Weissleder R. Targeted nanoparticles for imaging incipient pancreatic ductal adenocarcinoma. *PLoS Med* 2008, 5:e85.

15. Kirsch DG, Dinulescu DM, Miller JB, Grimm J, Santiago PM, Young NP, Nielsen GP, Quade BJ, Chaber CJ, Schultz CP, et al. A spatially and temporally restricted mouse model of soft tissue sarcoma. *Nat Med* 2007, 13:992–997.

16. Weissleder R, Pittet MJ. Imaging in the era of molecular oncology. *Nature* 2008, 452:580–589.

17. Goluch ED, Nam JM, Georganopoulou DG, Chiesl TN, Shaikh KA, Ryu KS, Barron AE, Mirkin CA, Liu C. A bio-barcode assay for on-chip attomolar-sensitivity protein detection. *Lab Chip* 2006, 6:1293–1299.

18. Thaxton CS, Daniel WL, Giljohann DA, Thomas AD, Mirkin CA. Templated spherical high density lipoprotein nanoparticles. *J Am Chem Soc* 2009, 131:1384–1385.

19. Agnew HD, Rohde RD, Millward SW, Nag A, Yeo WS, Hein JE, Pitram SM, Tariq AA, Burns VM, Krom RJ, et al. Iterative in situ click chemistry creates antibody-like protein-capture agents. *Angew Chem Intl Ed Engl* 2009, 48:4944–8.

20. Fan R, Vermesh O, Srivastava A, Yen BKH, Qin LD, Ahmad H, Kwong GA, Liu CC, Gould J, Hood L, et al. Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. *Nat Biotech* 2008, 26:1373–1378.

21. Zavaleta CL, Smith BR, Walton I, Doering W, Davis G, Shojaei B, Natan MJ, Gambhir SS. Multiplexed imaging of surface enhanced raman scattering nanotags in living mice using noninvasive raman spectroscopy. *Proc Nat Acad Sci U S A* 2009, 106:13511–6.

22. De la Zerda A, Zavaleta C, Keren S, Vaithilingam S, Bodapati S, Liu Z, Levi J, Smith BR, Ma TJ, Oralkan O, et al. Carbon nanotubes as photoacoustic molecular imaging agents in living mice. *Nat Nanotechnol* 2008, 3:557–562.

23. Calderon-Colon X, Geng H, Gao B, An L, Cao G, Zhou O. A carbon nanotube field emission cathode with high current density and long-term stability. *Nanotechnology* 2009, 20:325707.

24. Qian X, Zhou X, Nie S. Surface-enhanced raman nanoparticle beacons based on bioconjugated gold nanocrystals and long range plasmonic coupling. *J Am Chem Soc* 2008, 130:14934–5.

25. Smith AM, Mohs AM, Nie S. Tuning the optical and electronic properties of colloidal nanocrystals by lattice strain. *Nat Nanotechnol* 2009, 4:56–63.

26. Duan H, Nie S. Cell-penetrating quantum dots based on multivalent and endosome-disrupting surface coatings. *JACS* 2007, 129:3333–3338.

27. Majoros IJ, Williams CR, Baker JR. Current dendrimer applications in cancer diagnosis and therapy. *Curr Top Med Chem* 2008, 8:1165–1179.

28. Shi X, Wang SH, Swanson SD, Ge S, Cao Z, Van Antwerp ME, Landmark KJ, Baker JR. Dendrimer-functionalized shell-crosslinked iron oxide nanoparticles for *in vivo* magnetic resonance imaging of tumors. *Adv Mat* 2008, 20:1671–1678.

29. Olson ES, Aguilera TA, Jiang T, Ellies LG, Nguyen QT, Wong EH, Grossaf LA, Tsien RY. *In vivo* characterization of activatable cell penetrating peptides for targeting protease activity in cancer. *Integr. Biol* 2009, 1:382–393.

30. Davis M. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. *Mol Pharma* 2009, 6:659–668.

31. Davis M. Design and development of IT-101, a cyclodextrin-containing polymer conjugate of camptothecin. *Adv Drug Delivery Rev* 2009, 61:1189–1192.

32. Schluep T, Hwang J, Hildebrandt IJ, Czernin J, Choi CH, Alabi CA, Mack BC, Davis ME. Pharmacokinetics and tumor dynamics of the nanoparticle IT-101 from PET imaging and tumor histological measurements. *Proc Nat Acad Sci U S A* 2009, 106:11394–11399.

33. Radu CG, Shu CJ, Nair-Gill E, Shelly SM, Barrio JR, Satyamurthy N, Phelps ME, Witte ON. Molecular imaging of lymphoid organs and immune activation by positron emission tomography with a new [^{18}F]-labeled 2′-deoxycytidine analog. *Nat Med* 2008, 14:783–788.

34. Tumeh PC, Radu CG, Ribas A. PET imaging of cancer immunotherapy. *J Nucl Med* 2008, 49:865–868.

35. Laing RE, Walter MA, Campbell DO, Herschman HR, Satyamurthy N, Phelps ME, Czernin J, Witte ON, Radu CG. Noninvasive prediction of tumor responses to gemcitabine using positron emission tomography. *Proc Nat Acad Sci U S A* 2009, 106:2847–2852.

36. Lijowski M, Caruthers S, Hu G, Zhang H, Scott MJ, Williams T, Erpelding T, Schmieder AH, Kiefer G, Gulyas G, et al. High sensitivity: high-resolution SPECT-CT/MR molecular imaging of angiogenesis in the vx2 model. *Invest Radiol* 2009, 44:15–22.

37. Winter PM, Schmieder AH, Caruthers SD, Keene JL, Zhang H, Wickline SA, Lanza GM. Minute dosages of alpha(nu)beta3-targeted fumagillin nanoparticles impair vx-2 tumor angiogenesis and development in rabbits. *FASEB J* 2008, 22:2758–2767.

38. Schmieder AH, Caruthers SD, Zhang H, Williams TA, Robertson JD, Wickline SA, Lanza GM. Three-dimensional MR mapping of angiogenesis with alpha5beta1(alpha nu beta3)-targeted theranostic nanoparticles in the mda-mb-435 xenograft mouse model. *FASEB J* 2008, 22:4179–4189.

39. Salvador-Morales C, Gao W, Ghatalia P, Murshed F, Aizu W, Langer R, Farokhzad OC. Multifunctional nanoparticles for prostate cancer therapy. *Expert Rev Anticancer Ther* 2009, 9:211–221.

40. Zhang L, Gu F, Chan J, Wang A, Langer R, Farokhzad O. Nanoparticles in medicine: Therapeutic

applications and developments. *Clin Pharmacol Therap* 2008, 83:761–769.

41. Veiseh O, Sun C, Fang C, Bhattarai N, Gunn J, Kievit F, Du K, Pullar B, Lee D, Ellenbogen RG, et al. Specific targeting of brain tumors with an optical/magnetic resonance imaging nanoprobe across the blood-brain barrier. *Cancer Res* 2009, 69: 6200–6207.
42. Veiseh O, Gunn JW, Kievit FM, Sun C, Fang C, Lee JS, Zhang M. Inhibition of tumor-cell invasion with chlorotoxin-bound superparamagnetic nanoparticles. *Small* 2009, 5:256–264.
43. Sun C, Veiseh O, Gunn J, Fang C, Hansen S, Lee D, Sze R, Ellenbogen RG, Olson J, Zhang M. *In vivo* MRI detection of gliomas by chlorotoxin-conjugated superparamagnetic nanoprobes. *Small* 2008, 4:372–379.

Appendix 5: Key Cancer Nanotechnology Publications

1. Bailey RC, Kwong GA, Radu CG, Witte ON, and Heath JR. DNA-encoded antibody libraries: A unified platform for multiplexed cell sorting and detection of genes and proteins. *J Am Chem Soc*, 2007, 129: p. 1959-1967.

2. Davis ME, Zuckerman JE, Choi CHJ, Seligson D, Tolcher A, Alabi CA, Yen Y, Heidel JD, and Ribas A. Evidence of RNAi in humans from systematically administered siRNA via targeted nanoparticles. *Nature*, 2010, 467: p. 1067-1070.

3. Davis M. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: From concept to clinic. *Molecular Pharmaceutics*, 2009, 6(3): p. 659-668.

4. Davis M. Design and development of IT-101, a cyclodextrin-containing polymer conjugate of camptothecin. *Adv Drug Deliv Rev*, 2009, 61(13): p. 1189-1192.

5. Fan R, Vermesh O, Srivastava A, Yen BKH, Qin LD, Ahmad H, Kwong GA, Liu CC, Gould J, Hood L, and Heath JR. Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. *Nature Biotechnology*, 2008, 26(12): p. 1373-1378.

6. Gaster R, Hall D, Nielsen C, Osterfeld S, Yu H, Mach K, Wilson RJ, Murmann B, Liao JC, Gambhir SS, and Wang SX. Matrix-insensitive protein assays push the limits of biosensors in medicine. *Nature Medicine*, 2009, 15(11): p. 1327-U130.

7. Giljohann D and Mirkin C. Drivers of biodiagnostic development. *Nature*, 2009, 462(7272): p. 461-464.

8. Gratton SE, Williams SS, Napier ME, Pohlhaus PD, Zhou Z, Wiles KB, Maynor BW, Shen C, Olafsen T, Samulski ET, and Desimone JM. The pursuit of a scalable nanofabrication platform for use in material and life science applications. *Acc Chem Res*, 2008, 41(12): p. 1685-1695.

9. Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, and DeSimone JM. The effect of particle design on cellular internalization pathways. *Proc Natl Acad Sci U S A*, 2008, 105: p. 11613-11618.

10. Green MR, Manikhas GM, Orlov S, Afanasyev B, Makhson AM, Bhar P, and Hawkins MJ. Abraxane, a novel Cremophor-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer. *Ann Oncol*, 2006, 17(8): p. 1263-1268.

11. Gu F, Zhang L, Teply BA, Mann N, Wang A, Radovic-Moreno AF, Langer R, and Farokhzad OC. Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. *Proc Natl Acad Sci U S A*, 2008, 105(7): p. 2586-2591.

12. Heath JR and Davis ME. Nanotechnology and cancer. *Annu Rev Med*, 2007, 59: p. 251-265.

13. Hawkins MJ, Soon-Shiong P, and Desai N. Protein nanoparticles as drug carriers in clinical medicine. *Adv Drug Deliv Rev*, 2008, 60(8): p. 876-885.

14. Lee H, Yoon T, Figueiredo J, Swirski F, and Weissleder R. Rapid detection and profiling of cancer cells in fine-needle aspirates. *Proc Natl Acad Sci U S A*, 2009, 106(30): p. 12459-12464.

15. Mahmud G, Campbell CJ, Bishop KJM, Komarova YA, Chaga O, Soh S, Huda S, Kandere-Grzybowska K, and Grzybowski BA. Directing cell motions on micropatterned ratchets. *Nat Physics*, 2009, 5: p. 606-612.

16. Maltz JS, Sprenger F, Fuerst J, Paidi A, Fadler F, and Bani-Hashemi AR. Fixed gantry tomosynthesis system for radiation therapy image guidance based on a multiple source x-ray tube with carbon nanotube cathodes. *Med Phys*, 2009, 36: p. 1624-1636.

17. Park J-H, von Maltzahn G, Xu MJ, Fogal V, Kotamraju VR, Ruoslahti E, Bhatia SN, and Sailor MJ. Cooperative nanomaterial system to sensitize, target and treat tumors. *Proc Natl Acad U S A*, 2009, 107(3): p. 981-986.

18. Radu CG, Shu CJ, Nair-Gill E, Shelly SM, Barrio JR, Satyamurthy N, Phelps ME, and Witte ON. Molecular imaging of lymphoid organs and immune activation by positron emission tomography with a new [18F]-labeled 2'-deoxycytidine analog. *Nat Med*, 2008, 14(7): p. 783-788.

19. Salvador-Morales C, Gao WW, Ghatalia P, Murshed F, Aizu W, Langer R, and Farokhzad OC, Multifunctional nanoparticles for prostate cancer therapy. *Expert Rev Anticancer Therapy*, 2009, 9 (2): p. 211-221.

20. Sequist LV, Nagrath S, Toner M, Haber DA, and Lynch TJ. The CTC-chip: An exciting new tool to detect circulating tumor cells in lung cancer patients. *J Thorac Oncol*, 2009, 4(3):281-283.

21. Shi X, Wang SH, Swanson SD, Ge S, Cao Z, Van Antwerp ME, Landmark KJ, and Baker JR, Dendrimer-functionalized shell-crosslinked iron oxide nanoparticles for in vivo magnetic resonance imaging of tumors. *Advanced Materials*, 2008, 20(9): p. 1671-1678.

22. Teesalu T, Sugahara KN, Kotamraju VR, and Ruoslahti E. C-end rule peptides mediate neuropilin-1-dependent cell, vascular, and tissue penetration. *Proc Natl Acad Sci*, 2009, 106: p. 16157-16162.

23. Thaxton CS, Elghanian R, Thomas AD, Stoeva SI, Lee JS, Smith ND, Schaeffer AJ, Klocker H, Horninger W, Bartsch G, and Mirkin CA. Nanoparticle-based bio-barcode assay redefines "undetectable" PSA and biochemical recurrence after radical prostatectomy. *Proc Natl Acad Sci U S A*, 2008, 105(7): p. 2586-2591.

24. Weissleder R. Molecular imaging in cancer. *Science*, 2006, 312: p. 1168-1171.

25. Zheng D, Seferos DS, Giljohann DA, Patel PC, and Mirkin CA. Aptamer nano-flares for molecular detection in living cells. *Nano Lett*, 2009, 9: p. 3258-3261.

26. Zhang L, Gu F, Chan J, Wang A, Langer R, and Farokhzad O. Nanoparticles in medicine: Therapeutic applications and developments. *Clin Pharmacol and Therap*, 2008, 83(5): p. 761-769.

Appendix 6: Listing of Grant Awards in Phases I and II of the ANC Program

Phase I: Centers for Cancer Nanotechnology Excellence (CCNEs) (U54)		
Institution	**Principal Investigators**	**Scientific Focus**
Carolina Center of Cancer Nanotechnology Excellence		
University of North Carolina	Rudolph Juliano Joseph DeSimone	To fabricate "smart," or targeted, nanoparticles and other nanodevices for cancer therapy and imaging
Center for Cancer Nanotechnology Excellence Focused on Therapy Response [Awarded in February 2006]		
Stanford University	Sanjiv Sam Gambhir	To develop nanotechnology-enabled diagnostic tools to advance cancer detection and therapy techniques
Nanosystems Biology Cancer Center		
California Institute of Technology	Jim Heath Leroy Hood Michael Phelps	To develop and validate tools for early detection and stratification of cancer through rapid and quantitative measurement of panels of serum and tissue-based biomarkers
Nanomaterials for Cancer Diagnostics and Therapeutics CCNE		
Northwestern University	Chad Mirkin	To design and test nanomaterials and nanodevices for highly sensitive in vitro detection platforms
MIT-Harvard Center of Cancer Nanotechnology Excellence		
MIT-Harvard	Robert Langer Ralph Weissleder	To develop diversified nanoplatforms for targeted therapy, diagnostics, noninvasive imaging, and molecular sensing
Emory-Georgia Tech Nanotechnology Center for Personalized and Predictive Oncology		
Emory University & Georgia Institute of Technology	Shuming Nie	To innovate and accelerate the development of nanoparticles for cancer molecular imaging, molecular profiling, and personalized therapy
Center of Nanotechnology for Treatment, Understanding, and Monitoring of Cancer		
University of California, San Diego	Sadik Esener	To develop smart, multifunctional, all-in-one platform device solutions capable of targeting tumors and delivering payloads of therapeutics
Siteman Center of Cancer Nanotechnology Excellence		
Washington University in St. Louis	Samuel Wickline	To develop nanoparticles for in vivo imaging and drug delivery, with special emphasis on translational medicine

Phase I: Cancer Nanotechnology Platform Partnerships (CNPPs) (R01)		
Institution	**Principal Investigators**	**Research Title**
Northeastern University	Mansoor Amiji	Nanotherapeutic Strategy for Multidrug Resistant Tumors
University of Michigan	James Baker, Jr.	DNA-linked Dendrimer Nanoparticle Systems for Cancer Diagnosis and Treatment
Virginia Commonwealth University	Panos Fatouros	Metallofullerene Nanoplatform for Imaging and Treating Infiltrative Tumor
University of California, San Francisco	Douglas Hanahan	Detecting Cancer Early With Targeted Nano-probes for Vascular Signatures
Massachusetts General Hospital	Tayyaba Hasan	Photodestruction of Ovarian Cancer: ErbB3 Targeted Aptamer-Nanoparticle Conjugate
University of Missouri, Columbia	Kattesh Katti	Hybrid Nanoparticles in Imaging and Therapy of Prostate Cancer
University of Texas M.D. Anderson Cancer Center	Chun Li	Near-Infrared Fluorescence Nanoparticles for Targeted Optical Imaging
Massachusetts Institute of Technology	Scott Manalis	Integrated System for Cancer Biomarker Detection
Roswell Park Cancer Institute	Allan Oseroff (Deceased) Ravindra Pandey	Novel Cancer Nanotechnology Platforms for Photodynamic Therapy and Imaging
State University of New York, Buffalo	Paras Prasad	Multifunctional Nanoparticles in Diagnosis and Therapy of Pancreatic Cancer
The Sidney Kimmel Cancer Center	Jan Schnitzer	Nanotechnology Platform for Targeting Solid Tumors
University of Washington	Miqin Zhang	Nanotechnology Platform for Pediatric Brain Cancer Imaging and Therapy

Phase II: Centers for Cancer Nanotechnology Excellence (CCNEs) (U54)		
Institution	**Principal Investigators**	**Scientific Focus**
Center for Cancer Nanotechnology Excellence and Translation		
Stanford University	Sanjiv Sam Gambhir Shan Wang	To design and implement novel in vitro diagnostic devices and verification of their performance using in vivo imaging to monitor lung cancer therapy and for earlier detection of ovarian cancer
Center for Cancer Nanotechnology Excellence at Johns Hopkins		
Johns Hopkins University	Peter Searson Martin Pomper	To develop and integrate nanotechnology-based in vitro assays, targeted chemotherapy, and immunotherapy for diagnosis, therapy, and post-therapy monitoring of lung and pancreatic cancer
Texas Center for Cancer Nanomedicine		
The University of Texas Health Science Center	Mauro Ferrari Anil Sood G. Lopez-Berestein	To develop and apply a diverse array of nanoplatforms for new therapeutics, methodologies for reliable monitoring of therapeutic efficacy, early detection approaches from biological fluids and advances in imaging, and cancer-prevention protocols for ovarian and pancreatic cancers
Nanosystems Biology Cancer Center 2		
California Institute of Technology	Jim Heath Leroy Hood Michael Phelps	To develop and validate tools for early detection, diagnosis, and therapy of melanoma, glioblastoma, and ovarian cancers through in vitro diagnostics, in vivo molecular imaging, and targeted therapies, including adoptive T cell immunotherapies and siRNA delivery
Nanomaterials for Cancer Diagnostics and Therapeutics CCNE		
Northwestern University	Chad Mirkin Steven Rosen	To develop novel nanoscale technologies including highly innovative "nanoflares" for the detection of circulating cancer stem cells and the development of model matrices for elucidation of cancer biology. These technologies have potential clinical utility for brain, pancreatic, and breast cancer detection, diagnosis, and treatment
MIT-Harvard Center of Cancer Nanotechnology Excellence		
MIT-Harvard	Robert Langer Ralph Weissleder	To develop and translate to the clinic a diversified portfolio of nanoscale devices for targeted drug and siRNA delivery, diagnostics, noninvasive imaging, and molecular sensing for better diagnosis and treatment of melanoma and prostate and colon cancer
Dartmouth Center for Cancer Nanotechnology Excellence		
Dartmouth College	Ian Baker	To develop and use novel antibody-targeted magnetic iron/iron oxide nanoparticles, which can be excited by alternate magnetic fields to induce localized hyperthermia in breast and ovarian cancer cells
Carolina Center of Cancer Nanotechnology Excellence		
University of North Carolina	Joseph DeSimone Joel Tepper	To develop innovative and significant core technologies, PRINT nanoparticles, and carbon nanotube-based x-ray sources, for cancer therapy and early detection of lung, brain, and breast cancer
Center for Translational Cancer Nanomedicine		
Northeastern University	Vladimir Torchilin Nahum Goldberg	To develop and characterize nanopreparations that will be tested in vitro and in vivo for their ability to kill tumor cells, with a particular focus on lung, ovarian, and pancreatic cancer

Phase II: Cancer Nanotechnology Platform Partnerships (CNPPs) (U01)		
Institution	**Principal Investigators**	**Research Title**
University of Nebraska Medical Center	Alexander Kabanov	High Capacity Nanocarriers for Cancer Therapeutics
Cedars-Sinai Medical Center	Julia Ljubimova	Nanoconjugate Based on Polymalic Acid for Brain Tumor Treatment
University of Utah	Marc Porter	Magnetoresistive Sensor Platform for Parallel Cancer Marker Detection
Children's Hospital Los Angeles	Fatih Uckun	Targeting SKY Kinase in B-Lineage ALL with CD-19 Specific C-61 Nanoparticles
University of North Carolina	Wenbin Lin	Nanoscale Metal-organic Frameworks for Imaging and Therapy of Pancreatic Cancer
Emory University	Lily Yang	Theranostic Nanoparticles for Targeting Treatment of Pancreatic Cancer
University of Cincinnati	Peixuan Guo	RNA Nanotechnology in Cancer Therapy
Northeastern University	Mansoor Amiji	Combinatorial-designed Nano-platforms to Overcome Tumor Resistance
Emory University	Dong Shin	Toxicity and Efficacy of Gold Nanoparticle Photothermal Therapy in Cancer
Northwestern University	Thomas O'Halloran	Tumor Targeted Nanobins for the Treatment of Metastatic Breast and Ovarian Cancer
University of New Mexico	Cheryl Willman	Peptide Directed Protocells and Virus-like Particles – New Nanoparticle Platforms for Targeted Cellular Delivery of Multicomponent Cargo
Rice University	Naomi Halas	Preclinical Platform for Theranostic Nanoparticles in Pancreatic Cancer

Phase II: Cancer Nanotechnology Training Centers (CNTCs) (R25)		
Institution	**Principal Investigators**	**Scientific Focus**
Midwest Cancer Nanotechnology Training Center		
University of Illinois Urbana-Champaign	Rashid Bashir	To educate the next generation of cancer nanotechnologists by creating a highly interdisciplinary environment which educates and empowers the students and postdoctoral engineers, physical scientists, and biologists in the areas of ex vivo diagnostic nanotechnology, in vivo imaging nanotechnology, therapeutic nanotechnology, and mechanobiology.
Boston University Cross-Disciplinary Training in Nanotechnology for Cancer		
Boston University	Bennett B. Goldberg	To apply nanotechnology in the training of pre- and postdoctoral fellows for early cancer detection/cancer prevention, through identification of rare circulating tumor cells, and to use proteomics to detect nuclear matrix proteins and new biomarkers for screening of early-stage tumors. Also, nanowires and nanocantilever arrays are among the leading approaches under development for the early detection of precancerous and malignant lesions from biological fluids.
The Johns Hopkins Cancer Nanotechnology Training Center		
Johns Hopkins University	Denis Wirtz	To develop two training programs for graduate students – one track focused on nanotechnology for cancer biology and the other focused on cancer diagnostics and therapeutics.
UCSD Cancer Nanotechnology Training Center		
University of California, San Diego	Robert F. Mattrey	To provide training in cancer nanotechnology to predoctoral students, postdoctoral researchers, and physicians. The program offers tailored tracks for physical scientists/engineers and biological/life scientists and a well-developed plan for minority recruitment and retention.
Integrative Cancer Nanoscience and Microsystems Training Center		
University of New Mexico	Janet M. Oliver	To accelerate recruitment of interdisciplinary graduate students and postdoctoral fellows, and development of interdisciplinary teams that combines novel nanoprobes with in vitro fluorescence and EM to address altered membrane organization and vesicular trafficking in cancer cells; a team that develops and applies nano- and microdevices for DNA sequencing and analysis of chromatin remodeling in cancer; a team that generates novel probes and instruments for in vivo cancer detection; and a team that focuses on cancer drug discovery and the synthesis of multifunctional nanoprobes for targeted drug delivery.
The University of Kentucky Cancer Nanotechnology Training Center		
University of Kentucky	Bradley D. Anderson	To develop cancer nanotechnology projects composed of multidisciplinary focus area teams with the goal of training future researchers, including minority and women in the field of cancer nanotechnology in the areas of early detection and diagnosis in lung, colon, and ovarian cancer; treatment of gastrointestinal tumors and metastases; lung cancer treatment; and glioma therapy.

Phase II: Pathway to Independence Awards in Cancer Nanotechnology (K99/R00s)		
Institution	**Principal Investigators**	**Research Title**
Emory University	Aaron M. Mohs	Nanotechnology for Minimally Invasive Cancer Detection and Resection
Stanford University	Andrew M. Smith	Next-Generation Quantum Dots for Molecular and Cellular Imaging of Cancer
Duke University	Mingnan Chen	Inhibition of Metastasis-Initiating Cells by Chimeric Polypeptide Nanoparticles
University of California, San Diego	Andrew P. Goodwin	Enzyme-Responsive Nanoemulsions as Tumor-Specific Ultrasound Contrast Agents
NIBIB/NIH	Jin Xie	Nanoplatform Based, Combinational Therapy against Breast Cancer Stem Cells
Wake Forest University Health Sciences	Ravi N. Singh	Tumor Targeting and Diagnostic Applications of Glycosylated Nanotubes
Massachusetts General Hospital	Prakash R. Rai	Theranostic Nanomedicine for Breast Cancer Prevention and Image-Guided Therapy

The Cancer Genome Atlas (TCGA) Program

1. Mission

The Cancer Genome Atlas (TCGA) is a comprehensive and coordinated effort to accelerate our understanding of the molecular basis of cancer through the application of genome analysis technologies, including large-scale genome sequencing. The mission of TCGA is to improve our ability to diagnose, treat, and prevent cancer through the development of a complete understanding of the genetic changes that drive cancer initiation and development. TCGA plans to fully characterize *all* of the genomic alterations in most types of cancer. The multidimensional data will be made available to the global cancer research community, along with open source bioinformatics tools and analysis approaches, through a patient-protected TCGA data portal. Based on progress in the past decade, it is expected that a new knowledge base of the comprehensive molecular changes in cancer types and subtypes will inform a new generation of research by individual investigators and drive discoveries that will provide new, more effective cancer interventions.

To fulfill the vision, the National Cancer Institute and the National Human Genome Research Institute launched TCGA program in two phases. The pilot project assessed and validated the research teams, support functions, specifically sample quality and acquisition, and next-generation sequencing technologies to determine the feasibility of a full-scale effort to systematically explore the entire spectrum of genomic changes involved in human cancers. The pilot program informed Phase II of TCGA and the program has expanded significantly to molecularly characterize and sequence more than 20 additional cancers over the next 5 years. The ability to achieve this aggressive goal was strengthened by the addition of Recovery Act funds that are being used primarily to accelerate sample collection for the 5-year program and to support additional sequencing through the NHGRI's Genome Sequencing Centers.

2. Program History

TCGA was proposed as a major initiative to the National Cancer Advisory Board (NCAB) in a February 2005 report from an NCAB ad hoc Working Group on Biomedical Technology. The report, coauthored by Drs. Lee Hartwell and Eric Lander, recommended the initiation of a bold technology-based project with the aim of obtaining a comprehensive understanding of the genomic alterations that underlie all major cancers. This recommendation was formalized into a concept document and ultimately published as an RFA to the cancer research community. The RFA was unanimously approved in November of 2005, along with potential RFPs (contracts) to develop support for a Biospecimen Core Resource and Data Coordinating Center. The Genome Characterization Centers were awarded in 2006 for 3 years at a level of approximately $50M for the period of the pilot program. NHGRI committed a similar level of funding ($50M) to support the sequencing efforts for TCGA pilot program. The BSA reviewed TCGA progress on a regular basis, and following a report to the BSA in 2008, it was agreed that NCI would release an RFA for Phase II of TCGA. The reissuance in the spring of 2009 requested proposals to characterize 20 different tumor types as part of a Phase II. Twelve awards were made by the NCI in August of 2009 that included both Genome Characterization Centers and Genome Data Analysis Centers. The addition of Recovery Act funds enabled the NCI to develop needed additional functional support for TCGA through the addition of a second BCR and expansion of the Data Coordinating Center. In addition, the Recovery Act funding is targeted for support of prospectively acquiring as many samples as possible to complete the genomic characterization of 20 tumors. In summary, significant milestones in the development of TCGA through the NCI's review and approval processes consisted of the following;

Summary of TCGA Approvals:

September 29, 2005: NCI Executive Committee Initial Pilot Concept Review and Approval

November 2, 2005: NCI Board of Scientific Advisors; Unanimous Approval of Pilot

November 6, 2008: NCI Board of Scientific Advisors; Approved Reissuance for Phase II

3. The Cancer Genome Atlas Program

Background

TCGA Pilot Project was launched in 2006. A collaboration between the NCI and the National Human Genome Research Institute, TCGA was initiated as a 3-year pilot project to determine the feasibility of cataloging the genomic alterations associated with a small number of different human cancers. The pilot project focused mainly on three tumor types (glioblastoma multiforme [GBM], serous cystadenocarcinoma of the ovary, and squamous carcinoma of the lung). The goal was to assess the technical feasibility and clinical relevance of conducting a comprehensive analysis of the associated genomic alterations. The pilot project demonstrated that cancer-associated genes and genomic regions can be identified by combining diverse information from genome analyses (miRNA and gene expression, promoter methylation, SNP analysis, copy number and genomic rearrangements, and targeted sequencing) with tumor biology and clinical data, and that the sequencing of selected regions can be conducted efficiently and cost-effectively. Achievement of the pilot project goals has set the stage for the next phase of TCGA, which promises to rapidly and efficiently generate analogous genomic data for all major cancer types and subtypes. The comprehensiveness and rate of progress of TCGA will depend on both optimization of technical issues and resource availability.

TCGA Phase II marked an expansion of TCGA to twenty additional tumor types beyond those studied in the pilot and involved a restructuring that applied three key "lessons learned" during TCGA Pilot project. To ensure that the results generated from the Characterizaton and Sequencing Centers could be interpreted from a variety of technology platforms, the centers chose to utilize high-quality molecular analytes; perform experiments utilizing strict standardized protocols; and deposit the results in structured formats. The last lesson strongly influenced the ability of the various

analytical groups to extract meaningful results from the genomic data generated. The goal of TCGA, Phase II is to comprehensively characterize 500 cases of at least 20 tumor types, or 20,000 samples, over the next 5 years.

TCGA Strategy

TCGA was developed as a pilot to determine the feasibility of a large-scale cancer research program designed to understand the underlying genomic somatic alterations that lead to cancer. Initially, opponents were concerned that TCGA would not be able to reliably distinguish signal from noise and therefore would fail to develop the rigorous dataset the community requires. Therefore TCGA's strategy was, and remains, an evidence-based program that is defined by not only its breadth (of both tumor types and characterization platforms), but also its depth (500 cases of each type) and the quality of its data. TCGA dataset must be statistically rigorous; the target of 500 was derived from statistical analysis of the number of cases that would be required to identify recurrent genomic alterations that would occur only in 3%-5% of patient samples. TCGA has established a demanding set of biospecimen requirements to ensure that TCGA data are able to differentiate the genomic alterations that lead to tumorigenesis as opposed to those that may be artifacts of treatment contaminating cell types, or even evidence of a secondary malignancy. Finally, TCGA's sample requirements are based on the objective to leave "no platform behind." Simply, one of the major strengths of TCGA dataset is the ability to integrate data from across many platforms for every sample. Analytes from *every* case are run on *every* platform, without exception.

Structure of TCGA

To accomplish its goals, TCGA supports multidisciplinary teams of investigators and associated institutions that collectively provide molecular and clinical data, as well as inform strategies for sequencing and expertise for data analysis. The progress in understanding some cancer-associated molecular alterations and the accompanying advances in technology suggest that it is now possible to obtain comprehensive genomic information from multiple tumor types to catalog most, if not all, of the genomic changes associated with cancer. Ultimately, in collaboration with and in support of the NCI's extensive program of individual projects in cancer research, such efforts are expected to accelerate the identification of markers for prevention and diagnosis and novel targets for the development of therapeutic drugs, as well as provide the basis for a refined clinical understanding of patient stratification for therapy.

TCGA Research Network includes the following major organizational and functional components:

- *Biospecimen Core Resources (BCRs):* The BCRs serve as the tissue processing centers and provide the molecular analytes for the project. Standard operating procedures have been developed for clinical data collection, sample collection, pathological examination, biomolecule (e.g., DNA and RNA) extractions, quality control, laboratory data collection, and biomolecule distribution to the Genome Characterization Centers and the Genome Sequencing Centers. The samples are required to have patient informed consent for the public release of data or an IRB waiver.

- *Genome Characterization Centers (GCCs):* As a part of TCGA Pilot Project Research Network (see below), the CGCCs conducted high-throughput comprehensive genome-wide analyses using validated technologies (e.g., gene expression profiling, detection of chromosomal segment copy numbers alteration) to reveal the spectrum of genomic changes that exist in human tumors and to identify genomic regions for further characterization by the Network's Genome Sequencing Centers. Technologies were optimized by each CGCC to increase the rate, sensitivity, and specificity of production. During Phase II, TCGA will perform gene and miRNA profiling using RNA-seq, Affymetrix SNP 6.0 arrays to generate SNP and purity/ploidy data, Illumina Infinium arrays for promoter methylation, and low-pass second-generation sequencing for large-scale genome rearrangements and copy number validation.

- *Genome Sequencing Centers (GSCs):* High-throughput sequencing analysis of tumor DNA provided by the BCRs was performed by the NHGRI large-scale sequencing centers. Sanger sequencing was initially used

to target 601 candidate genes. In February 2009 sequencing expanded to 6,000 cancer-associated genes. Coverage challenges using capture reagents and the desire to push to state of the art led the GSCs to shift to second-generation technologies in the fall of 2009. Ten percent of cases will be analyzed by whole genome sequencing with the remaining 90% analyzed by whole exome sequencing.

- *Genome Data Analysis Centers (GDACs):* One of the major hurdles to overcome in conducting a comprehensive genomic level analysis of cancer stems from the limitations of current bioinformatic approaches to deal with large and complex datasets. There is also a need for meta-analysis tools that could be used for integrative analyses of genomic level alteration data (derived from different platforms) with clinical and non-genomic data. As part of TCGA – Phase II, the NCI is supporting new and innovative approaches to data analysis and visualization of genomic level alterations through the creation of seven GDACs, which work cooperatively to produce a bioinformatic data analysis pipeline that will provide the community with structured analysis at regular intervals. All of these data will be publicly available.

- *Data Management:* Following TCGA network Data Sharing Plan, data generated by TCGA components are deposited into public databases as soon as they are validated, in general within a few weeks of generation. The information generated by TCGA Research Network is centrally managed to develop data standards and controlled vocabularies for each new technology. The goal of this approach is to establish an informatics infrastructure for data exchange between components of the project and a central repository, create portals for basic and clinical researchers to easily access the data, and to encourage new computational approaches to analyze the data. Another key function of the bioinformatics component is to provide a secure network and means to protect the integrity and security of research and clinical data. All of the data generated have been made publicly available via tools and infrastructure that are compatible with the cancer Biomedical Informatics Grid (caBIG).

The following diagram provides an overview of the different laboratories that have been part of TCGA through the pilot and/or Phase II.

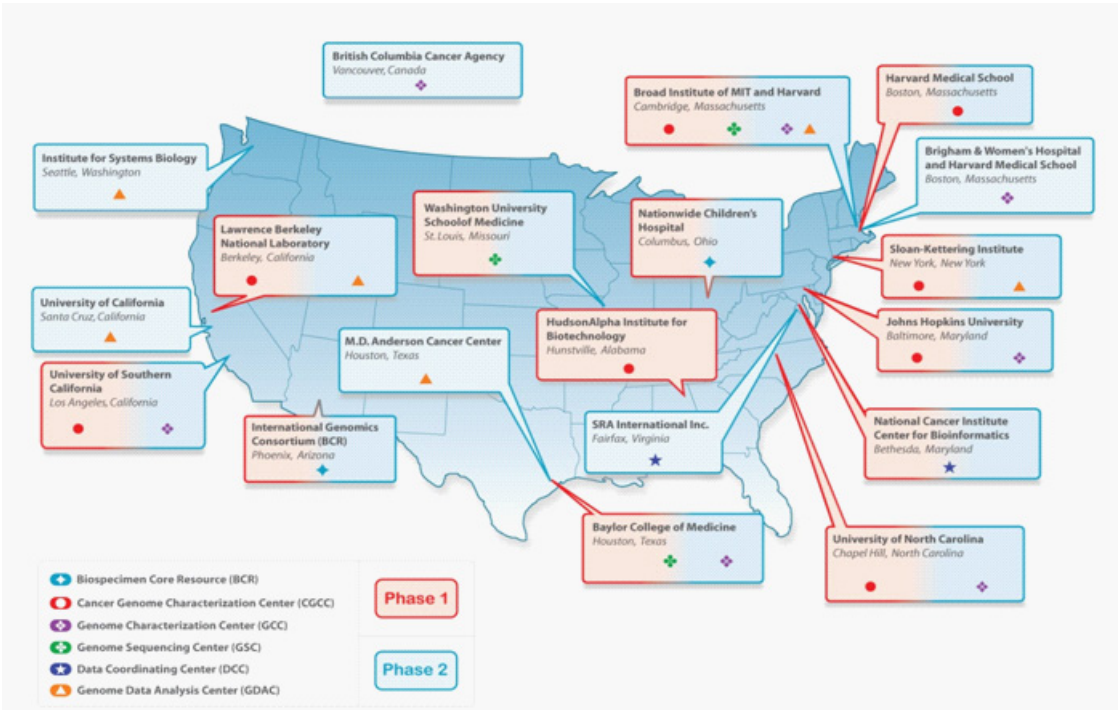


Figure 1. Centers Involved in TCGA Pilot and Phase II.

Examples of TCGA Achievements

- TGCA Research Network has clearly demonstrated that team science is critical for success in a project of this scope. Coupling the innovation spurred by independent investigators driving specific components of data generation and analysis with the ability to leverage the synergy of a network, TCGA has generated a dataset that is beyond anything produced in the community to date, and in months and years to come will continue to evolve and improve. TCGA not only has demonstrated feasibility of large-scale cancer genomics programs, but also has served as a model for both smaller scale programs and even international efforts, like the International Cancer Genome Consortium (ICGC).

- Forthcoming publication to include 500 cases of serous ovarian cancer reveals the significant differences in the genomes between ovarian and GBM, with the former characterized by unstable genomes manifested in the very large number of rearrangements and amplifications (figure 2). These data provide a clear depiction of the dramatic differences between tumor types as evidenced by the predominant types of genomic alteration present.

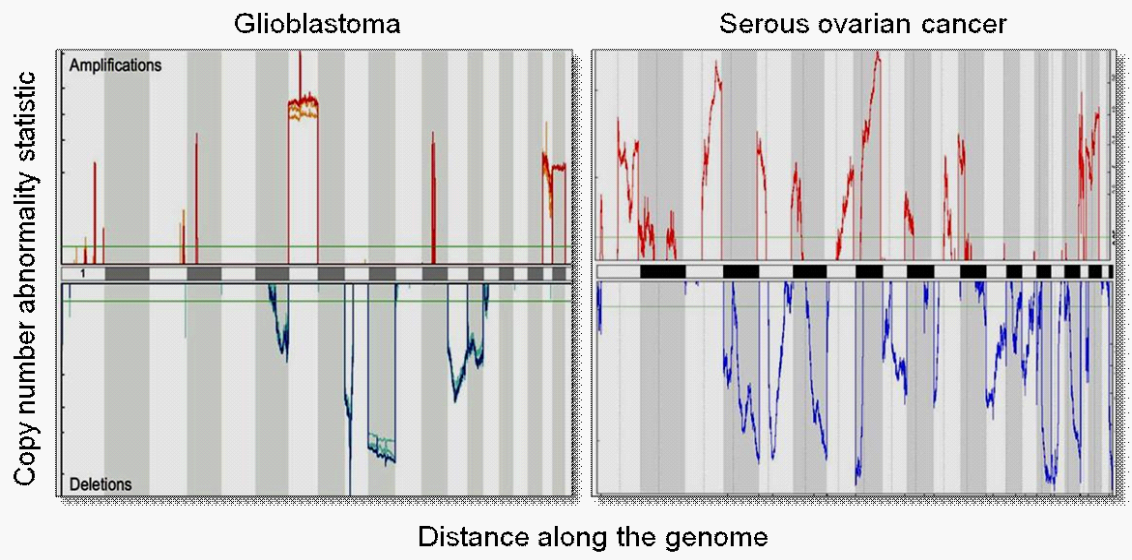


Figure 2. Comparison of copy number alterations between glioblastoma and ovarian cancer reveals a dramatic difference between the relatively stable GBM genomes and the highly amplified (red) and deleted (blue) ovarian cancer genomes.

- TCGA's success will be measured by how the project serves the biomedical research community. The first publication of TCGA has already demonstrated the value of such a rigorous dataset, with more than 300 publications already in the literature utilizing or citing TCGA dataset. Moreover, the R01 community is already relying on the data to make novel discoveries and submit new grant ideas. While it is too early to tell whether the relationship is entirely causal, the number of Type 1 grant applications for GBM and ovarian cancer research has risen significantly over the period of data generation and release (figure 3).

- TCGA has successfully established working groups for each tumor project, incorporating individuals from within TCGA community as well as from more diverse groups. These multidisciplinary teams are composed of members with oncology, pathology, surgical, clinical, and bioinformatic expertise, and, together with TCGA Program Office and TCGA Principal Investigators, these groups demonstrate how both funded and volunteer experts can work together to add value to a large-scale research project like TCGA that engages and is dependent on the research communities.

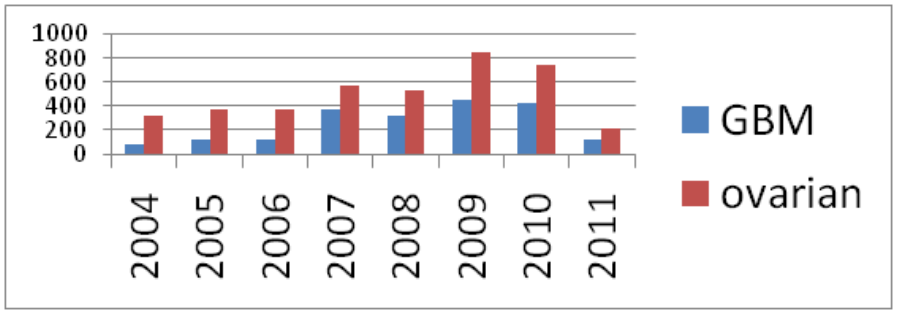
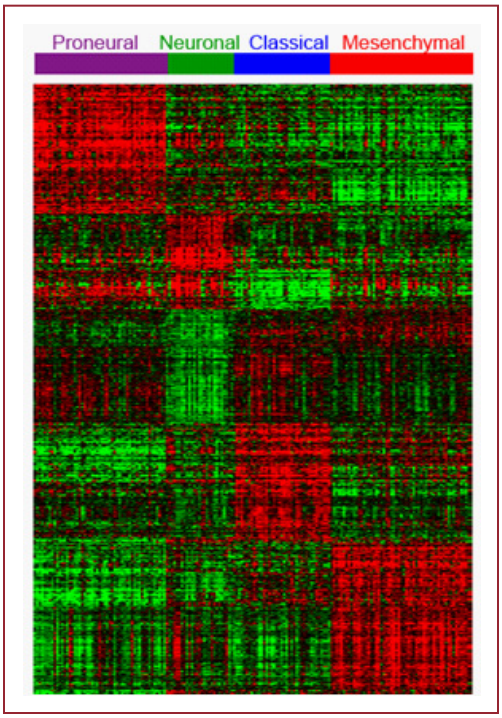


Figure 3. Type 1 grant applications mentioning GBM or ovarian cancer in the title or abstract have risen in number over the time period when data were released from TCGA into the public domain with the number of GBM applications almost quadrupling within just two cycles of the first TCGA publication.

- Analysis of TCGA data reveals that signal can indeed be differentiated from "noise," one of the primary concerns established by the community early on. The high bar set for sample quality, including percentage of tumor nuclei, drove data quality and the ultimate ability to discover beyond the "streetlamps."
- TCGA pilot data have already been used as foundational elements in the formation of new companies to use TCGA data for discovery and target development.

Examples of Scientific and Clinical Achievements

- Inaugural publication characterizing the tumor genomes of 206 GBM patients and identifying specific genes and core biological pathways commonly altered in these tumors. The network identified a possible mechanism for temozolomide resistance; the mechanism involves epigenetic modifications that impact the cell's DNA repair capabilities, pointing to the importance of the methylation platform in TCGA's pipeline. Specifically, the current standard of care for GBM is treatment with temozolomide. O-6-methylguanine-DNA methyltransferase (MGMT) exhibited promoter methylation in most treated cases and this MGMT inactivation correlated with a "hypermutated" phenotype, i.e., statistically increased mutation rates in mis-match repair genes. Inactive MMR genes would then be unable to repair the damage of the alkylating temozolomide and instead of initiating apoptosis the cells survive, suggesting a potential for translational impact for GBM clinical management.



- Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Correlation between the four expression subtypes of GBM with clinical outcome data, including responsiveness to standard therapy, revealed that the response to aggressive therapy differs by subtype, with the greatest benefit in the Classical subtype and no benefit in the Proneural subtype. The ability to correlate expression subtypes with a treatment response suggests that patients could be stratified based on their expression subtype and potentially spared the highly toxic therapy that is unlikely to have any positive impact on outcome.

- A CpG island methylator phenotype (CIMP) was identified in a subset of GBM patients. CIMP patients were generally younger and clustered with the proneural phenotype identified by expression studies. Not associated with MGMT methylation, CIMP is tightly linked to IDH1 mutations and is more frequent in lower grade gliomas. Of particular interest was the significant survival differential between the proneural CIMP+ and CIMP- patients (figure 4B), with CIMP- patients behaving more like other expression subtypes without a survival benefit.

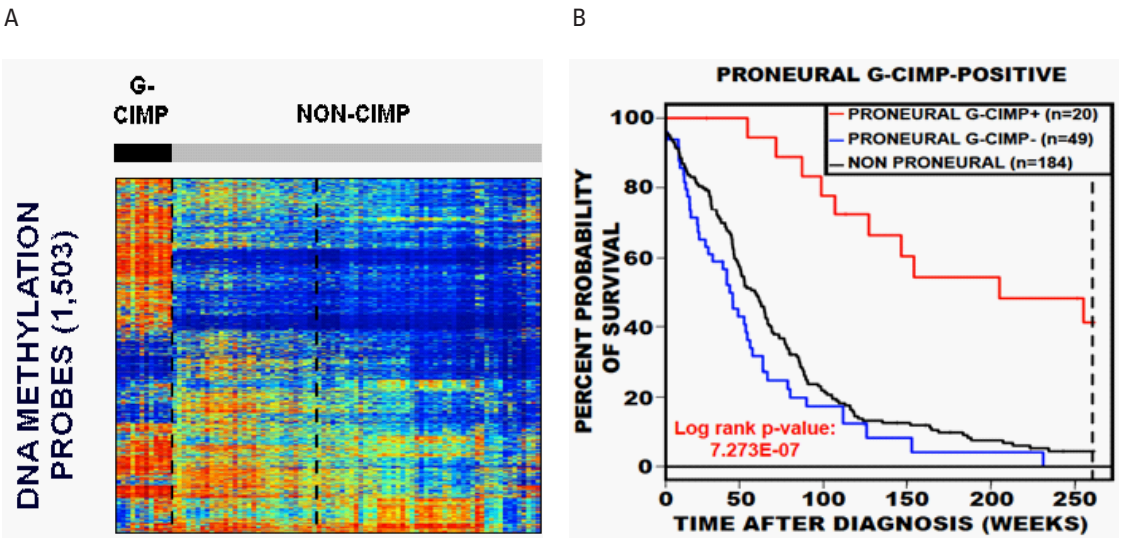


Figure 4. CpG island methylator phenotype (CIMP) defines a subgroup of glioma patients with improved survival outcomes. (A) Methylation profiles revealing the G-CIMP profile of a subset of glioma patients based on 1,503 methylation probes. (B) Comparison of long-term survival of patients in the proneural expression group with (red) and without (blue) G-CIMP.

Goals for Remainder of TCGA Funding Period (ends on 8/30/2014)

- A major goal for the remaining period is to organize the participating entities in TCGA Research Network in a manner that facilitates concurrent analysis and publication of multiple tumor types.

During the pilot TCGA was able to develop the team of researchers over time, with leaders for each cancer type studied. In the pilot program, the ability to concentrate on one tumor project at a time allowed multiple analysts to work on each tumor type, with more than 80 people named on the ovarian cancer analysis working group alone. Sequential management of each tumor is no longer possible, with 17 active working groups and more than a dozen tumor types already entering the BCR pipelines simultaneously.

Therefore, TCGA has recently instituted a tumor project model for tumor project management as outlined in figure 5.

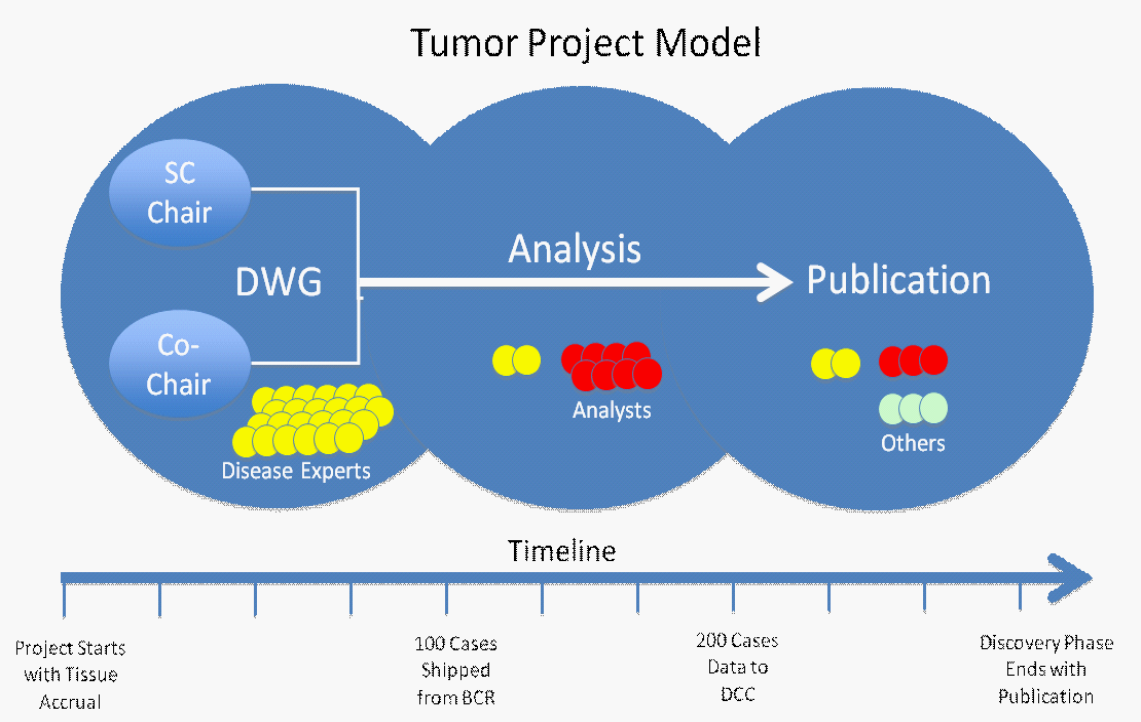


Figure 5. Tumor Project Model. In this new model the tumor projects continue to be led by a SC PI as in the pilot, but the number of analysts is kept small and focused in order to optimize participation of each individual. This should be possible because of the routine data production provided in an automated fashion by the GDACs. Note that a subset of external disease experts (yellow circles) remain important contributors throughout the process.

Over the past 5 months, TCGA has developed the capacity to collect more than 14 tumor types. This has required the development of a Disease Working Group (DWG) for each of the 20 tumor types. DWGs have three main roles in TCGA pipeline:

1. Provide disease-specific expertise required for the development of the clinical data collection forms;
2. Identify tissue source sites that could provide samples based on TCGA sample collection criteria; and
3. Provide groups of individuals to assist in the analysis and ultimate writing of the first comprehensive paper on a particular tumor. A list of DWGs and chairpersons is shown below.

Cancer Type	External Co-Chair	Steering Committee Co-Chair
Breast carcinoma	Matthew Ellis	Chuck Perou
Glioblastoma multiforme	Cameron Brennan	Lynda Chin
Lower grade gliomas	Dan Brat	Al Yung
Colon adenocarcinoma	Joel Tepper	Raju Kucherlapati
Hepatocellular carcinoma	To be determined	David Wheeler
Pancreatic ductal adenocarcinoma	Ralph Hruban	Stacey Gabriel
Stomach adenocarcinoma	To be determined	Peter Laird
Rectal	Joel Tepper	Raju Kucherlapati
Ovarian serous cystadenocarcinoma	Doug Levine	Joe Gray
Cervical cancer (squamous)	Janet Rader	Gordon Mills

Cancer Type	External Co-Chair	Steering Committee Co-Chair
Uterine corpus (endometrial carcinoma)	Doug Levine	Elaine Mardis
Head and neck squamous cell carcinoma	Adel El-Nagger Jennifer Grandis	Neil Hayes
Thyroid carcinoma	Thomas Giordano	Gad Getz
Acute myeloid leukemia	Tim Ley	Richard Wilson
Diffuse large B cell lymphoma	Louis Staudt	Marco Marra
Multiple myeloma	Daniel Auclair Joan Levy	Stacey Gabriel
Cutaneous melanoma	Jeff Gershenwald	Lynda Chin
Lung squamous cell carcinoma Lung adenocarcinoma	Ramaswamy Govindan	Matthew Meyerson Steve Baylin
Renal cell carcinoma	Michael Blue	Richard Gibbs
Renal papillary carcinoma	Marston Linehan	Paul Spellman
Bladder non-papillary carcinoma	Seth Lerner	John Weinstein
Prostate adenocarcinoma	Phil Kantoff	Chris Sander
Soft tissue sarcoma	Alexander Lazar Raphael Pollock Sam Singer	Marc Ladanyi

- Another goal is to leverage Recovery Act funds to enable the project to create a backlog of samples at the BCR and establish a series of pilot studies designed to determine how TCGA sample accrual specifications can be adjusted.

TCGA received a significant investment from Recovery Act funds. These funds are providing the capacity for TCGA to accelerate tissue accrual and the supporting capacity needed to achieve the project's goals of complete characterization of 20 tumor types during the 5 years of the project. The following are examples of how the $153.5M of NCI Recovery Act funding is being applied to accelerate the achievement of TCGA's 5-year goals:

Tissue Accrual: Tissue accrual is being significantly increased by the investment of $42M in Recovery Act funding. TCGA established a series of tissue acquisition requirements described in five Requests for Quotations (RFQs) and one Request for Proposals (RFP). The infusion of Recovery Act funds will make a major difference in the rate of tissue accrual, but will still not be sufficient to acquire rare and difficult-to-process tumors, which will require significant time beyond the Recovery Act-funded period.

These RFQs and RFPs represent a three-pronged approach to enable rapid acquisition of existing samples from known, reputable academic and commercial biobanks and focused accrual of prospectively collected specimens.

1. *Commercial Biobanks:* Commercial entities are generally equipped with personnel who can be assigned and reassigned to filling custom biospecimen accrual criteria and are therefore considered a source of materials for initial filling of the biospecimen accrual pipeline as additional academic centers are brought online.

2. *Academic Retrospective Biobanks:* More than 100 different academic centers representing over 160 different Principal Investigators have been interviewed and their collections assessed for TCGA's needs. Similar to the challenges experienced during the early years of accrual, many source sites fail to have significant sample numbers owing to the lack of case-matched samples of blood or DNA from blood and

paucity of untreated, primary cases owing primarily to the standard of care in the United States. In addition, many samples do not meet the requirements of TCGA in regard to tumor size and purity.

3. *Academic/Commercial Prospective Collection:* In addition to the retrospective samples, funded through purchase orders mandated by the RFQ mechanisms, prospective tissue collection networks will be funded to accrue specific tumor types needed by TCGA depending on the performance of the retrospective collections.

Sequencing: TCGA has adopted cutting-edge second-generation sequencing technologies to complete Phase II tumor projects. In order to achieve the throughput required by the expansion to 20 tumor types, the three sequencing centers are predicted to need at least $80M in additional funding beyond what was already allocated. NCI is dedicating $45M of its Recovery Act funds toward this requirement. The NCI Office of Acquisitions (OA) is currently in negotiations with three centers, and awards are expected to be made by the end of August 2010.

Biospecimen Core Resources (BCRs): Two BCRs are currently operational for TCGA and are actively providing TCGA with deliverables that include shipment of samples from individual tissue source sites (TSSs) to the BCR, pathology review, molecular analyte preparation, and clinical data collection. The capacity of the centers will be funded at a rate equivalent of a quadrupling of the initial funding levels of the pilot, utilizing approximately $38M of Recovery Act funds with 400 cases/month per BCR being processed, enabling as many as 200 cases shipped to the centers per month.

Data Coordinating Center (DCC): The DCC for TCGA is responsible for accepting, validating, and providing all finalized data to the community through the data portal. Over time, the DCC has been required to incorporate many more data formats and types than originally anticipated. Together with the incredible increase in data being submitted to the DCC in recent months, the DCC was underperforming due to a lack of funding. The support of additional $8M allows for expansion of the DCC to the levels required for a program of this size, enabling the contractor to serve a true coordinating function as opposed to a data storage function.

Quality Management System (QMS): During the pilot phase, TCGA generated 7 terabytes (TB) of data, spanning biospecimen information, patient information, and genome, exome, and epigenetic data, for both tumor and normal specimens per patient. The ability to ensure data completeness associated with each specimen required a coordinated effort across numerous institutions and information systems. This coordination has become increasingly difficult as the program has not yet implemented a system to monitor data quality in real time. The value of the data generated in TCGA will lie in its quality, as all subsequent analyses and research outcomes will be dependent on the quality of the information generated. The QMS will serve as the basis by which the quality of all aspects of TCGA can be monitored, to be funded through the Recovery Act at approximately $7M.

The Future of TCGA

TCGA will create the most rigorous, complete public dataset on more than 20 human cancer types ever assembled and make the data publicly available to drive discovery of new targets and enable drug and diagnostics development. TCGA is on target to continue producing the highest quality cancer genomics datasets, and the combination of retrospective and prospective tissue accrual will continue to provide the program with the nucleic acids needed to optimize data development and output. The data-sharing policy of TCGA is integrated throughout the network, with the data provided to the public pre-publication. TCGA research network will provide comprehensive genomics data on 10,000 clinically annotated specimens by the end of the 5-year performance period of the project.

Now that TCGA has expanded to 20 additional tumor types, the separation between TCGA and pediatric cancer genomics is seemingly even more arbitrary since many of the tumors under investigation by TCGA occur in both pediatric (under 18) and adult populations at reasonable frequencies. Recent data on GBMs in pediatric cases and the recent publicized efforts by St. Jude to characterize 600 pediatric cancer genomes suggest that TCGA could make valuable headway into many of these diseases by including pediatric cases. Inclusion of pediatric cases will require TCGA to revisit some

of its policies and identify data elements that are currently treated differently between adult and pediatric genomics projects.

As more and more investigators across the globe are able to gain expertise in genomic technologies, TCGA, as a member of the International Cancer Genome Consortium, will be able to develop even stronger collaborations. TCGA intends to strengthen existing relationships with China and Korea, where existing partnerships are developing around GBM, gastric, and pancreatic cancers. TCGA also receives samples that meet TCGA specifications from institutes in more than 10 countries worldwide. Increasingly, due to the differences in standard of care, TCGA will need to collaborate to acquire meaningful samples from targeted countries.

TCGA is actively working with the Clinical Proteomic Technologies for Cancer (CPTC) program to validate candidate targets at the protein level. Moreover, additional programs at the NCI, including the Cancer Target Discovery and Development (CTD^2) Network in the Office of Cancer Genomics (OCG), fund investigators to perform functional screens (including RNAi and small molecule) to identify targets from TCGA and other large-scale cancer genomics datasets that are biologically relevant to tumorigenesis. TCGA plans to collaborate more formally with these groups to better leverage the valuable samples and data from TCGA.

Finally, TCGA will increase its efforts to provide additional high-value datasets to individual investigators and teams of scientists based on analysis of mouse models of human cancer. This program will interface with the Mouse Models of Human Cancer and other programs.

Examples of TCGA Partnerships

TCGA has long been a partner with the Office of Biorepositories and Biospecimen Research (OBBR). Previously, its Director and Deputy Director served on TCGA Project Team before OBBR launched caHUB. It was through the work of the OBBR that TCGA adopted the biospecimen criteria that are currently in use. The rigorous, evidence-based philosophy of OBBR has served TCGA and the cancer research community. The tissue accrual core for TCGA is collaborating with caHUB to identify prospective network sites that could serve both programs, thus better leveraging the infrastructure supported through TCGA contracts.

Clinical Proteomic Technologies for Cancer (CPTC): The dataset provided by TCGA to the scientific community is the most comprehensive of its kind to date. From these data, scientists are actively identifying potential targets from purely genomic data. These targets, however, remain to be validated at the functional level. Therefore, in the most recent CPTC-supported RFA, it was announced that TCGA samples will be used whenever possible for discovery proteomics.

TCGA's Informatics Team will be partnering with the Office of Physical Sciences-Oncology to cross-test unique non-linear analysis programs developed by investigators within the Physical Sciences-Oncology Centers (PS-OCs) currently utilizing TCGA datasets.

TCGA is actively partnering with the senior scientific leadership of the Global Viral Forecasting Initiative (GVFI) to investigate the contribution of viral integration in the development of glioblastoma multiforme (GBM). Over the next 6 months, TCGA will share residual tissue samples with GVFI investigators to interrogate the GBM genome for evidence of viral signatures.

Appendix: Current Staffing

Joseph Vockley, Ph.D., Director, TCGA Program

Dr. Vockley received his Ph.D. degree in molecular genetics from the University of Delaware, after which he completed a clinical genetics residency and a postdoctoral fellowship at the University of California, Los Angeles. Dr. Vockley came to the NCI after serving as the laboratory/business director for the Biological and Chemical Defense Division of Science Applications International Corporation (SAIC). His work there focused on the development of bioinformatic software and a microarray-based detection system for the simultaneous identification and characterization of microbial threat agents, emerging threats, and genetically engineered microorganisms. Prior to working for SAIC, Dr. Vockley was Vice President of Genomics at Gene Logic, Inc., where he directed the construction of a large-scale gene expression microarray database. Previously, he was a senior scientist at SmithKline Pharmaceuticals, where he worked on the discovery of diagnostic and therapeutic targets for cancer.

Kenna M. Shaw, Ph.D., Scientific Projects Manager

In her role as a projects manager for TCGA, Dr. Shaw oversees and manages tissue acquisition, clinical data, and pilot programs related to sample accrual. After completing a Fulbright fellowship developing science curricula in Chile, Dr. Shaw received her doctorate in cell and developmental biology from Harvard Medical School, where she examined the pathways involved in breast ductal morphogenesis. She then served as an American Cancer Society-funded postdoctoral fellow working at NICHD in zebrafish genomics. Dr. Shaw has been the Principal Investigator for a National Science Foundation program on science education, and prior to coming to the NCI she directed an open-access educational publishing venture for Nature Publishing Group.

Greg Eley, Ph.D., Scientific Program Manager

Over the past decade, Dr. Eley has served as a technology and management consultant with a major consulting firm, where he supported the Federal, academic, and commercial entities. His focus was on biomedical program management, technology evaluation, and biomedical information integration. Among his many engagements, Dr. Eley has served as the technical manager for multiple human performance programs at the Defense Advanced Research Projects Agency (DARPA), supported national security programs within the Defense Intelligence Agency, served as the Workspace Lead for the caBIG Tissue Banks and Pathology Tools Workspace, and led engagements in support of pharmaceutical technology and information integration. Dr. Eley received his Ph.D. degree in biomedical sciences with a focus on tumor biology from the Mayo Clinic and Foundation in Rochester, Minnesota.

Laura Dillon, M.S., Health Scientist, NCI/NIH

Prior to joining TCGA Project Office at NCI, where she coordinates the Tumor Project Working Groups, Ms. Dillon worked as a regulatory project manager for the Biotechnology Manufacturing Team in the FDA's Office of Compliance and as a program analyst for the National Human Genome Research Institute's ENCODE Project. She earned a bachelor's degree in biology and political science from the University of Richmond and a master's degree in biotechnology studies from the University of Maryland. Her previous research experiences focused on phosphate transport in the human bacterial pathogen *Shigella flexneri* and on the effects of delta 9-tetrahydrocannabinol on the human immune system.

Mae Avenilla, M.B.A. Program Analyst

Ms. Avenilla recently joined TCGA Program Office to support finance and contract development issues. She has extensive private sector experience in the areas of finance, audit, project management, process reengineering, and systems implementation. Prior to joining NIH in October 2009, she had worked with MedImmune, LLC, Marriott International, KPMG, and Del Monte Foods. She obtained a B.S. degree in business administration from the University of California, Berkeley, and an M.B.A. degree from Pennsylvania State University.

Catherine Evans, Ph.D., Health Communications Intern

Dr. Evans recently graduated from the University of Michigan in Ann Arbor with a Ph.D. degree in neuroscience. Her doctoral thesis work focused on PET imaging of the neurochemistry and neuroanatomy of placebo analgesia. Specifically, she examined hormonal and genetic contributions to individual differences in placebo responding. In addition to her doctoral work, Dr. Evans contributed to the University of Michigan Medical School's research news magazine, in which she wrote about the latest research findings within the University of Michigan medical community.

Office of Cancer Genomics (OCG)

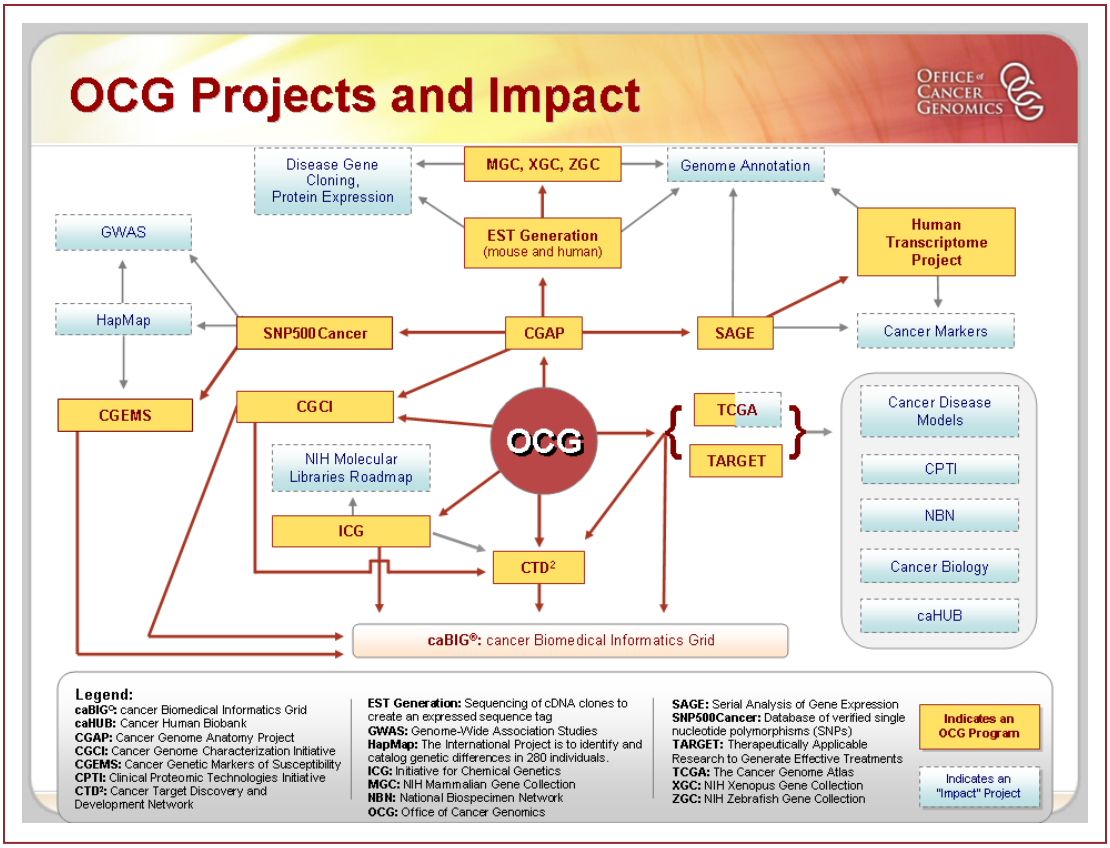
1. Mission and Goals

The mission of the NCI's Office of Cancer Genomics (OCG) is to enhance the understanding of the molecular mechanisms of cancer and to advance and accelerate the science and technology to efficiently translate the genomics data to improve cancer prevention, early detection, diagnosis, and treatment.

Created in 1996, the OCG interfaces genomics, chemical genetics, and cancer research through the establishment of information platforms, material resources, and technology infrastructure. Different cancer types originate from a wide range of genetic mutations within an organ or tissue which defines the molecular subtypes. Efforts to understand cancer etiology of each subtype requires comprehensive and systematic approaches. The OCG has made numerous contributions to the research enterprise during its first decade and a half, including development of databases of cancer characterization and chemical genetics data, development of bioinformatic tools to interpret the data, supporting the development of novel technologies for cancer characterization, and making cDNA clones from seven species freely available.

The OCG helps the scientific community to overcome technological challenges by supporting research to improve molecular characterization methods and their throughput; evaluate the various novel DNA sequencing technologies; improve the detection of epigenetic changes; and develop new analytical protocols to correlate disease state with the intricate network of molecular interactions in a cancer.

The large-scale genomic datasets require the development of new technologies to rapidly translate them into patient-based therapeutics with concomitant predictive markers. The aim is to bridge the technology transfer gap, "the valley of death," in drug discovery and make progress possible; an analogy can be made with the development of molecular characterization (including sequencing) technologies that have been so propitious to the understanding of cancer-causing mechanisms. OCG is supporting the development of the process and making it publicly available to accelerate innovations in drug discovery.



2. Programs

2.1 The Therapeutically Applicable Research to Generate Effective Treatments (TARGET) Initiative

Background

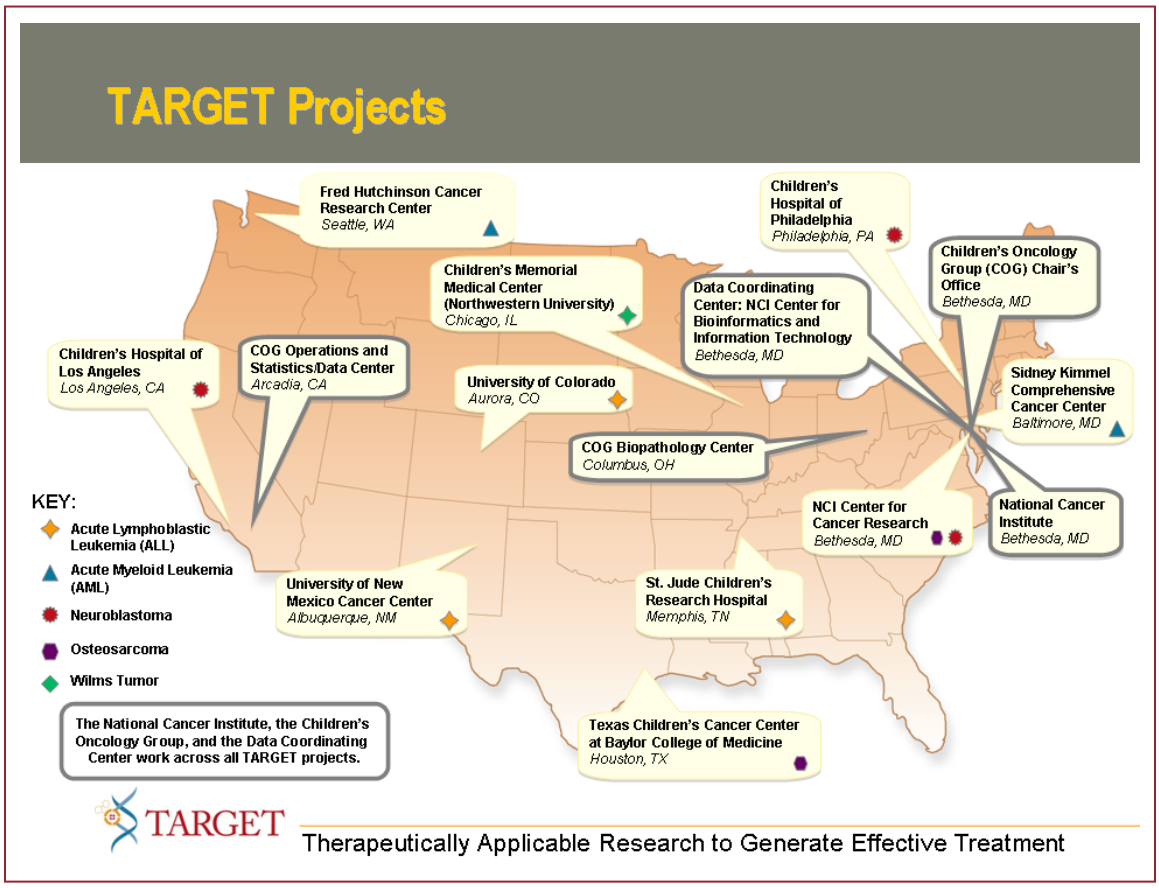
The objectives of the TARGET Initiative stem directly from the recommendations of a workshop sponsored by the American Cancer Society (ACS) and the National Cancer Institute (NCI) in May 2005 entitled "Childhood Cancer Targeted Therapeutics Workshop." The TARGET Initiative has also received input from the NCI's Board of Scientific Advisors (BSA). A subcommittee of the BSA, chaired by Dr. Joe Gray, was established to provide oversight for the TARGET Initiative. The BSA TARGET subcommittee met primarily during the initial phases of the project, beginning in November 2006, and provided valuable feedback and advice on its scientific direction. The results from the TARGET Initiative have also been presented to the full BSA meetings, most recently in March 2009, and the project team received positive feedback from the members. The project is managed as a collaboration by OCG and Dr. Malcolm Smith, Deputy Branch Chief, Pediatric Oncology, Cancer Therapy Evaluation Program (CTEP). Dr. Smith is the Project Director of the Children's Oncology Group.

Goals

TARGET is designed to use the tools of modern genetics for the discovery of valid therapeutic targets in childhood cancers so that new, more effective treatments can be rapidly developed. The Initiative incorporates in real time the lessons learned from, as well as the tools and technologies developed for, the Cancer Genome Characterization Initiative (CGCI) and The Cancer Genome Atlas (TCGA) with resources (e.g., clinical trial infrastructure) and scientific expertise of the Children's Oncology Group (COG). The TARGET projects utilize comprehensive genomic characterization methods on clinically well-annotated tissues from the COG clinical or biological trials. The participation of the COG and the leading researchers for each cancer type also allows a rapid translation of the data into the clinic.

Program Components

The ultimate goal of TARGET is to provide the scientific insight and resources that will reduce the devastating burden of cancer for children and their families. To fulfill this mission, each project is led by a senior investigator with extensive expertise in the clinical or basic science of the disease. The generated data are stored in a central facility, Data Coordinating Center (DCC), managed by the NCI. The current projects within the initiative and the principal investigators are:



TARGET Project and Principal Investigators

Acute Lymphoblastic Leukemia (ALL)	Steve Hunger – The Children's Hospital, University of Colorado, Denver
Acute Myeloid Leukemia (AML)	Bob Arceci – The Sidney Kimmel Comprehensive Cancer Center Soheil Meshinchi – Fred Hutchinson Cancer Research Center
Neuroblastoma (NBL)	John Maris – The Children's Hospital of Philadelphia Bob Seeger – Children's Hospital of Los Angeles Javed Khan – NCI Center for Cancer Research
Osteosarcoma (OS)	Ching Lau – Texas Children's Cancer Center Paul Meltzer – NCI Center for Cancer Research
Wilms Tumor (WT)	Elizabeth Perlman – Children's Memorial Medical Center

Aims

The overall goal of the TARGET initiative is to provide a detailed molecular map of high-risk pediatric cancers. The cancers and cohorts were chosen based on the poor outcome and associated toxicity of currently available treatments, the unique accessibility of study material and clinical outcome data through the COG, and the expertise and coordinated efforts of the investigators. The Initiative includes molecular characterization by chip-based as well as second- and third-generation sequencing technologies to define the alterations in tumor transcriptomes, exomes, genomes, and epigenomes for childhood malignancies. Between 100 and 200 cases per cancer will be comprehensively characterized. For some of the cancers, relapse specimens will be characterized in parallel when they are available. The data generated from TARGET will be publicly available for the worldwide research community to investigate, analyze, and integrate, thereby enhancing the likelihood of finding more effective treatments for pediatric cancers.

The tumor types were chosen based on three criteria: (1) The need for improved treatment options for many children with these diagnoses. (2) The existence of ongoing NCI-supported projects for limited analysis of the cancers which allowed cost-efficiency of the molecular characterizations (e.g., mRNA and miRNA profiling, chromosome copy number alterations and translocations, methylation alteration, and mutation detection). (3) The availability of clinically annotated, high-quality human tissue collections that met TARGET's strict scientific, technical, and ethical requirements. Each cancer is studied by a team so that many, if not all, of the scientific and institutional components (clinical trials, molecular characterization, statistics, etc.) are available to achieve the Initiative's goals. Each group meets at least once per month (some meet semi-monthly) by teleconference to discuss progress and problems, identify solutions, and formulate updated research plans based on the results obtained. The project has a SharePoint site for documents and information exchange. The first TARGET face-to-face Steering Committee of all five projects was held in May 2010 and the next meeting is planned for December 2010. Two Center for Cancer Research (CCR) laboratories (Drs. Khan's and Meltzer's) participate in NBL and OS respectively by contributing second-generation sequencing for a subset of the samples. The NBL project team has a collaboration with the Broad Institute (BI) to get second-generation sequencing of the "full" exome for ~90 cases (and controls and the analysis of the data).

The NCI provides the bioinformatic infrastructure by hosting the Data Coordinating Center (DCC) that will allow the analyses of the dataset generated with tools used in TCGA, e.g., NCI's Cancer Molecular Analysis and Cancer Genome Workbench, BI's GenePattern (NCI Center for Bioinformatics/TCGA funded to make its analytical software usable within the DCC format) as well as using other standard methods (e.g., unsupervised hierarchical clustering, etc.). The TARGET DCC was established as a separate entity with pediatric cancer-only Data Use Limitation (DUL) as per opinion from the NIH Ethics Program. In addition, the projects will have access to the analytical methods developed in TCGA by the Genome Data Analysis Centers (GDACs) when they are deposited into TCGA DCC. The identical DCC database formats will also allow the easy integration with the adult data generated in TCGA and CGCI.

TARGET will include two analytical pilots, one for AML through a local (Seattle) collaboration with Dr. Steve Friend and SAGE Bionetworks. The specifics are yet to be defined. The other pilot will be in NBL with Andrea Califano through OCG facilitation. Specifically, Dr. Califano has applied for approval to use the funds for an outreach project of his NCIIT in silico analysis contract to use his methods to discover master regulators, modulators, etc. of cancer development (ARACHNE, MINDy, etc.). The NBL researchers will test the bioinformatic predictions to validate the potential drug and/or marker candidates.

Funding

The TARGET Initiative received $25M in Recovery Act funds in September 2009 for 2 years. Five million dollars was allocated for the tissue processing and molecular characterization, mostly with chip-based methods; $19.5 million is for second- and third-generation sequencing of either exomes or genomes through the SAIC contract mechanism and $0.5M for the database infrastructure. The sequencing contracts include an option to evaluate the quality of the deliverables in a 6-month period to ensure that the best possible technology and cost-efficiency are utilized in a timely fashion by the Initiative.

TARGET Pilot and Results

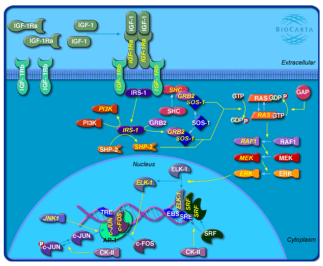
The TARGET Initiative started as a pilot and involved the molecular characterization of high-risk ALL and NBL, specifically gene expression, chromosome segment copy number profiling (CNA), targeted sequencing (sequencing of all exons with Sanger chemistry, including 3′ & 5′ UTRs after PCR amplification of genomic DNA with Sanger chemistry), as well as whole transcriptome second-generation sequencing on a small number of cases. The data are publicly available.

Progress from the ALL project is summarized herein. The pilot project characterized cases from a high-risk cohort (P9906) with mature outcome data who were uniformly treated by an "augmented BFM" regimen and which did not include the known high-risk phenotypes or genotypes (BCR-ABL minus, hypodiploid minus). The key members of the ALL group include Gregory Reaman (COG Chair); Stephen Hunger (Chair, COG ALL Committee and TARGET HR ALL Pilot PI), William Carroll (immediate past chair, COG ALL Committee), Mignon Loh (Vice Chair for Biology, COG ALL Committee), Meenakshi Devidas (COG ALL Lead Statistician), James Downing (Scientific Director, St. Jude Children's Research Hospital [SJCRH]), Charles Mullighan, Mary Relling (Chair, Pharmaceutical Sciences SJCRH), Cheryl Willman (Director and CEO, University of New Mexico [UNM] Cancer Center), and Richard Harvey, UNM. The NCI staff included Malcolm Smith (Associate Branch Chief, Pediatric Oncology), James Jacobson (Acting Associate Director, Cancer Diagnosis Program), Daniela Gerhard (Director, OCG), and Jinghui Zhang (Staff Scientist, Center for Bioinformatics); Dr. Zhang joined SJCRH in January 2010.

A selection of some of the findings are highlighted below:

- Supervised clustering resulted in a molecular risk classifier to predict event-free survival and minimal residual disease.
- Unsupervised clustering discovered a new high-risk subtype of pediatric ALL with a well-defined expression signature which is "kinase-like."
  - Targeted sequencing in these cases identified mutations in JAK2 and JAK1.
  - When the JAK2 or JAK1 genes with these mutations are introduced into mouse cell lines of the appropriate lineage, the cells become transformed. This phenotype can be suppressed by a small-molecule inhibitor.
  - A number of these cases had activation lesions (usually translocations), which involved CRLF2 (cytokine receptor-like factor 2).
- The data confirmed and expanded upon preliminary finding that the B-cell maturation pathway is important (PAX5 mutations and IKZF1 deletions or mutations).
- 78% of the cases with mutations in JAK2 or JAK1 as well as genomic change (deletion or mutation) in IKZF1 relapse within 4 years.
- Four manuscripts were published, one is under review (see below), and two to three more will be submitted in the next 1-3 months.
- Targeted sequencing of 125 genes, which were selected as candidates from the analysis of the expression and CNA data, found enrichment of mutations in genes of the RAS pathway, a number of which are not mutually exclusive. The final dataset was obtained last week and a manuscript of the results will be submitted for publication in August.

NCI ALL TARGET Sequencing Update:
125 Genes in 187 Samples

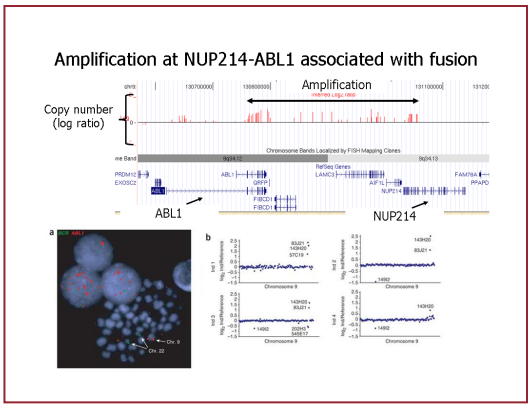


11% - JAK Kinase Mutations

25% of Samples have RAS Family Mutations (N-RAS, K-RAS)

42% of samples have mutations in the FLT3/ RAS Pathway: RAS, FLT3, NF1, PTPN11

- The whole transcriptome sequencing of 10 cases of high-risk ALL identified translocations in 9/10 cases, of which 4 were previously unknown in B-cell ALL. For example, NUP214-ABL1 fusion was previously known to occur only in T-cell ALL, yet it was found in one of the 10 cases and then confirmed to be present in 3 others. Validation of the SNV is in progress and the goal is to have a manuscript submitted in autumn.



The ALL research team members are following up the results:

- They submitted an application to study the predictive power of the supervised and unsupervised classifiers using a TaqMan assay in a clinical trial starting 2011.
  - A subgroup has a Recovery Act-funded project which will develop the technologies for the assay.
- They developed a single agent phase I trial of JAK2 inhibitor (Incyte Inc.). It was approved by CTEP and awaiting final contract sign-off by the company.
  - If the safety profile looks good in phase I, they are discussing the development of a phase II trial of chemotherapy and drug to start in 2011.

Publications

1. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LAA, Miller CB, Ma J, Liu W, Cheng C, Schulman B, Harvey R, Chen I-M, Clifford R, Carroll WL, Reaman G, Bowman WP, Devidas M, Gerhard DS, Yang W, Relling MV, Shurtleff SA, Campana D, Borowitz MJ, Pui C-H, Smith M, Hunger SP, Willman C, Downing JR. Deletion of IKZF1 (Ikaros) is associated with poor prognosis in acute lymphoblastic leukemia. *New England Journal of Medicine*, 360: 470-480, 2009. Epub 2009 Jan 7. PMID: 19129520

2. Mullighan* CG, Zhang* J, Harvey* RC, Collins-Underwood JR, Schulman BA, Phillips LA, Tasian SK, Loh ML, Su X, Liu W, Devidas M, Atlas SR, Chen I-M, Clifford RJ, Gerhard DS, Carroll WL, Reaman GH, Smith M, Downing# JR, Hunger# SP, Willman# CL. JAK mutations in high-risk childhood acute lymphoblastic leukemia. *Proc Natl Acad Sci USA*, 106: 9414-9418, 2009. Epub 2009 May 20. *CGM, JZ, and RCH contributed equally; #JRD, SPH, and CLW contributed equally. PMID: 19470474

3. Kang H, Chen I-M, Wilson CS, Bedrick EJ, Harvey RC, Atlas SR, Devidas M, Mullighan CG, Wang X, Murphy M, Ar K, Wharton W, Borowitz MJ, Bowman WP, Bhojwani D, Carroll WL, Camitta B, Reaman GH, Smith MA, Downing JR, Hunger SP, Willman CL. Gene expression classifiers for relapse free survival and minimal residual disease improve risk classification and outcome prediction in pediatric B-precursor acute lymphoblastic leukemia. *Blood*, 115: 1394-1405, 2010. Epub 2009 Oct 30. PMID: 19880498

4. Harvey* RC, Mullighan*# CG, Chen I-M, Wharton W, Mikhail FM, Carroll AJ, Kang H, Liu W, Dobbin KK, Smith MA, Carroll WL, Devidas M, Bowman WP, Camitta B, Reaman GH, Hunger# SP, Downing JR, Willman# CL. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, hispanic/latino ethnicity and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. *Blood*, 115: 5312-5321, 2010. *RCH and CGM contributed equally; #CGM, SPH, and CLW are corresponding authors. Epub 2010 Feb 4. PMID: 20139093

5. Harvey RC, Mullighan CG, Wang X, Dobbin KK, Davidson GS, Bedrick EJ, Chen I-M, Atlas SR, Kang H, Ar K, Wilson CS, Wharton W, Murphy M, Devidas M, Carroll AJ, Borowitz MJ, Bowman WP, Downing JR, Relling M, Yang J, Bhojwani D, Carroll WL, Camitta B, Reaman GH, Smith M, Hunger SP, Willman CL. Identification of

novel cluster groups in pediatric higher risk B-precursor acute lymphoblastic leukemia with gene expression profiling: Correlation with genome-wide DNA copy number alterations, clinical characteristics and outcome. Submitted and under review

Funding

The TARGET Pilot was funded by the CTEP SPECS program and OCG CGAP. The latter mechanism contributed a total of $3.6M over 3 years for contract sequencing of the NBL and ALL samples.

2.2 Cancer Genome Anatomy Project/Cancer Genome Characterization Initiative (CGAP/CGCI)

Background

The OCG supports research to improve sequence-based molecular characterization methods and their throughput and evaluate the various novel techniques of DNA sequencing. There are two projects ongoing which were initiated in 2008 and were selected by an expert scientific panel which was convened by SAIC. The data generated are submitted to the DCC and NCBI databases and are publicly available under the access regulations developed for NIH-supported projects, such as GWAS, TCGA, etc.

Goals

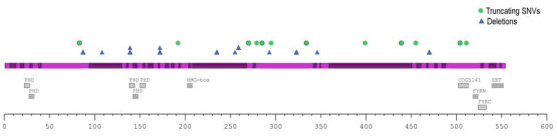
Project 1 evaluates whole transcriptome sequencing with second-generation technology on 92 cases of Diffuse Large B-Cell Lymphoma (DLBCL) and the analysis of the resulting data within the biological context of the disease. Project 2 compares second-generation vs. Sanger sequencing of ~240k amplicons/case of PCR-amplified exon products from genomic DNA of 23 cases of pediatric medulloblastomas in the discovery screen. The candidate mutations were sequenced in 80 cases (the prevalence screen) and the complete data analyzed to discover driver mutations.

Results

The results from the DLBCL project are highlighted here. DLBCL is the most common type of non-Hodgkin lymphoma. Previous molecular characterization by a number of laboratories stratified the cases into four subtypes, germinal-center, activated-B cell (ABC, involves the activation of NF-kB), mediastinal, and others. The whole transcriptome data led to the discovery of mutations in expressed genes and evidence of other genomic alterations including, but not limited to, translocations, insertions, and deletions. The team discovered that the histone modification pathway is important in DLBCL, a surprising finding given the DLBCL has been extensively studied by a number of investigators.

In addition, the investigators are developing bioinformatic algorithms to quantitate the sequencing data into a measure of transcript levels. One of the challenges has been how to incorporate alternative splicing into the model. Work is ongoing and the preliminary results look promising.

Widespread Distributed Somatic Mutation in MLL2



Lymphoma type	FL	DLBCL	DLBCLcell-lines	Centroblast
Truncation	16	4	5	0
Indel with frameshift	18	7	6	0
Splice site	4	2	0	0
SNV	6	4	2	0
Any mutation	29 / 36	14 / 37	10 / 17	0/8
Percentage	81%	38%	59%	0%

R. Morin

Morin et al. (see below) published the EZH2 results earlier this year. They are currently following up a finding that MLL2 is also mutated in a large number of samples (see figure); the mutations are spread out across the gene and most are truncating. This is perplexing since EZH2 trimethylates K27 and thereby causes the reduction of transcription while MLL2 methylates K4 and causes activation of transcription.

Publication

Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, Paul JE, Boyle M, Woolcock BW, Kuchenbauer F, Yap D, Humphries RK, Griffith OL, Shah S, Zhu H, Kimbara M, Shashkin P, Charlot JF, Tcherpakov M, Corbett R, Tam A, Varhol R, Smailus D, Moksa M, Zhao Y, Delaney A, Qian H, Birol I, Schein J, Moore R, Holt R, Horsman DE, Connors JM, Jones S, Aparicio S, Hirst M, Gascoyne RD, Marra MA. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet*, 2010 Feb;42(2): 181-185. Epub 2010 Jan 17

HIV+ Tumor Molecular Characterization Project (H+TMCP)

The project was established in August 2009 and is a joint effort of the OCG and the Office of HIV and AIDS Malignancy (OHAM).

Goals

The goals are to characterize human immunodeficiency virus (HIV)-associated cancers (obtained from HIV-infected patients) and compare them to the same types of cancers from patients without HIV infection and determine the molecular causes of the disease.

Background

Approximately 1.1 million people living in the United States are infected with HIV. The global prevalence of HIV infection is approximately 40 million. Persons infected with HIV have an elevated risk of cancer and mortality, and cancer is a leading cause of death among people with HIV/AIDS. Certain cancers, but not others, are increased in patients with HIV infection. Even though many HIV-associated cancers have a viral etiology, and immunodeficiency is believed to provide a permissive environment for viral oncogenesis, many questions remain about how these tumors form. Surprisingly, these tumors have not been extensively molecularly characterized and very little sequencing has been done. For example, a recent search in PubMed did not find any manuscripts with sequencing (even of a small number of candidate genes) in lung cancers from HIV+ patients.

Research Plan

OCG and OHAM organized a number of teleconferences in the past 6 months with HIV experts to discuss the tumor selection based on increased incidence, severity of disease, tissue availability, etc. The rationale for the tumors selected is as follows:

- *Diffuse Large B-Cell Lymphoma (DLBCL):* Incidence of DLBCL is significantly increased among HIV+ patients, a trend that continues to rise despite highly active antiretroviral therapy (HAART). A significant proportion of the cases are not known to be caused by an oncogenic virus, and there are questions about both the pathogenesis and high rate of incidence.
- *Lung:* Lung cancer incidence is significantly increased among HIV+ patients, including those on HAART. The HIV+ patients are heavy smokers, yet the lung tumors are almost exclusively non-small cell adenocarcinomas, suggesting a different biological etiology as compared to their non HIV+ counterpart.
- *HPV-Related Cancer:* This project will characterize a human papilloma virus (HPV)-derived cancer, either cervical or anal, as yet to be determined. The selection criteria include tissue availability (control as well as tumor) based on prevalence and logistic parameters.

The highlights of the progress made to date include the development of standard operating procedures for tissue collection, evaluation, processing, etc. (all lessons learned from TCGA and TARGET). The clinicians, investigators, and/or institutions providing care for the HIV+ cancer patients who can contribute the high-quality tissues and participate in the project(s) have been identified. We are working with the SPORE project director, and among the investigators who will participate in H+TMCP and provide tissues are those funded by SPORE grants; this allows for efficient utilization

of NCI's resources. We have two MTAs in place and will test the entire pipeline of the process, including the molecular characterization by sequencing, for the cases which pass quality control, next month. The DCC, and the regulatory infrastructure, is already in place for deposition of the data so that it can be shared with the global research community.

The molecular characterization will consist of second-generation sequencing of 100 cases from each HIV+ tumor type (paired tumor and germline DNA) and transcriptome. These platforms allow discovery of mutations in both coding and non-coding genomic regions, as well as determination of gene expression profiles and genomic alterations (including translocations, insertions, and deletions). The contract-based sequencing provides the project with flexibility to evaluate results and, if the QC data warrant it, make changes in technology and specifics of the sequencing methods utilized. Comparing tumors of cancer patients both with and without HIV infection will provide insight into the potential function of this virus in certain cancers; for example, CGCI is generating data on non-HIV DLBCL patients. For comparison, the ethnicity and, if possible the gender and age of diagnosis, will be matched. TCGA is characterizing lung cancer and that dataset will be used for the comparison component with the same requirement for careful matching of ethnicity, gender, and age of diagnosis.

Each cancer type will have a group of experts (clinicians who see HIV+ patients with cancer, experts in molecular characterizations of cancers, etc.) who will participate in the analyses and generation of the publication. For DLBCL, more than six researchers have provided a verbal commitment of their role in the project. The analytical tools that have been developed for the other NCI-supported projects will be available as well.

Funding
$3.5M/year, up to 5 years

2.3 Cancer Target Discovery and Development (CTD^2) Network

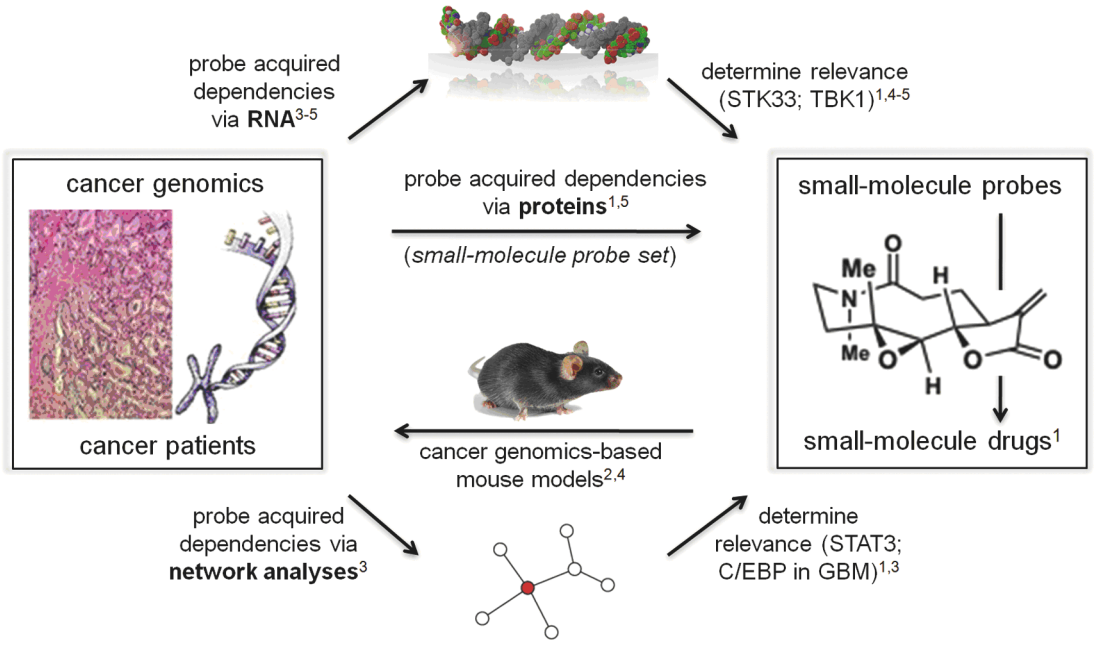
Background
The comprehensive characterization data from tumor transcriptomes and genomes generated by the CGCI, TARGET, and TCGA (the last project is a collaboration with NHGRI) are producing detailed information on the repertoire of alterations in a variety of tumors. The next 5 to 10 years will result in a compendium of all possible changes from the projects listed above, as well as the recently initiated International Cancer Genome Consortium (ICGC). The initial results clearly demonstrate that individual genes and alterations are not the drivers of cancer; rather, cancer arises from the alterations of one or more biochemical pathways. In addition, it is not yet clear (a) whether oncogenic drivers essential for tumor initiation are the same for progression as for metastasis; (b) how, if at all, the mutation load (i.e., the mutations in genes which are not "drivers" for one of the steps, but could be facilitators of future phenotypes of the tumor) impacts on cancer's etiology.

Goals
A new approach to the discovery of cancer therapeutics is emerging that begins with the cancer patient. It requires relating the genetic features of cancers to acquired gene and pathway dependencies and identifying small-molecule therapeutics that target them. The Cancer Target Discovery and Development (CTD^2, http://ocg.cancer.gov/programs/tddn.asp) network is an ARRA-funded pilot to develop new scientific approaches to accelerate the translation of the genomic discoveries into new treatments. The network emphasizes interaction of laboratories with complementary and unique expertise, including bioinformatics, genome-wide loss of function screening and targeted gain-of-function candidate gene validations, judicious use of mouse-based screens, and small-molecule high-throughput screens.

Progress

In April 2009, NCI issued a RC2 RFA and in August 2009 convened a special review panel. The network was selected based on the priority scores and the goals of the NCI as stated in the announcement. The five centers are:

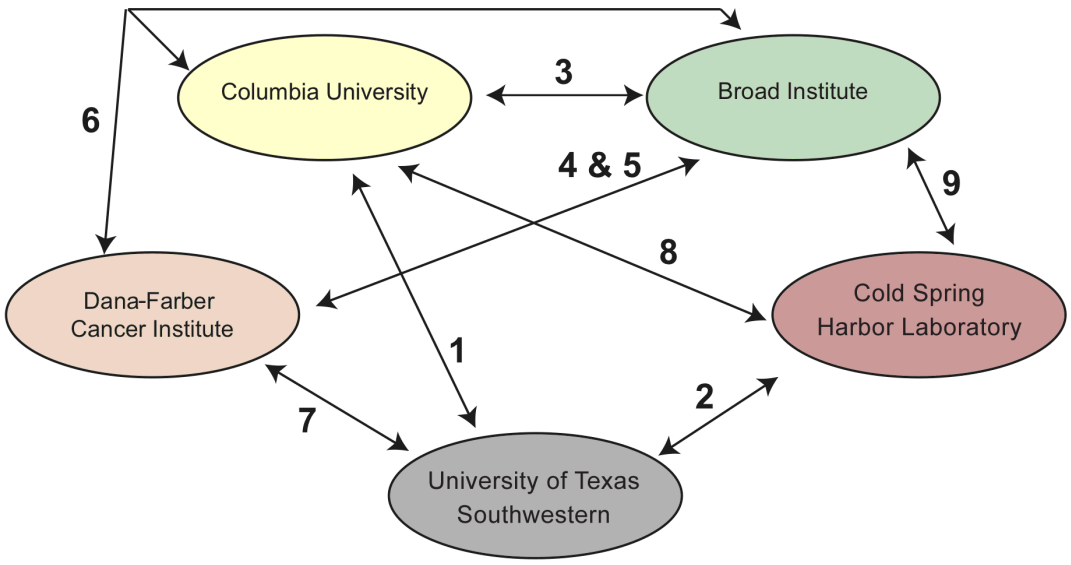
Project Title:	Targeting Causal Cancer Genes with Small Molecules
Affiliation:	Broad Institute, Cambridge, Massachusetts
Principal Investigator:	Stuart Schreiber, Ph.D.
Project Title:	Cold Spring Harbor Laboratory
Affiliation:	Cold Spring Harbor Laboratory, Long Island, New York
Principal Investigator:	Scott Powers, Ph.D., co-PI: Scott Lowe, Ph.D
Project Title:	Systems Biology of Tumor Progression and Drug Resistance
Affiliation:	Columbia University, New York, New York
Principal Investigator:	Andrea Califano, Ph.D.
Project Title:	Functional Annotation of Cancer Genomes: TCGA, Glioblastoma and Ovarian Cancer
Affiliation:	Dana-Farber Cancer Institute, Boston, Massachusetts
Principal Investigator:	William Hahn, M.D., Ph.D., Lynda Chin, M.D., and Ronald DePinho, M.D.
Project Title:	A Concerted Attack on Patient Specific Oncogenic Vulnerabilities in Lung Cancer
Affiliation:	University of Texas Southwestern Medical Center, Dallas, Texas
Principal Investigator:	Michael Roth, Ph.D., co-PIs: Michael White, Ph.D., and John Minna, M.D.



The Cancer Target Discovery and Development (CTD²) Network of the NCI aims to relate the genetic features of cancers to acquired cancer dependencies and to identify small molecules that target the dependencies (superscript numbers refer to [1]Broad Institute of Harvard and MIT; [2]Cold Spring Harbor Laboratory; [3]Columbia University; [4]Dana-Farber Cancer Institute; [5]University of Texas Southwestern Medical Center at Dallas).

The mission of the CTD^2 Network is to decode cancer genotypes so as to read out acquired pathway and oncogene addictions of the specific tumor subtypes, and to identify small molecules that target these dependencies. The CTD^2 Network is probing the consequences of the cancer-present alterations on the dependencies or co-dependencies different cancers have on specific oncogenes or their interacting genes ("oncogene addiction" and "non-oncogene co-dependencies"). Cataloguing these Achilles' heels and linking them to the causal genetic alterations will be critically important for therapies that are tailored to individual patients, including combination therapies aimed at targeting multiple such dependencies at once. It will also be important for anticipating resistance mechanisms and identifying clinical biomarkers.

The Network has a monthly Steering Committee telecon (attended by the PIs and their key personnel) to discuss progress in center-based projects and identify topics of common interest that can be pursued. The Center PIs agreed to a data-sharing policy that is in parallel with that for genome projects, although they would like to be able to evaluate the quality of the data (which entails doing analyses that become part of their publication). The network has an IT subcommittee (each Center has at least one cheminformatic specialist as a member, together with NCI's NCIIT and OCG staff) which is working out the issues of data formats, definition of metadata, etc. The expectation is that the first dataset will be available next month and the process of submission and retrieval tested.



(1) Collaborative integration of proteomic, chemical, and genetic data for response network construction together with empirical validation of predicted molecular pressure points to build tumorigenic response networks in non-small cell lung cancer. (2) Construction of genetic archetypes as a meaningful disease subtype classifier in non-small cell lung cancer by leveraging copy number variation data from an extensive panel of tumors and cell lines. These will be used to predict and test subtype-selective chemical and genetic perturbations. (3) Collaboration to discover and characterize small molecule probes that target STAT3 and CEBPβ/δ, two synergistic transcriptional master regulators associated with the mesenchymal subtype of GBM. This screen is directed toward the identification of direct inhibitors of these transcription factors. (4) Discovery and characterization of small molecules that target a tumor cell dependency amplified in ovarian cancer (ID4). (5) Discovery and characterization of small molecules that target a kinase encoded by a gene discovered as synthetic lethal with k-RAS in RNAi screens (TBK1). (6) Columbia will develop regulatory models of ovarian cancer, using reverse-engineering algorithms and TCGA data. These models will be interrogated for master regulators of tumorigenesis and cis-platinum sensitivity. The Dana-Farber and Broad teams will validate the targets and identify small-molecule inhibitors that can be used to abrogate their activity in vivo. (7) Established a focused collaboration combining copy number variation and whole genome siRNA screens to pinpoint acquired dependencies in melanoma. (8) Based on master regulator analysis for ovarian cancer, Columbia will assist the CSHL team on the prioritization of the key drivers of the neoplastic phenotype derived from their genome-wide gene-silencing screen in the mouse. CSHL will perform functional validation of high-likelihood, high-impact regulators using their transplantable mouse model system. (9) Discovery and characterization of small-molecule probes that target a highly validated tumor cell dependency discovered in AML.

The key members of each Center met face to face in January 2010 and committed to initiating collaborative projects to emphasize the creativity of each Center and add intellectual value. They developed nine projects (CTD^2 Network SharePoint and figure):

CTD^2 Collaborative Projects

Publication
The Cancer Target Discovery and Development Network: Towards patient-based cancer therapeutics, *Nature Biotechnology*, accepted for publication, 2010

Funding
$12M total/year for 2 years with Recovery Act funds

Action Item
The Recovery Act funding will end on September 30, 2011. An informational presentation to the NCI BSA in June 2010 elicited great enthusiasm by the members. If this concept is to continue as part of NCI's portfolio, a new RFA needs to be issued by the latter part of autumn, which means approval at the November BSA. It is an aggressive timeline, but can be achieved. The OCG requires the NCI Director's approval to start the process.

2.4 Projects Recently Completed

Initiative for Chemical Genetics (ICG)

Background
In February 2002 the NCI funded ICG to enable public research to accelerate the discovery of small-molecule probes. ICG was led by Dr. Stuart L. Schreiber and was a public-access research facility consisting of an integrated team of synthetic and analytical chemists, assay developers, high-throughput screening (HTS) engineers, computational scientists, and software developers. The HTS concept was adopted by the NIH and became the basis of the Molecular Libraries and Imaging Roadmap project, whose pilot phase was funded in the fall of 2005 and expanded 3 years later. ICG also set the stage for the Recovery Act-funded RC2 RFA to develop a program in innovating and accelerating the science of progression from high-density genomic data to functional validation of candidate genes (for treatment or prognostic or diagnostic markers) and small-molecule (chemical or biological) screening to identify targets and their modulators for future drug discovery. The program is the CTD^2 Network and is described above.

Highlight of Scientific Accomplishments
Synthetic chemistry has enabled the creation of large collections of complex and diverse small molecules, patterned after natural products, which are tested for the ability to induce specific biological phenotypes. The ICG provided a systematic approach to study biology using such small molecules, to develop new screening tools and compounds, and to accelerate the development of new cancer strategies and therapies. The ICG focused on a number of deliverables, including biological assays, chemical libraries, a repository of chemical probes, and a scientific database. Discoveries made through the ICG program have resulted in more than 100 publications and 17 patents.

Biological Assays. About 130 unique, individual biological projects were analyzed through the ICG. Many of the screens have been developed by external investigators using assistance and supplies provided by the ICG. All results were deposited into ChemBank no later than 1 year after the completion of the screen. The group is investigating how the results can also be submitted into NCBI's PubChem.

Chemical Synthesis. The ICG synthesized more than 10,000 novel compounds and acquired unique molecules from external academic sources.

Scientific Database. ChemBank is an online database and includes chemical data on 700,000 small molecules, a subset of which has also been characterized further using additional assays. Investigators can use ChemBank's tools to query and analyze available data and export raw information for subsequent analysis. ChemBank enables researchers to identify promising drug candidates for further development and to gain new knowledge of human disease.

Publications which highlight important scientific advancements, the first on new technologies which were applied to a challenging target in cancer and the second on how creative technology development is used to support novel drug discovery:

1. Stanton BZ, Peng LF, Maloof N, Nakai K, Wang X, Duffner JL, Taveras KM, Hyman JM, Lee SW, Koehler AN, Chen JK, Fox JL, Mandinova A, Schreiber SL. A small molecule that binds Hedgehog and blocks its signaling in human cells. *Nat Chem Biol,* 5(3): 154-156, 2009. PMID: 19151731; PMCID: PMC2770933

2. Ong SE, Schenone M, Margolin AA, Li X, Do K, Doud MK, Mani DR, Kuai L, Wang X, Wood JL, Tolliday NJ, Koehler AN, Marcaurelle LA, Golub TR, Gould RJ, Schreiber SL, Carr SA. Identifying the proteins to which small-molecule probes and drugs bind in cells. *Proc Natl Acad Sci U S A,* 106(12): 4617-4622, 2009. PMID 19255428; PMCID PMC2649954

Mammalian Gene Collection (MGC)

Background

MGC was a trans-NIH project initiated in 1999 and led by the NCI and NHGRI to generate one full-length cDNA for each of the mouse and human genes. Projects to generate a partial set of cDNAs for other species were added along the way and with various partnership structures, but the pipeline and/or the clone generation process was identical. The project was managed through the NCI SAIC subcontracts mechanism, which was essential for its completion.

Scientific Accomplishments

The characteristics of the MGC have been published and access to the clone lists can be found at mgc.nci.nih.gov. Drs. Temple (NHGRI) and Gerhard wrote a manual instructing how to find and obtain the clones (see MGC home page).

Upon the departure of Dr. S. Klein from NICHD, OCG became the lead on cloning *Xenopus* cDNAs (*tropicalis* and *laevis*). In addition to the utility of the cDNA clones for the study of *Xenopus* biology, the clones were important for the understanding of the genome. Specifically, due to the highly repeated structure of the *X. tropicalis* genome, the clones generated by XGC were necessary to provide scaffold for the assembly of the genome sequence; the results were published earlier this year (see below).

Finally, Drs. Temple and Gerhard provide input to the ORFeome Collaboration (http://www.orfeomecollaboration.org/) an international good-will collaborative effort to convert the human MGC clones into ORF-only fully sequenced constructs in an "entry" vector. The clones are available through 15 distributors worldwide. The expectation is that this will be completed by the end of 2010. Two CTD^2 Centers will use the ORF clones from OC in their gain-of-function screen.

Publications

1. MGC Project Team, Temple G, Gerhard DS, Rasooly R, Feingold EA, Good PJ, Robinson C, et al. The completion of the mammalian gene collection (MGC). *Genome Res*, Dec;19(12):2324-2333, 2009. Epub 2009 Sep 18. PMID: 19767417

2. Hellsten U, Harland RM, Gilchrist MJ, Hendrix D, Jurka J, Kapitonov V, et al. The genome of the western clawed frog *Xenopus tropicalis*. *Science*, 328:633-636, 2010. PMID: 20431018

3. Future Activities and Vision

The OCG has been at the forefront of the rapid application and development of novel genomic and translational technologies, and their utilization for the understanding of cancer etiology and application of the findings to the patient. The OCG will continue to support innovation and the facilitation of extramural research. The complexity of cancer at the cellular, tissue, organ, genetic, and environment levels requires integration of population-based genetics with molecular characterization of cancer tissues (somatic changes) and environmental exposures (diet, medication, air, etc.). This integration needs to happen rapidly to ensure efficient use of the datasets generated by the various cancer subspecialties, and OCG is well poised to facilitate that process.

The NCI is a large institute and requires a nontrivial effort to interact and keep abreast of progress with other components, not only within CSSI, but also the Divisions, CCR, etc. We think that the effort is essential to minimize duplication of projects and optimize the utilization of available resources. To keep communication channels open, the OCG invites representatives from other divisions to the face-to-face steering committee meetings and in turn OCG staff participates in their meetings. OCG members participate in trans-NCI or trans-NIH groups on topics of tissue accrual, genetics, genomics, data release, data access, and drug development. For example, Dr. Gerhard was one of the leaders of the CGEMS project and at the request of the DCEG transitioned her role, as well as the management of the Web site that OCG developed, to them. However, she remains engaged through discussions and attendance at workshops.

Opportunities: Selected Examples

1. TARGET
    - OCG facilitated the interaction of one TARGET project with a CTD^2 Center to identify novel targets and/or markers which can be followed up for development of novel therapies and associated predictive or prognostic markers.
    - The initiative includes two CCR collaborators in the NBL and OS projects, who in turn will keep the rest of the CCR groups informed about the progress and lessons learned.
    - We invited the CTEP and SPECS project officers to participate in the projects of their interest. Drs. Smith and Gerhard answer questions and present updates as needed. The goal is to ensure transparency and elimination of duplication. In the future, there may be interest in developing translation initiatives that utilize the TARGET data.
    - TARGET has initiated a partnership with a nonprofit bioinformatics organization, SAGE Bionetworks. They will collaborate with the AML research project to integrate all data generated within TARGET as well as published data to determine new pathways causing the disease. The outcome of this collaboration will inform other NCI-funded large-scale molecular characterization projects about the methodologies and will make them rapidly available for use as the data formats will already have been established.
    - We have initiated a collaboration with Dr. Park of the National Cancer Center of Korea to study pediatric OS. TARGET is providing the standard operating protocols (SOPs) for all aspects of the project to Korea so that the tissues and the molecular analytes are of highest quality. They will follow up the OS's project findings to determine whether the molecular drivers of cancer development are different or the same as found in the population studied in TARGET; this will define the contribution of the genetic background and/or the environmental exposures. In addition, we facilitated their contact with the COG leadership, and a representative will attend the next COG meeting, which may lead to their international membership.
2. H+TMCP
    - We have initiated a discussion with a private foundation to study a total of ~100 cases of Burkitt's lymphoma, with and without HIV infection using sequence-based molecular characterization. Dr. L. Staudt is on its

scientific board and they proposed to work with NCI through the FNIH. This interaction is at an early stage and there is an opportunity to get funding as well as their participation in collecting samples in the United States, Europe, and Africa. The interaction would be of benefit to the scientific community and the foundation, a "win-win" for all. The foundation funds would ensure that a fourth HIV+ cancer is studied and the advisory board will provide the scientific leadership to analyze the data and follow up with translation of the results. The project would use the SOPs NCI already tested for DLBCL and other cancers to ensure that the quality of the tissues, molecular analytes, and data generated is high. Finally, the data will be made available through the CGCI DCC and would be accessible to the scientific community.

- The study of HIV+ lung cancer will be synergistic with TCGA's efforts in non-HIV lung cancer. The DCC infrastructure will make it possible to compare the molecular changes in the two tumor types effortlessly. In addition, we are interacting with the NCI lung SPORE program (from the project officer to the PIs) to obtain the tissues which will be characterized, thereby conserving tissue as well as monetary resources. One of the SPORE PIs is actively engaged in leading the analyses and the translation of the findings once the data are generated.

3. CTD^2
   - *NCI Experimental Therapeutics (NExT) program.* The OCG director sits on the Senior Management Group established for NExT by Dr. Doroshow. These are monthly meetings whose main function is to exchange information and discuss strategies and identify solutions to problems that may have been encountered with the NExT leadership and staff. The meetings provide opportunities to identify possible overlaps and exchange lessons learned. In addition, OCG staff was asked a few months ago to serve on one of the Special Emphasis Panels that was evaluating one round of functional biology center proposals submitted in response to an RFA.

   - *Molecular Libraries Initiative (MLI).* Two of the OCG staff participate on the MLI project team, and one is also a Science Officer. This interaction has benefited both MLI and OCG chemical genetics programs and the NCI leadership as it ensures information exchange, reduces the possibilities of duplication, and encourages the application of results (positive and negative) for current and future projects. For example, the experience in PubChem and ChemBank was used to define the data formats, etc. for CTD^2. Tools developed for mining the data by all these projects will be easily accessible.

4. Interactions Across the NCI and NIH

   One example of a trans-NCI interaction is the organization of a workshop which was conceived in the OCG and developed together with the Division of Cancer Control and Population Sciences (DCCPS) and Division of Cancer Epidemiology and Genetics (DCEG), with input from extramural investigators, titled "Integrating Knowledge of Inherited Genetic Variation and Acquired Somatic Alterations in Cancer Tissues: Promising Scientific Opportunities." The December 2009 meeting was attended by extramural and intramural investigators with expertise in either genome-wide association studies (GWAS), the study of somatic large-scale comprehensive genetic alterations in tumors, or bioinformatics. NCI and other funders are currently conducting and/or supporting major collaborative studies to characterize the genomes of many cancers. The studies address both cancer germ-line variation and somatic mutations. Its objectives were to:

   - Identify the most promising scientific opportunities to integrate knowledge of human constitutional genomic variation with somatic genomic alteration in cancer tissue.
   - Identify analytic methods, tools, and pipelines that exist or need to be developed to analyze and integrate constitutional and somatic data and make them accessible to researchers.
   - Identify resources, such as tissue, informatics, and funding, that exist or are needed to allow researchers and clinicians to efficiently access, integrate, analyze, and query these data.
   - Identify resources, such as datasets and metadata, that exist or are needed to make the results applicable for use in the clinical setting, for example, for patient treatment, classification, and stratification.

Appendix: Current Staffing

Daniela S. Gerhard, Ph.D., Director, OCG

Dr. Gerhard moved to the OCG/NCI in 2002. Her research interests include the identification of somatic mutations in cancer, determination of genetic risk factors in cancer, identification of which pathways are the same and which are unique in sporadic vs. inherited cancers, the integration of the results from somatic and genetic studies to elucidate the various components of cancer etiology, and the development of new scientific methods to rapidly translate large-scale genomic data into patient-based therapeutics with concomitant predictive markers. She participated in the Cancer Genetic Markers of Susceptibility project, which has published on large whole genome association studies (GWASs) of prostate and breast cancer that identified candidate loci that influence the development of risk; these were the first GWAS data that were made freely available to other researchers to use. Dr. Gerhard received her baccalaureate degree in biochemistry from Barnard College and her Ph.D. degree in genetics and molecular biology from Cornell University Medical School. She did a postdoctoral fellowship in the laboratory of Dr. Housman at Massachusetts Institute of Technology, developing and implementing novel tools that were used in genetic mapping of human diseases and the identification of genes causing cancer. She headed a laboratory for 16 years in the Department of Genetics at the Washington University School of Medicine in St. Louis. She participates in a number of NCI and NIH committees dealing with genomic policies, patient protection, and data sharing, is a reviewer for a number of journals, and has published more than 80 manuscripts, 8 chapters, and reviews.

Jean Claude Zenklusen, Ph.D., Scientific Program Director, OCG

Dr. Zenklusen is a scientific program director in the NCI Office or Cancer Genomics and manages the HIV+ Tumor Molecular Characterization initiative and participates in the Cancer Target Discovery and Development network. In addition, he acts as a Science Officer in the NIH Molecular Libraries and Imaging (MLI) program, helping to guide and complete high-throughput small molecules screening that results in high-quality chemical probes to enhance our understanding of biology. In this role, he provides information about the efforts funded by MLI to ensure that OCG's projects do not overlap. Dr. Zenklusen received his master's degree in chemistry from the University of Buenos Aires, and his doctorate degree in cancer biology and genetics from The University of Texas; his thesis project focused on the discovery of two novel tumor suppressor genes on human chromosome 7. He was an NIH Intramural Fellow at the National Human Genome Research Institute and then joined the Neuro-Oncology Branch at NCI as a Staff Scientist in the laboratory of Dr. Howard Fine, where he developed and managed the Glioma Molecular Diagnostic Initiative (GMDI) and its database, the Repository of Molecular Brain Neoplasia Data (Rembrandt).

Jaime M. Guidry Auvil, Ph.D., Scientific Project Manager, OCG

Dr. Guidry Auvil is a scientific project manager for the TARGET Initiative and oversees the administrative and supportive tasks that arise with monitoring the progress of data generation, submission, and analysis by each group representing the five cancers studied. In addition, she acts as a communications and education liaison for the OCG, by creating awareness about office initiatives, research findings, and the impact of these efforts to enhance the understanding of genomics in cancer biology. Dr. Guidry Auvil earned her bachelor of science degree with honors from Wake Forest University with a premedical focus, minoring in psychology and chemistry. Dr. Guidry Auvil graduated magna cum laude to obtain her doctorate in tumor biology from Georgetown University. Dr. Guidry Auvil's thesis work and subsequent postdoctoral fellowship focused on characterizing the role of mesenchymal stem cell marker, cadherin-11, in aggressive cancers. Her work led to the discovery of a novel small-molecule inhibitor, which is currently moving toward early-phase clinical trials. Prior to returning to graduate school, Dr. Guidry Auvil gained experience in the biotechnology field performing molecular and cell biology research for a Phase III clinical trial for an AIDS vaccine.

Two part-time (<10% each) contractors assist the OCG staff with tissue-related issues, including the alignment with processes developed for TCGA and TARGET, and meeting minutes.

Future personnel

OCG will be joined in September by an AAAS fellow, Dr. Bougham.

Office of Cancer Clinical Proteomics Research (OCCPR)

1. Mission and Goals

The Office of Cancer Clinical Proteomics Research was established to develop a robust science-based foundation for proteomics to enable the development and ultimately the clinical application of protein biomarkers to inform and support the development of molecularly based cancer diagnostics and therapeutics.

To achieve this mission, after a great deal of due diligence with the extramural scientific community, the NCI established a program, the Clinical Proteomics Technologies for Cancer initiative. Phase I of the initiative sought to develop and standardize proteomics technologies, create standard operating procedures to ensure reproducibility across laboratories, and create high-quality reagents. Phase II of the program will address more comprehensive goals with plans to:

- Characterize the proteomic component of cancer-related biospecimens, informed by defined genomic changes in specific cancers
- Develop technologies and procedures that provide accurate, reproducible, and precise measurements of cancer-related proteins and limit variability
- Discover and verify biomarkers for further qualification studies
- Develop publicly available datasets, reagents, standards, and computational tools that help define proteomic states

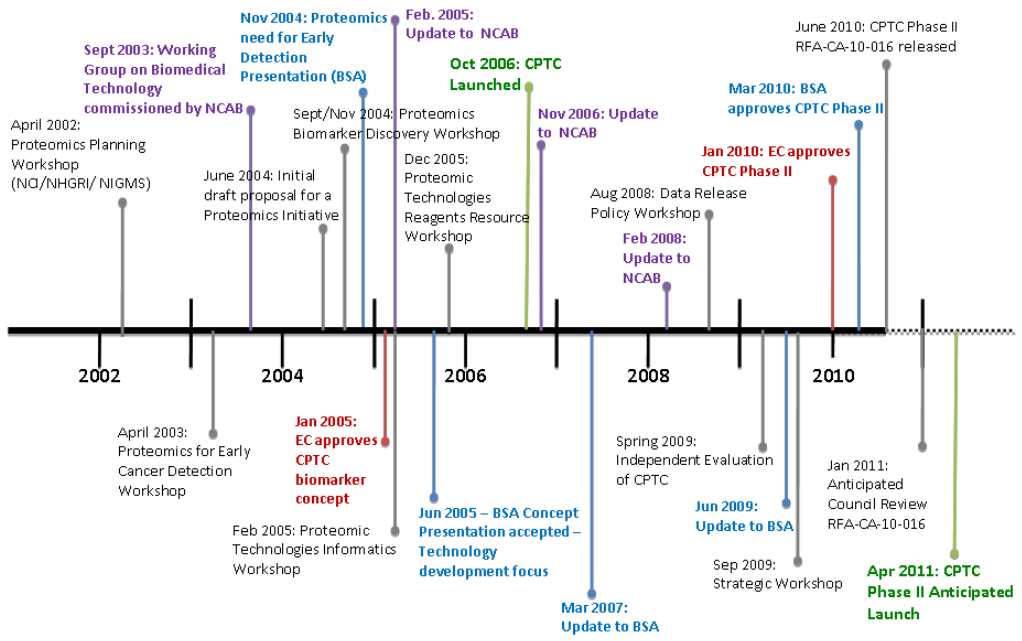


Figure 1. Timeline of CPTC Development Process.

2. Development Process

NCI's Clinical Proteomic Technologies for Cancer initiative (CPTC) was recommended to the NCI by the National Cancer Advisory Board's Working Group on Biomedical Technology. This working group, commissioned in 2003 and chaired by Drs. Eric Lander and Lee Hartwell, provided the framework for a molecular diagnostic development program that utilized recent technological advances for detecting proteins in patient samples. The working group emphasized the importance of team science, streamlined sample collection, data standards, robust informatics platforms, and the availability of well-characterized reagents. In response to this recommendation, NCI held a series of workshops (figure 1) that led to the development of the CPTC initiative.

The NCI Executive Committee approved this original concept for the CPTC encompassing biomarker discovery and technology development. In interactive reviews with the NCI Board of Scientific Advisors (BSA), it was recommended that the program focus primarily on proteomic technology assessment/development. These recommendations were incorporated into the concept and the BSA unanimously approved the CPTC initiative to address the lack of reproducibility and transferability of measurement technologies across laboratories and lack of quality reagents for the cancer research community in June 2005.

After 4 years of work by CPTC teams, an independent evaluation of the CPTC program was conducted in 2009. The evaluation was followed by a workshop in September 2009 entitled "Implementation of a New Cancer Biomarker Development Pipeline," which was held to seek input from the extramural proteomics research community on ideas that they felt would be of most value to the field as CPTC moved into Phase II of the initiative. Based on recommendations from this and previous workshops, a concept for the reissuance of the CPTC initiative was developed. The NCI Executive Committee approved this concept in January 2010. In March 2010, the BSA unanimously approved the concept to include a greater emphasis on discovering biomarkers and an increased openness to technology platforms beyond mass spectrometry. The corresponding RFA (RFA-CA-10-016) was released in late June 2010, with an application receipt date of September 29, 2010. Anticipated council review date is January 2011, with an earliest anticipated start date of April 2011.

3. Program(s)

(a) Background for the Program

Background

Proteomics is the field of research concerned with the multiplex measurement of proteins and peptides. Early in the 21st century, two events sparked widespread interest in proteomics. First, the completion of a working draft of the Human Genome Project in June 2000 provided the blueprint for amino acid sequences in human proteins, opening proteomics as the next unexplored frontier for biology. Under the leadership of Drs. Hartwell and Barker, NCI initiated a process to thoroughly examine the field of proteomic biomarkers and proteomics overall beginning in 2003. Concomitant with this process, a number of publications, including a *Lancet*[1] report of a successful early detection mass spectrometry-based blood test for ovarian cancer (sensitivity of 100% and specificity of 95%), provided significant impetus to the field.

As part of its due diligence, the NCI convened a series of workshops aimed at harnessing this area of science (first workshop held in April 2002, involving the NCI, National Human Genome Research Institute (NHGRI), and National Institute of General Medical Sciences (NIGMS); figure 1). Based on input from the research community, the NCI concluded that although proteomics held great promise for biomarker development, there was a great deal of work to do. One of the issues identified at an earlier symposium ("Defining the Mandate of Proteomics in the Post-Genomics Era") was that while current technologies for proteomic measurements have reached various degrees of maturity, none was fully mature.[2] The broader scientific field encountered further setbacks related to technological variability, sample preparation, and study design. NCI held additional workshops (from 2004 to 2005) and developed a responsive concept to address many of the identified issues, which was presented to the BSA. Through interactions with the BSA, the proposed proteomics-based biomarker discovery program was redefined to address measurement accuracy and reproducibility issues associated with technologies and the lack of reproducibility across laboratories. The NCI's CPTC initiative was approved by the BSA in June 2005 and launched in October 2006. CPTC was charged to address the lack of reproducibility and transferability of measurement technologies (mass spectrometry) across laboratories and lack of quality reagents.

The Need for New Technologies in Cancer Biomarker Discovery

Historically, cancer protein biomarkers have been discovered in body fluids and tumor tissues (or cell lines) using 2D-gel separations or by identifying immunogenic antigens on cancer cells. Conventional approaches have successfully produced nine FDA-approved, blood-based cancer biomarkers to date, most of which are used to monitor treatment.[3] The number of new protein biomarkers achieving FDA approval has trended downwards for the past decade to a point where only 0-3 new markers are approved per year (across all diseases).[4] This disappointing downward trend suggests that conventional approaches have contributed all that they can and there is a need to implement new approaches and new technologies to discover novel protein biomarkers of clinical relevance.

A typical protein biomarker pipeline involves a discovery phase followed by a qualification (clinical validation) stage. Modern "-omics" experiments are capable of producing thousands of candidate biomarkers; however, most of these potential candidates do not have commercially available enzyme-linked immunosorbent assay (ELISAs) for qualification studies. Thus, at high expense ($100,000's-$1,000,000's) and long lead times (1-2 years) validation ELISAs are developed

1 Petricoin III EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, Mills GB, Simone C, Fishman DA, Kohn EC, Liotta LA. (2002). Use of Proteomic Patterns in Serum to Identify Ovarian Cancer. *The Lancet*, 359 :572-577.

2 "National Research Council Steering Committee. Defining the Mandate of Proteomics in the Post-Genomics Era: Workshop Report. (October 1, 2002). *Molecular & Cellular Proteomics*, 1, 763-780.

3 Ludwig JA, Weinstein JN. (2005). Biomarkers in cancer staging, prognosis and treatment selection. *Nat Rev Cancer*, 5 :845-856.

4 Anderson N.L. (2010 Feb). The clinical plasma proteome: a survey of clinical assays for proteins in plasma and serum. *Clin Chem*, 56(2):177-185. Epub 2009 Nov 2.

for only a small fraction of potential candidates.[5] Alleviating this rate-limiting step will allow evaluation of more candidates and thus inform the discovery and prioritization steps of those candidates most likely to reach success.

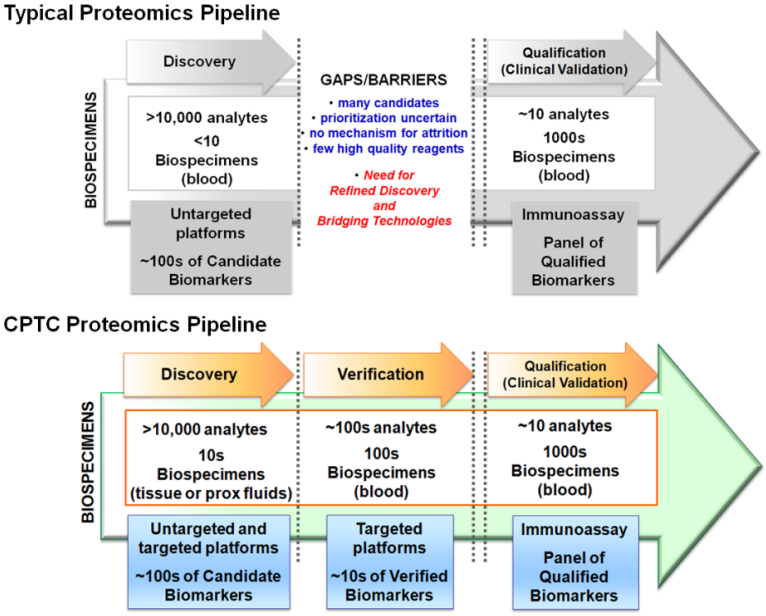


Figure 2. CPTC Proteomics Pipeline.

The first 4 years of CPTC have shown the effectiveness of this initiative to address the long-standing problems of measurement variability issues in proteomics resulting in large part from analytical platforms and addressing the need for a more reliable and efficient proteomics workflow. In this setting, CPTC investigators developed a new pipeline that addresses the variability of mass spectrometric technologies through the use of metrics and standards, including the lack of a coherent connection between biomarker discoveries with well-established methods for qualification studies (figure 2).

Clinical Proteomic Technology Assessment for Cancer (CPTAC) network investigators achieved a major milestone using targeted mass spectrometric quantitative assays to reproducibly credential discovered targets. Known as the "verification" stage (*gap* between biomarker discovery and qualification), this approach takes into account that very few protein candidates will ever meet the bar of a clinically useful biomarker – one that positively impacts patient care. Verification allows for the effective testing of a large number of candidate biomarkers using targeted, quantitative assays, which are commonly multiplexed and suitable for examination of a larger number of biospecimens to ensure appropriate statistical power. This approach offers a unique opportunity to credential large sets of biomarker candidates prior to costly qualification studies.

CPTC Reissuance

In the spring of 2009, an independent evaluation of the initiative was commissioned by the Office of the Director, NIH, for which initial support from two NIH institutes was required (letters of support provided by the proteomic directors at National Center for Research Resources [NCRR] and National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK]). The evaluation was performed by an outside firm (ICF Macro), whose evaluation of the CPTC initiative focused on processes and outputs with particular attention to program design, effectiveness of NCI program management, and strategies toward promoting collaborations and cohesion in the network. The interviews involved several groups: investigators, trainees, proteomics experts not participating in the initiative, program staff from NCI, NIH, and staff from other Federal agencies. An evaluation advisory committee (composed of four trans-NCI staff, one member from NCRR, one unaffiliated academic, and one CPTC member) was formed to examine and determine the merit of the independent program evaluation performed by ICF Macro. The committee's assessment of the program and its recommendations for the continuation of CPTC were used in the development of the RFA for the reissuance of CPTC (RFA-CA-10-016). Briefly, the committee noted:

5 Wang P, Whiteaker JR, Paulovich AG. (2009). The evolving role of mass spectrometry in cancer biomarker discovery. *Cancer Biol Ther,* 8(12):1083-1094.

- A successful beginning of an integrated network that operates through a joint principal investigator–NCI governance structure resulted in the achievement of significant milestones, the development of multidisciplinary teams, numerous scientific achievements, and renewed promise in the field of proteomics;
- The need for the continuation and expansion of efforts between the FDA and CPTC in translating multiplex protein-based in vitro diagnostic technologies (and candidates) to the clinic;
- The need for the continuation of the CPTC initiative beyond the 5-year mark to build upon the infrastructure and quantitative protein biomarker development pipeline established during the first 3.5 years of the initiative.

In the fall of 2009, leaders and experts from academia, industry, and regulatory agencies were convened by the NCI at a workshop titled *Implementation of a New Cancer Biomarker Development Pipeline*. The workshop focused on how the NCI should implement a new protein biomarker development pipeline that builds upon the analytical reliability and efficiencies emerging from CPTC, and reflects the biology of cancer. A consensus among attendees was to pursue the analysis of tissue for their proteins using samples from TCGA in order to systematically explore the cancer proteome that translates from defined alterations in cancer genomes. The workshop attendees agreed that complementing TCGA's genomics pipeline with CPTC's proteomics pipeline could produce a unique continuum that will allow the cancer research community to begin connecting cancer genotype to phenotype. As a result, this workshop formed the basis for the reissuance of the CPTC initiative, which leverages the outputs from CPTC Phase I to systematically explore the functional cancer proteome that derives from defined alterations in the cancer genomes or other factors in order to discover and develop verified cancer biomarkers. An important goal of this next phase is to provide the cancer research community with comprehensive proteomic characterization data of several tumor types. The CPTC reissuance is designed to support efforts that will utilize data and biospecimens from high-throughput cancer genome characterization and sequencing programs such as TCGA to discover and verify markers that can be transitioned to clinical studies by other NCI programs.

(b) Program Description – Goals

The CPTC initiative, launched in 2006, is structured around three distinct programs focused on removing major barriers in proteomics in order to enable the accurate and reproducible identification and quantification of proteins that could drive high-value clinical biomarker qualification studies. Achieving these goals would provide a firm foundation for the field of discovery proteomics and enable the rational development of clinical biomarkers to address various needs in cancer management. The three programs are as follows:

- *Clinical Proteomic Technology Assessment for Cancer network* (CPTAC network; U24 mechanism): provides funds for a geographically dispersed network of five multidisciplinary teams to collaborate on research that would increase the understanding of experimental and analytical sources of error for existing technologies. This research is establishing a basis for proteomics science and development in the form of technology and other standards, metrics, and reference datasets.
- *Advanced Proteomic Platforms and Computational Sciences* (APPCS; R01, R21, R21/33 mechanisms): provides grants for 15 individual investigators to develop novel tools and algorithms related to improving the accuracy of proteomics technologies.
- *Proteomic Reagents and Resources component* (PRRC; RFP mechanism): provides high-quality, well-characterized reagents (antibodies), data, and standard reference materials for the research community.

CPTC Reissuance

The main organizational structure of the proposed reissuance will largely replicate the structure of the CPTC initiative. However, incorporating "lessons learned" from the first 4 years of the program, as well as inputs from outreach efforts

to the proteomics community and NCI community, the APPCS and CPTAC programs were merged to form Proteome Characterization Centers (PCCs) in order to further integrate research activities.

One of the main goals of the CPTC reissuance is to establish a network of PCCs that will leverage the achievements from the CPTC initiative to systematically explore the functional cancer proteome that derives from defined alterations in the cancer genome or other factors in order to discover and develop verified cancer biomarkers.

An important goal of CPTC is to provide the cancer research community with comprehensive proteomic characterization data of several tumor types. The CPTC reissuance is designed to support efforts that will utilize data and biospecimens from high-throughput cancer genome characterization and sequencing programs (e.g., TCGA) to discover and verify biomarkers that can be transitioned to clinical studies by other NCI programs, e.g., the NCI's Cancer Therapy Evaluation Program (CTEP), Early Detection Research Network (EDRN), Cooperative Groups, and broad cancer research communities.

Each proposed PCC is to have a Discovery Unit and a Verification Unit, which together will implement the two-step proteomics developmental pipeline. PCC's are also to have a technology development plan to improve technology/ platform(s) used in the Discovery and/or Verification Units. Goals for each component are as follows:

- *Biomarker Discovery Unit:* PCCs are to identify and characterize proteins from tumor and normal biospecimens (tissue). Within the context of hypothesis-driven cancer biology and potential clinical applications, discovered biomarkers will be prioritized for verification assay development.
- *Biomarker Verification Unit:* PCCs are to develop analytically validated, multiplex, quantitative assays for verifying the prioritized list of discovered biomarkers in blood (and tissue/proximal fluids when appropriate). These assays will be performed on a large number of clinically relevant biospecimens in order to ensure sufficient statistical power of the results. Proteins successfully passing this verification screen are anticipated to be considered high-value targets for translating into other initiatives involved in clinical qualification studies. These candidates will be made publicly available through a CPTC Data Center, as will the unbiased discovery data and assay details.

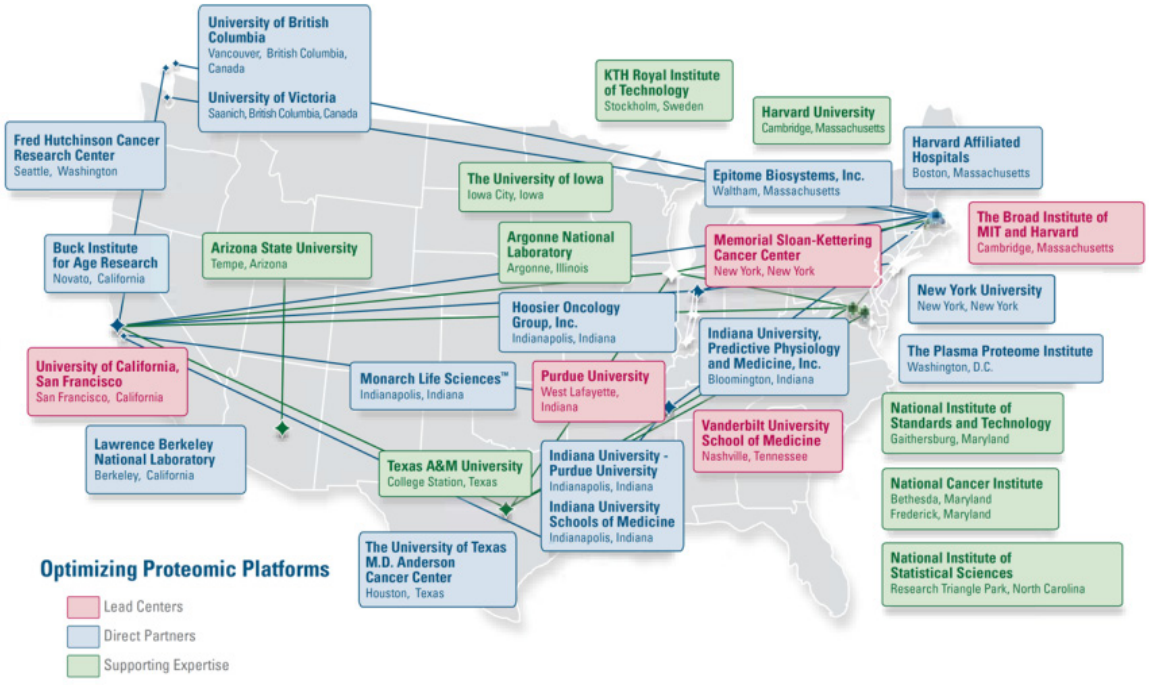


Figure 3. The CPTAC Research Network.

- *Technology Development:* PCCs are also expected to pursue analytical improvements on their proposed technologies for protein detection, identification, and quantification, actively leveraging the network environment.

(c) Funding History

CPTC Initiative

The CPTC initiative (phase I) awarded grants (5 U24, 4 R21, 3 R21/R33, and 8 R01) and contracts to support reagents and resources. Total project period (5 years), at an cost of $104M.

RFA-CA-07-012

This RFA was for the establishment of a network of CPTAC research teams that would serve as a network of proteomic technology assessment centers responsible for evaluating, comparing, optimizing, and standardizing proteomic platforms, methods, and applications across multiple sites (figure 3). RFA-CA-07-012 was posted on February 7, 2006, with applications reviewed July 19-20, 2006. Those elected for funding were selected in the order of the peer-review scores. The following applicant teams were approved by the NCI Executive Committee:

1. Broad Institute

Principal Investigator	Steve Carr – Broad Institute
Co-Investigators & Participating Institutions	Amanda Paulovich – Fred Hutchinson Cancer Research Center Leigh Anderson – Plasma Proteome Institute Steven Skates – Massachusetts General Hospital Terry Pearson – University of Victoria Julie Gralow – University of Washington Constance Lehman – University of Washington

Description: The overall strength of this team includes the evaluation of multiple reaction monitoring (MRM) mass spectrometric assays, including a novel enrichment technology known as SISCAPA, for the quantification of candidate-based protein markers in plasma.

2. Vanderbilt University Medical Center

Principal Investigator	Dan Liebler – Vanderbilt University
Co-Investigators & Participating Institutions	Carolos Arteaga – Vanderbilt University Dean Billheimer – Vanderbilt University David Carbone – Vanderbilt University Amy-Joan Ham – Vanderbilt University Gordon Mills – M.D. Anderson Cancer Center

Description: A unique strength of this proposal is the extensive comparison of untargeted mass spectrometric techniques (commonly known as shotgun-based assays), examining almost all aspects of known issues including throughput, dynamic range, quantitation, and peptide identification.

3. University of California, San Francisco

Principal Investigator	Susan Fisher – UCSF
Co-Investigators & Participating Institutions	Joe Gray – Lawrence Berkeley National Laboratory Steven Hall – UCSF Brad Gibson – Buck Institute Laura Esserman – UCSF John Conboy – LBNL/UCSF Jonas Almeida – M.D. Anderson Cancer Center

Description: Strengths of this application are the focus on the identification of genomic aberrations in protein splicing and analytical methodologies to assess post-translational modifications.

4. Purdue University

Principal Investigator	Fred Regnier – Purdue University
Co-Investigators & Participating Institutions	Jiri Adamec – Purdue University Xiang Zhang – Purdue University Christopher Sweeney – Indiana University School of Medicine Mu Wang – Indiana University School of Medicine Jake Chen – Indiana University School of Informatics Jacob Vinson – Hoosier Oncology Group

Description: The strength of this application focuses on the evaluation of high-throughput immunoaffinity and other separations technologies - central to overcoming the challenges in mass spectrometry-based proteomics.

5. Memorial Sloan-Kettering Cancer Center

Principal Investigator	Paul Tempst – MSKCC
Co-Investigators & Participating Institutions	Brett Carver – MSKCC James Eastham – MSKCC Hans Lilja – MSKCC Martin Fleisher – MSKCC David Fenyo – New York University Thomas Neubert – New York University

Description: The strength of this application is the development of robotic sample handlers to eliminate variability, and the evaluating of serum peptide pattern technologies and custom-designed protease assays.

RFA-CA-07-005

This RFA was for the development of innovative new tools and computational approaches for protein/peptide measurement. RFA-CA-07-005 was posted on December 8, 2005, with applications reviewed on July 26-27, 2006. In response to this funding opportunity, 68 applications were received, of which 39 were scored with a range of 149 to 242 and 15 were recommended for funding (range 149 to 221). The following applicant teams were approved by the NCI Executive Committee:

Innovative Technology Development (R21, R21/R33)	Computational Science Development (R01)
Institute for Systems Biology. PI: Daniel B. Martin *A New Platform to Screen Serum for Cancer Membrane Proteins*	Vanderbilt University. PI: David Tabb *New Proteomic Algorithms to Identify Mutant or Modified Proteins*
Northeastern University. PI: Barry Karger *Global Production of Disease-Specific Monoclonal Antibodies*	University of Michigan. PI: Alexey Nesvizhskii *Analysis and Statistical Validation of Proteomic Datasets*
University of California, Los Angeles. PI: Joseph Loo *Top-Down Mass Spectrometry of Salivary Fluids for Cancer Assessment*	College of William and Mary. PI: Dariya Malyarenko *Enhancement of MS Signal Processing Toward Improved Cancer Biomarker Discovery*
Emory University. PI: Junmin Peng *A Proteomics Approach to Ubiquitination*	MIT. PI: Denkanikota Mani *A Platform for Pattern-based Proteomic Biomarker Discovery*
University of Houston. PI: Xiaolian Gao *Proteomic Phosphopeptide Chip Technology for Protein Profiling*	University of Colorado at Boulder. PI: William Old *Computational Tools for Cancer Proteomics*
Battelle Pacific Northwest Laboratories. PI: Richard Smith *A Proteomics Platform for Quantitative, Ultra-High Throughput, and Ultra-Sensitive Biomarker Discovery*	Fred Hutchinson Cancer Res. Center. PI: Tim Randolph *Quantitative Methods for Spectral and Image Data in Proteomics Research*
Michigan State University. PI: Stephen P. Walton *Aptamer-Based Proteomic Analysis for Cancer Signatures*	University of Virginia. PI: Dennis Templeton *PICquant – An Integrated Platform for Biomarker Discovery*
	Georgetown University. PI: Nathan Edwards *Proteomic Characterization of Alternate Splicing and cSNP Protein Isoforms*

CPTC Reissuance

RFA-CA-10-016 was posted on June 25, 2010, with applications scheduled for review December 13-15, 2010. Council review is scheduled for January 2011, with an earliest anticipated start date of April 1, 2011. The approved funding will allow for (1) a sufficient number of Proteome Characterization Centers (PCCs) to advance proteomic science through the network, and (2) production of reagents and resources for the network as well as the greater cancer community. The budget is proposed as follows: 6 to 8 (U24) centers, and contracts to support reagents and resources. Estimated total project period (5 years) would be $87.5-132.5M.

(d) Composition of Program

Attaining the goals of the CPTC initiative, and specifically those of the U24 cooperative agreement, requires an actively managed research network. In addition to ordinary program duties, NCI Program Staff coordinates inter-laboratory studies, manages requests for CPTC reference materials, conducts annual site visits and organizes an annual PI meeting, and oversees the antibody-characterization program (part of the Reagents and Resources Core).

The scientific components of the CPTC (vide supra) are overseen by the Program Coordinating Committee (figure 4). This committee consists of both extramural investigators and NCI Program Staff. Activities include:

- Maintenance of the scientific vision of CPTC
- Prioritization of trans-network experiments
- Establishment and termination of task-oriented working groups (12 working groups developed)
- CPTC policy decisions (e.g., data sharing and joint publication)

Early in the initiative, the Program Coordinating Committee agreed to share their respective U24 grant applications with their fellow committee members. This transparency has led to the completion of 15 trans-network studies aimed at understanding the inter-laboratory variability of proteomic measurements, and faster adoption of robust methodologies within the network. Additionally, the antibody characterization pipeline has produced 112 antibodies, all of which are available to the research community.

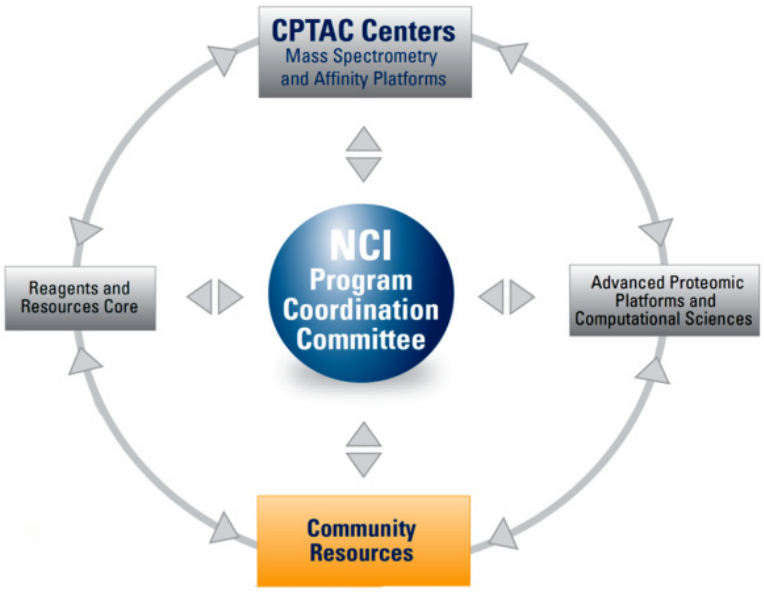


Figure 4. CPTC Initiative Structure.

Looking ahead, the CPTC reissuance will continue to utilize an integrative structure for the development of Proteome Characterization Centers (PCCs) that will characterize human cancers in the context of a proteomics pipeline (figure 5). A CPTC Steering Committee will serve the analogous role of the governing body in determining network priorities and policies. Subordinate to the Steering Committee, the Biomarker Candidate Selection Subcommittee will facilitate the transition of protein analytes from the discovery phase to the verification phase. These committees will consist of extramural investigators and NCI program staff. A CPTC Data Center will coordinate collection, curation, and distribution of data across the network and with the greater research community, and a CPTC Resource Center will coordinate distribution of biospecimens and other physical materials across the network.

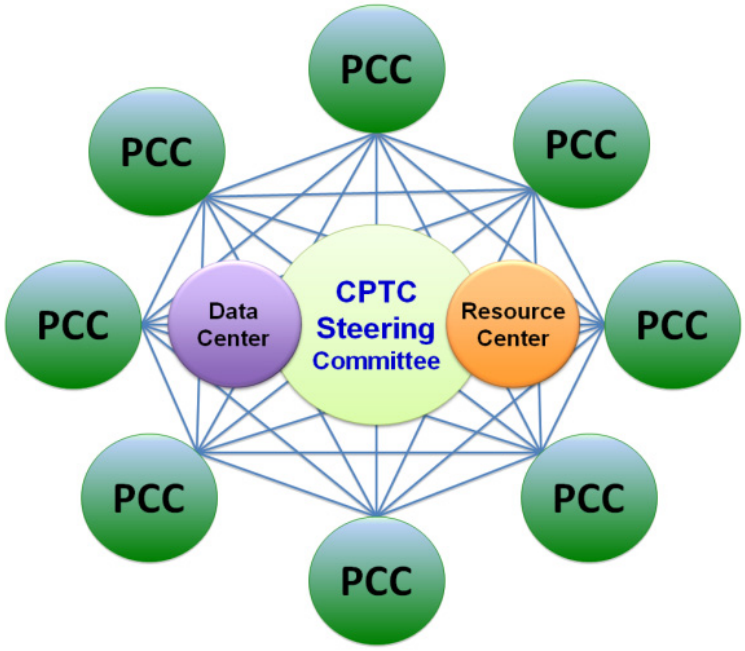


Figure 5. CPTC Reissuance Structure.

(e) Scientific Accomplishments to Date

When the CPTC launched in 2006, cancer biomarker research lacked a coherent pipeline connecting discovery with well-established methods for qualification (figure 2). The CPTAC network brought together a cadre of investigators who agreed to work together in developing the technology pieces necessary for a comprehensive proteomics pipeline. Investigators outlined three phases in such a pipeline: Discovery, Verification, and Qualification (clinical validation).[6] CPTAC investigators have focused on

6 Rifai N, Gillette MA, Carr SA. (2006). Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol*, 24(8):971-983.

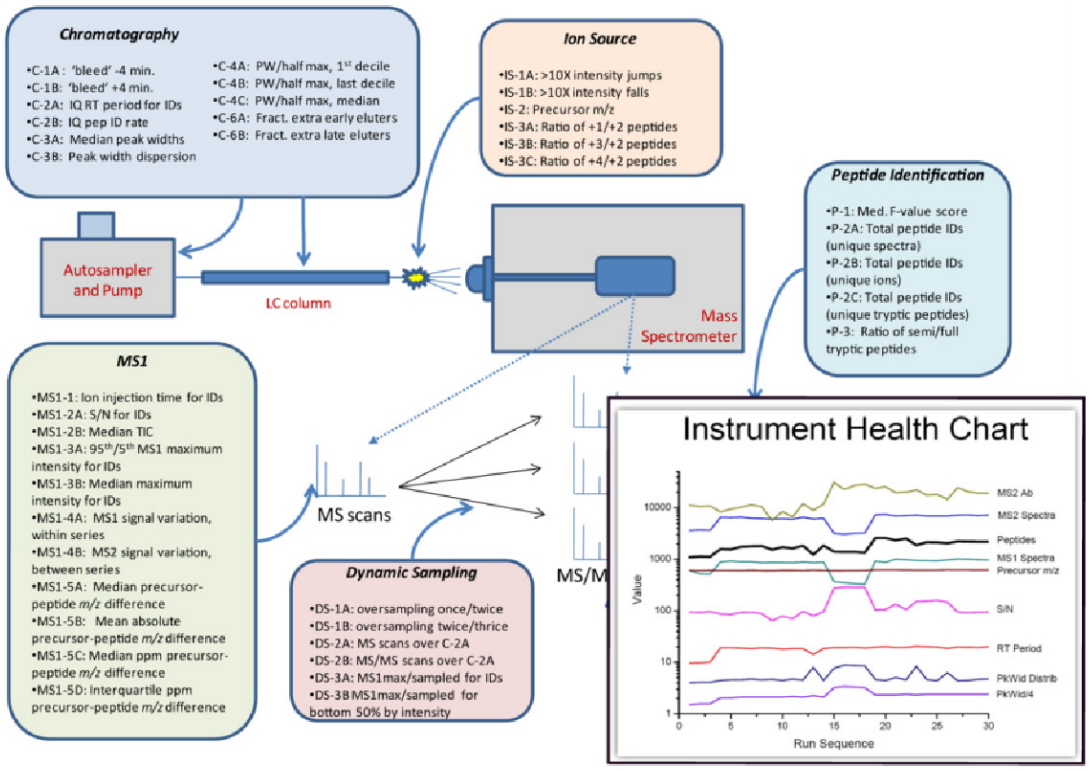


Figure 6. Performance Metrics for Liquid Chromatography-Tandem Mass Spectrometry Systems in Proteomics Analyses. Schematic representation of performance metrics mapped to LC-MS/MS system elements. *PW*, peak width; *IQ*, interquartile; *pep*, peptide; *ID*, identification; *Med.*, median; *ID'd*, identified; *Fract.*, fraction; *Num.*, number.

technology assessment of the first two of these phases. Better understanding of the analytical challenges inherent in each phase should improve the accuracy, reproducibility, and transferability of protein measurements.

The first 4 years of this program have shown the effectiveness of a multidisciplinary team approach in addressing the long-standing issues of variability and lack of repeatability in proteomic technologies. The network has developed workflows that address analytical issues of mass spectrometric methods and other technologies, enabling greater accuracy, quality, and confidence in proteomic measurements.

Selected key accomplishments of the initiative include:

Technology Assessment/Optimization (a multi-laboratory study to address the irreproducibility of mass spectrometry-based proteomics)

e.1 Discovery Phase - unbiased protein characterization

Discovery is the unbiased, semiquantitative process by which the differential expression of specific proteins between states is first defined. In the Discovery Phase, many technology platforms exist to globally identify proteins in a complex biological sample. Mass spectrometry-based proteomics methods have been used to generate many new biological insights, including proteins associated with organelles or signaling systems, the description of protein-protein interaction networks and their dynamic response to perturbations, and potentially the discovery of protein disease markers. Despite these attributes, proteomics methods have the reputation of being poorly reproducible, meaning that the set of proteins identified from identical samples in repeat analyses in the same laboratory or between laboratories are at least partially different. Several factors, such as systematic bias of different methods, stochastic

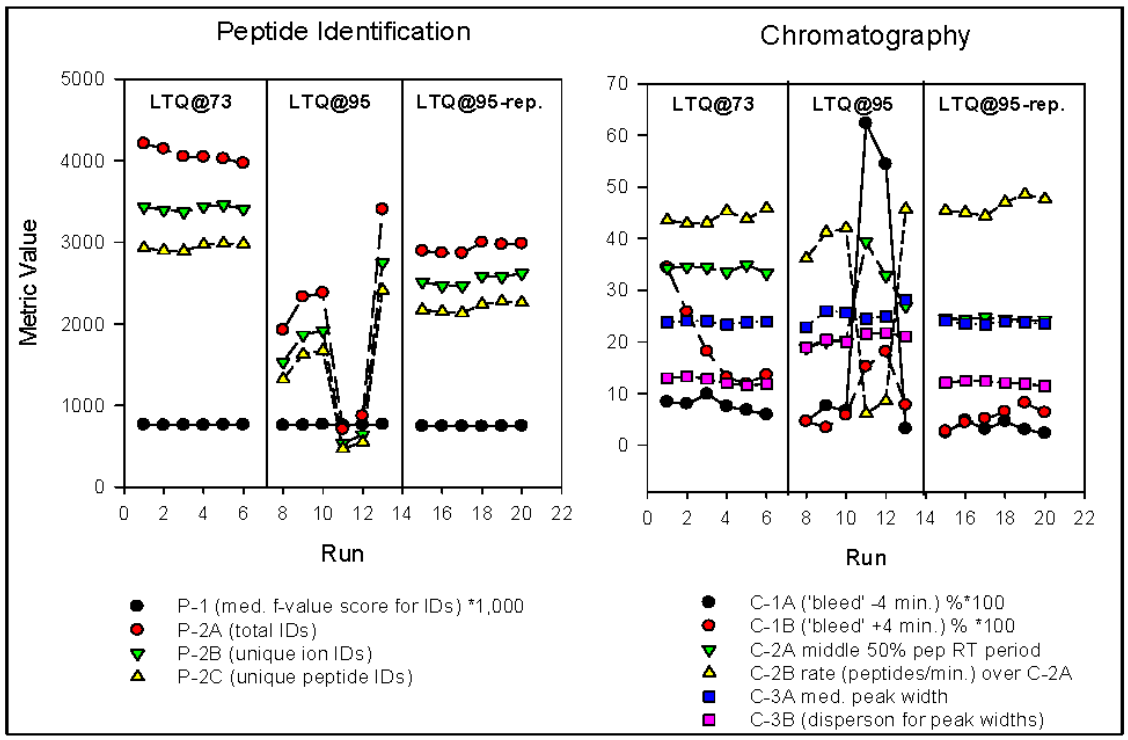


Figure 7. Performance Metrics for Six Replicate Analyses of a Tryptic Digest of the Yeast Reference Proteome on LTQ and Orbitrap Instruments.

sampling of proteomes in unbiased analyses, or erroneous protein identification by computational tools, could cause or contribute to this apparent poor reproducibility. To address these analytical issues in unbiased mass spectrometry, CPTAC investigators conducted interlaboratory (round robin) experiments designed to measure both optimal instrument performance and detection efficiency.

Development of Metrics to Quality Control Instrument Performance. To first address proper instrument performance, the CPTAC network along with the National Institute of Standards and Technology developed a quality control software tool (MassQC) that monitors and troubleshoots mass spectrometry instrument performance (figure 6).

A total of 46 system performance metrics for monitoring chromatographic and mass spectrometric performance parameters for peptide and protein identification have shown the capability of assessing interlaboratory analytical variation.[7] Specifically, this tool indicates which analytical system components vary the most between laboratories, allowing users to properly assess their research data. Application of these metrics enables rational, quantitative quality assessment for proteomics and other LC-MS/MS analytical applications in real time. In addition to this tool being openly available through the National Institute of Standards and Technology, it has been further developed into a commercial product by Proteome Software (www.massqc.com) for wider adoption by the proteomics community. An application of MassQC metrics on a CPTAC yeast digest is depicted in figure 7. Here, CPTAC laboratories used a yeast material that was digested for tryptic peptides and analyzed in six replicates on three LTQ and three Orbitrap mass spectrometer instruments in five laboratories. In the peptide identification panel on the left, the P-2A metric indicates total peptide identification. Laboratory denoted "LTQ@73" demonstrates six very consistent replicates (red dots). Laboratory "LTQ@95," however, shows three consistent replicates followed by a sudden drop in total peptide identifications.

7 Rudnick PA, Clauser KR, Kilpatrick LE, Tchekhovskoi DV, Neta P, Blonder N, Billheimer DD, Blackman RK, Bunk DM, Cardasis HL, Ham A-JL, Jaffe JD, Kinsinger CR, Mesri M, Neubert TA, Schilling B, Tabb DL, Tegeler TJ, Vega-Montoto L, Variyath AM, Wang M, Wang P, Whiteaker JR, Zimmerman LJ, Carr SA, Fisher SJ, Gibson BW, Paulovich AG, Regnier FE, Rodriguez H, Spiegelman C, Tempst P, Liebler DC, Stein SE. (2010). Performance metrics for evaluating liquid chromatography-tandem mass spectrometry systems in shotgun proteomics analyses. *Mol Cell Proteomics*, 9(2):225-241.

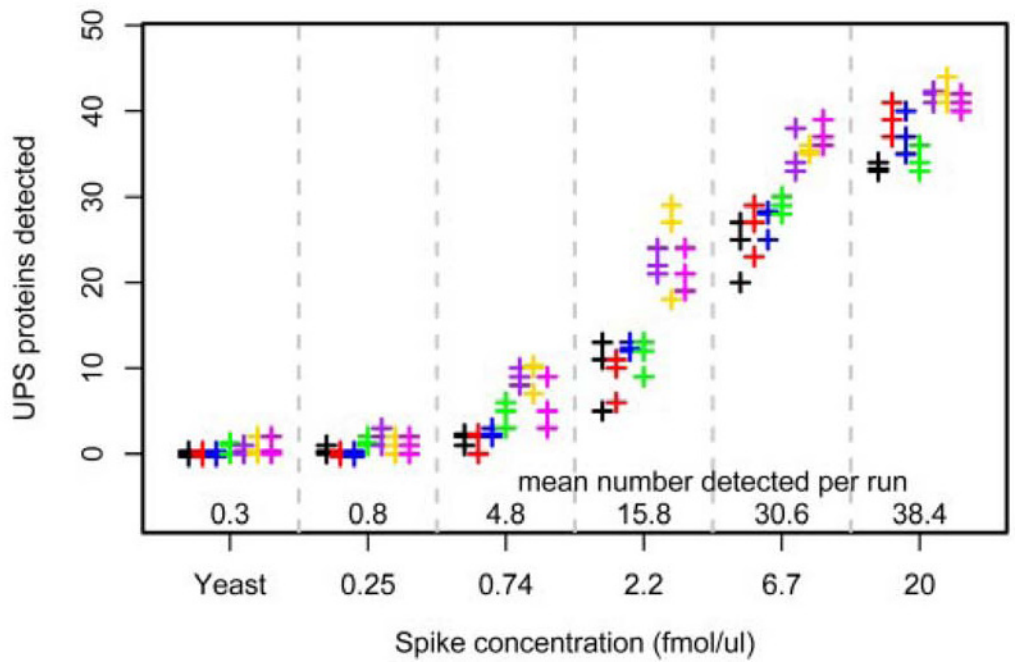


Figure 8. Interlaboratory Study on the Detection Efficiency of Human Proteins Spiked Into a Yeast Matrix Standard. This figure summarizes the detection of UPS1 (48 equimolar human protein mixture from Sigma) and yeast proteins in the spiking experiments. The result of each LC-MS/MS run is indicated by a "+" plotting symbol; colors denote different instruments. Protein detection is defined as observing two or more peptides mapping to the same protein (in a single LC run). On the x axis, "Spike concentration" refers to the concentration of the 48 equimolar human proteins (UPS1) spiked into the yeast matrix. This figure shows that the number of detected UPS1 proteins increases with increasing spike concentration and that the results are comparable between laboratories and platforms. For the lowest spike-in level (0.25 fmol/µl), none of the human proteins were detectable, whereas for the highest spike-in level (20 fmol/µl), laboratories were able to correctly identify 40 of 48 spiked-in proteins as differential.

Examining the panel of metrics (chromatography panel on the right) led immediately to the observation that the chromatography metrics show dramatic perturbations in column bleed, peak shape, and distribution characteristics. These differences were reflected in increased values for chromatographic bleed metrics C-1A and C-1B and decreased peptide identification rate (C-2B) and lowered numbers of peptide identifications. This enabled identification of an analytical malfunction – not changes attributed to a biological difference. Correction of the problem partially restored the metrics and peptide identifications to values comparable to the other systems (see "LTQ@95-rep").

Development of a Universal Reference Material for Benchmarking Instrument Detection Efficiency. Optimal performance of LC-MS/MS platforms is critical to generating high-quality proteomics data. Although individual laboratories have developed quality control samples, there is no widely available universal performance standard of biological complexity (and associated reference datasets) for benchmarking instrument detection efficiency for analysis of complex biological proteomes across different laboratories in the research community. Individual preparations of the yeast *Saccharomyces cerevisiae* proteome have been used extensively by laboratories in the proteomics community to characterize LC-MS platform performance. The yeast proteome is uniquely attractive as a biological reference standard because it is the most extensively characterized complex biological proteome and the only one associated with several large-scale studies estimating the abundance of all detectable proteins.

In this study, the CPTAC network developed and characterized a yeast reference standard (Reference Material 8323 available through the National Institute of Standards and Technology) for benchmarking the detection efficiency of an instrument for the analysis of complex biological proteomes across laboratories. An accompanying reference dataset demonstrating typical performance on commonly used discovery ion trap instruments (e.g., LTQ, Orbitrap) provides a

basis for laboratories to benchmark their own performance, to improve upon current methods, and to evaluate new platforms when developed. Additionally, the yeast reference, when spiked with human proteins of interest, can be used to benchmark the power of proteomics platforms for detection of differentially expressed proteins at levels of concentration representative in a complex matrix, thereby providing a metric to evaluate and minimize pre-analytical and analytical variation in comparative proteomics experiments (figure 8).[8]

e.2 Verification Phase - targeted/quantitative protein measurements

Reproducibility of Multiple Reaction Monitoring Mass Spectrometry (MRM-MS) Measurements. Given the stochastic nature of discovery platforms due to the undersampling of the mass spectrometer for complex biological samples, CPTAC investigators recognized the need for a technology that may be suitable for use in preclinical studies to rapidly screen large numbers of candidate protein biomarkers in the hundreds of patient samples necessary for verification, prior to making the investment in moving them forward into qualification studies that use ELISA (figure 2). Such a platform would be capable of confirming the differential expression of biomarker candidates in clinical samples that closely represent the population in which a corresponding clinical test would be deployed. The CPTAC investigators selected Multiple Reaction Monitoring mass spectrometry (MRM-MS) to fill this role.

MRM-MS uses a mass spectrometer to detect and quantify only those ions of a predetermined mass. This technology enjoys widespread use for quantitatively measuring small molecules (particularly in Pharma for monitoring drug metabolism and pharmacokinetics and to assay hormones, drugs, and their metabolites *(source 2009 ASMS Conference Proceedings)*. Prior to CPTAC, MRM-MS was not widely used in proteomics to measure peptide abundance in complex biological samples. Before MRM-MS could be widely adopted for verification studies, its analytical reproducibility for protein-based assays had to be demonstrated. CPTAC teams performed the first-of-its-kind, multi-institutional evaluation of MRM-MS for evaluating the transferability and robustness of the technology within and between laboratories. The basis was that if successful, MRM-MS could provide a robust assay for proteins for which acceptable affinity capture reagents are unavailable.

Using reference materials and standardized protocols, this study demonstrated that multiplex, quantitative MRM-MS-based assays can be configured and deployed in multiple laboratories to reproducibly measure proteins in plasma[9] (figure 9). With CV%'s at 21.9%, this initial study demonstrated that MRM-MS is a suitable technology for the verification phase – rapidly screening candidate protein biomarkers in preclinical studies (CV%'s of clinical assays are generally better than 15%, and the best are less than 5% - *source Steven Skates, biostatistician, Massachusetts General Hospital)*. Presently, CPTAC investigators are assessing this methodology's capability of generating multiplex assays against 100 peptides with sensitivities from µg/ml to ng/ml in non-depleted, non-fractionated plasma. In addition, an immunoaffinity-based MRM-MS technique using antibodies to enrich for peptides can significantly improve the sensitivity of detection and quantitation by ~1,000-fold compared to direct MRM-MS analysis.[10] Coupling immunoaffinity enrichment of signature peptides with MRM-MS has been shown to enhance targeted, quantitative analysis of proteins with sensitivities from ng/mL to pg/mL in plasma with CV%s less than <20% *(source - Broad Institute 2010 intra-lab report)*. A followup CPTAC inter-laboratory study has been started to build on these preliminary data.

8 Paulovich AG, Billheimer D, Ham AJ, Vega-Montoto L, Rudnick PA, Tabb DL, Wang P, Blackman RK, Bunk DM, Cardasis HL, Clauser KR, Kinsinger CR, Schilling B, Tegeler TJ, Variyath AM, Wang M, Whiteaker JR, Zimmerman LJ, Fenyo D, Carr SA, Fisher SJ, Gibson BW, Mesri M, Neubert TA, Regnier FE, Rodriguez H, Spiegelman C, Stein SE, Tempst P, Liebler DC. (2010). Interlaboratory Study Characterizing a Yeast Performance Standard for Benchmarking LC-MS Platform Performance. *Mol Cell Proteomics,* 9(2):242-254.

9 Addona TA, Abbatiello SE, Schilling B, Skates SJ, Mani DR, Bunk DM, Spiegelman CH, Zimmerman LJ, Ham AJ, Keshishian H, Hall SC, Allen S, Blackman RK, Borchers CH, Buck C, Cardasis HL, Cusack MP, Dodder NG, Gibson BW, Held JM, Hiltke T, Jackson A, Johansen EB, Kinsinger CR, Li J, Mesri M, Neubert TA, Niles RK, Pulsipher TC, Ransohoff D, Rodriguez H, Rudnick PA, Smith D, Tabb DL, Tegeler TJ, Variyath AM, Vega-Montoto LJ, Wahlander A, Waldemarson S, Wang M, Whiteaker JR, Zhao L, Anderson NL, Fisher SJ, Liebler DC, Paulovich AG, Regnier FE, Tempst P, Carr SA. (2009). Multi-site assessment of the precision and reproducibility of multiple reaction monitoring-based measurements of proteins in plasma. *Nat Biotechnol,* 27(7):633-641.

10 Anderson N, Jackson A, Smith D, Hardie D, Borchers C, Pearson TW. (2009). SISCAPA peptide enrichment on magnetic beads using an in-line bead trap device. *Molecular & Cellular Proteomics*, 8:995-1005.

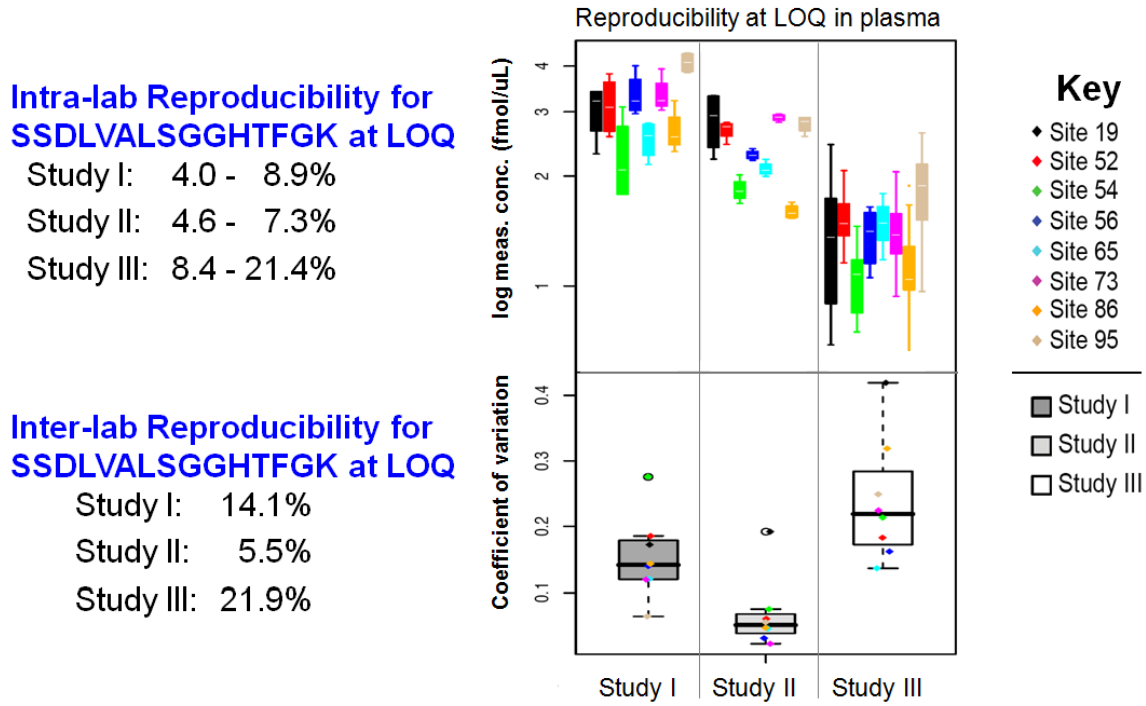


Figure 9. An Overview of CPTAC Interlaboratory Study on the Reproducibility of MRM-MS Analysis of Proteins in Plasma. The studies (I-III) sequentially introduced sources of variability in sample preparation and instrument analysis, thereby enabling assessment of their impact on the quantitative measurements. Study I and II samples were prepared centrally at the National Institute of Standards and Technology. In Study I, heavy (13C/15N) and light (12C) peptide standards were spiked into centrally prepared pooled, digested plasma and analyzed in 8 participating laboratories. In Study II, heavy and light peptide standards and corresponding centrally prepared digested proteins were spiked into centrally prepared pooled, digested plasma and analyzed in the 8 participating laboratories. In Study III, heavy and light peptide standards and corresponding intact proteins were spiked into plasma, with all sample handling performed at each site. In each study, plasma was non-depleted, non-fractionated, and samples were serially diluted across 9 different concentrations. The data show that intra- and inter-lab reproducibility for centrally prepared materials is less than 15% (Studies I and II). Sample handling at each site increased CV%'s to 21.9%, indicating that this a major contributor to variability. Data for the SSDLVALSGGHTFGK signature peptide are derived from HRP (Horseradish Peroxidase).

Technology Development. Investigators developed an innovative multi-channel nanoelectrospray emitter device for improving the sensitivity and throughput of analysis by mass spectrometry. This 19-channel emitter demonstrated an average of 11-fold sensitivity enhancement[11] (figure 10). Additionally, an LC-Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS) platform was developed to provide increased dynamic range for high-throughput proteomic studies.[12] In an evaluation of this platform, a complex tryptic digest of mouse plasma spiked with 20 reference peptides at varying concentrations was analyzed using both the traditional LC-Fourier Transform (FT)-MS platform with a 100-minute gradient and also the LC-IMS-TOF MS with a 15-minute gradient. In the LC-FT MS study, only 14 of the 20 spiked peptides with concentrations ≥100 ng/mL could be detected. In contrast, the LC-IMS-TOF MS platform was able to detect 19 of 20 spiked peptides at concentration levels down to 1 ng/mL. These technology developments led to faster sample analysis at greater sensitivity for MS platforms.

11 Kelly RT, Page JS, Zhao R, Qian WJ, Mottaz HM, Tang K, Smith RD. (2008). Capillary-based multi nanoelectrospray emitters: improvements in ion transmission efficiency and implementation with capillary reversed-phase LC-ESI-MS. *Anal Chem*, 80:143-149.

12 Baker ES, Livesay EA, Orton DJ, Moore RJ, Danielson WF 3rd, Prior DC, Ibrahim YM, LaMarche BL, Mayampurath AM, Schepmoes AA, Hopkins DF, Tang K, Smith RD, Belov ME. (2010). An LC-IMS-MS platform providing increased dynamic range for high-throughput proteomic studies. *J Proteome Res*, 9(2):997-1006.

Computational Tools Development. Researchers have focused on the development of bioinformatics tools for proteomics. Several noteworthy highlights include (a) CanProVar, a database (http://bioinfo.vanderbilt.edu/canprovar/) designed to store and display single amino acid alterations including both germline and somatic variations in the human proteome, especially those related to the genesis or development of human cancer based on the published literature including TCGA[13] and (b) Skyline (https://brendanx-uw1.gs.washington.edu/labkey/project/home/software/ Skyline/begin.view), whose interface simplifies the development of mass spectrometry methods and the analysis of data from targeted proteomics experiments performed using MRM.[14] Skyline is currently being installed approximately 150 times each month, and current data suggest it has about 500 dedicated users worldwide. In addition, researchers developed a public database of human protein-protein interactions mediated by phosphoprotein binding domains. This online interactive tool called PepCyber (http://www.pepcyber.org/PPEP/index.php) compiles several known databases into one central relational database.

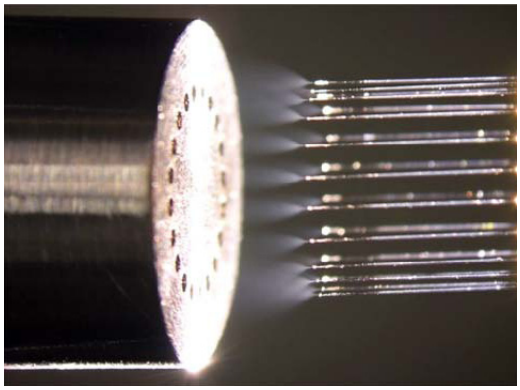


Figure 10. Photograph of an Array of 19 Emitters Positioned in Front of a Heated Multicapillary Inlet.

Additional Accomplishments

Regulatory Science and Clinical Chemistry Community. To empower the community with the process on how to correctly design studies that address analytical and clinical questions asked by the FDA on multiplex, protein-based assays, CPTAC investigators and the Office of In Vitro Diagnostics at the FDA developed mock 510(k) presubmissions on platforms being assessed through the CPTAC network. These first-of-their-kind analytical validation review documents illustrate the details involved in regulatory presubmissions and serve to benefit the global proteomics community in designing appropriate studies to support analytical and clinical claims, and to streamline the regulatory process by providing examples of submission formatting. These mock presubmissions, along with the comments from the FDA review staff, were published in a special issue of *Clinical Chemistry* (Feb. 2010)[15,16,17,18], the journal of the American Association for Clinical Chemistry (AACC) (figure 11).

These efforts on regulatory science recently earned a "leveraging/collaboration award" from the FDA in 2010. In addition, the AACC entered into a memorandum of understanding with the office (OCCPR) to coordinate efforts in educating/training clinical chemists on metrics and standards developed by CPTAC investigators for multiplex proteomic technologies.

13 Li J, Duncan DT, Zhang B. (2010). CanProVar: a human cancer proteome variation database. *Hum Mutat*, 31(3):219-228.

14 MacLean B, Tomazela DM, Shulman N, Chambers M, Finney GL, Frewen B, Kern R, Tabb DL, Liebler DC, MacCoss MJ. (2010). Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics,* 26(7):966-968.

15 Rodriguez H, Težak Z, Mesri M, Carr SA, Liebler DC, Fisher SJ, Tempst P, Hiltke T, Kessler LG, Kinsinger CR, Philip R, Ransohoff DF, Skates SJ, Regnier FE, Anderson NL, Mansfield E, on behalf of the Workshop Participants. (2010). Analytical validation of protein-based multiplex assays: a workshop report by the NCI-FDA Interagency Oncology Task Force on Molecular Diagnostics. *Clinical Chemistry,* Jan;56(2):237-243.

16 Regnier FE, Skates SJ, Mesri M, Rodriguez H, Težak Z, Kondratovich MV, Alterman MA, Levin JD, Roscoe D, Reilly E, Callaghan J, Kelm K, Brown D, Philip R, Carr SA, Liebler DC, Fisher SJ, Temps P, Hiltke T, Kessler LG, Kinsinger CR, Ransohoff DF, Mansfield E, Anderson NL. (2010). Protein-based multiplex assays: mock presubmissions to the U.S. Food and Drug Administration. *Clinical Chemistry,* Jan;56(2):165-171.

17 Anderson NL. (2010). PepCa10 510(k) Filing: "Mock 510(k)" for a multiplex diagnostic test using immunoaffinity mass spectrometry protein quantitation . Supplementary Material. *Clinical Chemistry,* Jan;56(2).

18 Regnier FE. (2010). A "mock 510(k)" for an immunological array platform for simultaneous assay of multiple glycoprotein isoforms. Supplementary Material. *Clinical Chemistry,* Jan;56(2).

Open Data Access Policies. To emulate the data-sharing path taken by the genomic community, as outlined in the Bermuda Principles, CPTC held an international summit in Amsterdam (2008) to solidify principles for proteomics open data access. The recommendations from this summit (known as the "The Amsterdam Principles") were published in the *Journal of Proteome Research* in 2009.[19] Recently, aspects of these principles were adopted by that journal as they plan to require raw datasets to be submitted in a public repository (CPTC's caTranche is suggested) for publication purposes. CPTC is currently assisting other journals in adopting these principles. A followup meeting is now planned for September 18, 2010, with a focus to develop quality metrics for data submission. NIH partners include NHLBI and NLM. This upcoming meeting is endorsed by *Molecular and Cellular Proteomics*.

Figure 11. Regulatory Science.

Community Datasets. Raw datasets generated by CPTC investigators are made accessible to the public through the caTranche data repository; http://cptac.tranche.proteomecommons.org. caTranche has served as the repository for the CPTC investigators and in 2009 became caBIG®-silver compliant.

Reference Materials. A yeast protein lysate community reference material is now available to the public for calibration of mass spectrometers by measuring proteins in a complex mixture (RM 8323 available through NIST: https://www-s.nist.gov/srmors/view_detail.cfm?srm=8323). A second reference material of an aqueous mixture of 400 peptides (RM 3952) to calibrate mass spectrometers that target peptide mixtures is scheduled for a release date in December 2010.

Community Reagents

- Antibodies: Antibodies developed through CPTC's Monoclonal Antibody Characterization program are made available to the public (http://antibodies.cancer.gov), as specified in an affinity reagents workshop held by the NCI.[20] All stages of the reagents pipeline utilize SOPs that are published on the reagents portal. The antibodies and their corresponding hybridomas are deposited at the Developmental Studies Hybridoma Bank (http://dshb.biology.uiowa.edu) at the University of Iowa and made available to the research community at a nominal cost (supernatant at $28/1.0 ml; concentrate at $45/0.1 ml).

- Antigens (proteins) are produced at Argonne National Laboratory (ANL) through an interagency agreement and serve a dual purpose. Proteins are ^{15}N-labeled for use as internal calibrants in mass spectrometry studies, while simultaneously used to generate monoclonal antibodies for affinity array studies. Proteins are publicly available to the research community at ANL (http://antigens.anl.gov).

19 Rodriguez H, Snyder M, Uhlén M, Andrews P, Beavis R, Borchers C, Chalkley RJ, Cho SY, Cottingham K, Dunn M, Dylag T, Edgar R, Hare P, Heck AJR, Hirsch RF, Kennedy K, Kolar P, Kraus HJ, Mallick P, Nesvizhskii A, Ping P, Pontén F, Yang L, Yates JR, Stein SE, Hermjakob H, Kinsinger CR, Apweiler R. (2009). Recommendations from the 2008 International Summit on Proteomics Data Release and Sharing Policy – The Amsterdam Principles. *Journal of Proteome Research*. 8:3689-3692.

20 Haab BB, Paulovich AG, Anderson NL, Clark AM, Downing GJ, Hermjacob H, Uhlen M. (2006). A reagent resource to identify proteins and peptides of interest for the cancer community: a workshop report. *Molecular & Cellular Proteomics*, 5(10):1996-2007.

Consensus Biospecimen SOP. CPTAC investigators developed a multisite SOP for collecting, processing, and storing plasma and tissue (in coordination with NCI's Office of Biorepositories and Biospecimens Research and the Best Practices for Biospecimen Resources); established a blood (plasma) repository collected prior to diagnosis, to avoid bias; developed a multisite biospecimen tracking database with pathology annotation; and developed a centralized biorepository (NCI-Frederick) with distribution SOPs.

Coordination With International Organizations.

- Korea Institute of Science and Technology (KIST): CPTC entered into a memorandum of understanding with the KIST to facilitate the coordination of CPTC's analytical proteomic workflows into Korea's Functional Proteomics Centers (FPC). KIST houses the FPC, one of the 21st Century Frontier Research and Development Initiatives of the Korean Ministry of Education, Science and Technology.

- European ProteomeBinders and Wellcome Trust: CPTC is coordinating efforts with these organizations on the development of community standard formats for the representation of protein affinity reagents.[21]

CPTC by the Numbers. Other accomplishments include 27 SOPs, 6 publicly available reference datasets, 2 analytical reference standards, 7 filed patents, 26 computational tools developed, 4 partnerships with Federal agencies and professional organizations, 11 partnerships with biotechnology companies (NCI-sponsored SBIR contracts), 12 leveraged funding activities, 112 well-characterized monoclonal antibodies against cancer-associated proteins, and over 190 peer-reviewed publications.

(f) Goals/Plan for Remaining Funding Period

In the remaining period of the CPTC initiative (year 5), more technology assessment studies (multi-institutional round-robin studies) will be performed, with peer-review publications anticipated in the summer of 2011. Remaining studies include:

- *Technology assessment study of 100-plex MRM assay in 13 laboratories.* The overall goal is to design a stable isotope dilution-MRM mass spectrometry assay for the precise relative quantitation of 100 cancer-relevant peptide targets in human plasma.

- *Technology assessment of anti-peptide antibodies (SISCAPA) MRM assays in 13 laboratories.* The overall goal of this study is to demonstrate the transferability of affinity enrichment MRM assays across laboratories. This study will also test a Bio-Rad SISCAPA kit, being developed in coordination with the CPTAC network in order to facilitate widespread adoption.

- *Tumor interlaboratory study.* The overall goal of the project is to apply CPTAC proteomic technology platforms in parallel to analyze a common set of well-characterized ovarian serous tumor specimens. This would be the first interlaboratory study of a human cancer tissue sample set using proteomics technologies and is similar in concept to TCGA.

- *Breast cancer patient plasma study.* This study will quantitate biomarker candidates in plasma samples collected from women with a breast lesion prior to a biopsy. Inter-lab variability of targeted platforms on clinical samples will be assessed.

21 Bourbeillon J, Orchard S, Benhar I, Borrebaeck C, de Daruvar A, Dübel S, Frank R, Gibson F, Gloriam D, Haslam N, Hiltker T, Humphrey-Smith I, Hust M, Juncker D, Koegl M, Konthur Z, Korn B, Krobitsch S, Muyldermans S, Nygren PA, Palcy S, Polic B, Rodriguez H, Sawyer A, Schlapshy M, Snyder M, Stoevesandt O, Taussig MJ, Templin M, Uhlen M, van der Maarel S, Wingren C, Hermjakob H, Sherman D. (2010). Minimum information about a protein affinity reagent (MIAPAR). *Nat Biotechnol,* Jul;28(7):650-653.

4. Recommended Future Vision for the Area Discussed

Science Vision

The overarching goal of the CPTC reissuance will be to improve the ability to diagnose, treat, and prevent cancers through a better understanding of the molecular basis of these diseases. The true value of TCGA efforts cannot really be fully realized without a complementary evaluation of the functional proteome of tumors. Efforts of CPTC in Phase I helped realign and restructure proteomic platforms (mass-spectrometry) and therefore the technology is now available to perform an informative proteome analysis across a large cohort of tumors. Delineating the effects of genomic aberrations in cancer on protein levels and function is the long-term scientific vision of the CPTC.

Despite great advances in the understanding of cancer, current diagnostic and prognostic methods are predominantly based on histopathology and a small number of DNA and protein biomarkers. Future clinical intervention needs to be supported by molecular data that will complement clinical data including tissue type, stage, grade, and size. Patients with similar tumor types often show significant heterogeneity in disease progression, clinical outcome, and response to therapy. Projects like TCGA have clearly shown that high-throughput molecular profiling of biological samples at the genomic, transcriptomic, and epigenomic levels contribute to the understanding of phenotypic variations and reveal many alterations and affected pathways. Though these multidimensional datasets are challenging to interpret, their utility in subtype definition and responses to therapy is now apparent. However, while genomic profiling provides an overview of the composition of the cellular genome, it does not always reveal how genomic changes can lead to disease. It is therefore advantageous to complement genomic characterization with proteomic studies, which have the potential to understand the dynamic processes that govern cellular biology.

To date, no systematic studies have yet been reported correlating detailed causative pathogenic gene mutations and corresponding translated proteins. The combination of proteomics with other global functional genomics approaches at the levels of genome and transcriptome (as envisioned in CPTC) can provide important bridges between genes, physiology, and pathology.

Programmatic Vision

CPTC will develop programs that incorporate input from the research community for the development and implementation of an effective protein biomarker pipeline for the discovery and verification of protein biomarkers. Achieving this requires improvements to protein measurement through the development and availability of standards, protocols, reagents, protein assays, analysis software, datasets, and technology platforms.

Moving forward, the CPTC will continue to drive advances in proteomics technology by partnering with investigators who are studying the role of proteins in cancer biology. Through integration of genomic analysis with proteomic analysis of the same sample, CPTC researchers will inform the search for cancer biomarkers with genomic evidence. Ultimately, CPTC will produce an improved proteome map of cancer biology, corroborating, or complementing, genomic findings in multiple tumor types; along with the rigorous protein assays and datasets provided by multiple laboratories. These outputs will provide new insights into cancer biology.

Future programmatic directions include (a) establishing a trans-NCI working group that coordinates efforts in the area of proteomics; the working group is to comprise representatives from respective divisions, be led by OCCPR, and report to the Office of the Director; and (b) developing a National Proteomics Strategy for the United States.

Appendix 1: Current Staffing

Henry Rodriguez, Ph.D., M.B.A., Office Director, OCCPR

Dr. Rodriguez is responsible for the oversight and development of OCCPR programs in clinical proteomics. This involves the Clinical Proteomic Technologies for Cancer initiative (CPTC), which also includes a monoclonal antibody characterization program. As Director, he oversees OCCPR and also evaluates the effectiveness of proteomics initiatives. Prior to the NCI, he served as Leader of the Cell and Tissue Measurements Group at the National Institute of Standards and Technology (NIST). There, he developed four metrology research programs: Gene Expression, Proteomics, Cell Imaging, and Bioinformatics. At NIST, he also served as a Program Analyst in the Office of the Director and as Principal Scientist in the DNA Damage and Repair program. He has authored more than 75 peer-reviewed publications and book chapters. He holds an M.S. degree in toxicology/chemistry (1986) from Florida International University, a Ph.D. degree in molecular and cellular biology (1992) from Boston University, and an M.B.A degree in finance and management (2003) from Johns Hopkins University School of Business.

Emily Boja, Ph.D., Program Manager, OCCPR

Dr. Boja directs regulatory affairs focusing on aspects of molecular diagnostic regulatory science with the U.S. Food and Drug Administration (FDA) and quantitative-based analysis of proteins and their post-translational modifications (phosphorylation, oxidation, and glycosylation, etc.) in complex biological systems. She provides leadership and oversight to the physical reference portfolio at NIST and also manages oversight of the U24 grant to the Broad Institute. Prior to the NCI, she served as Staff Scientist and Lead of Proteomics at the Laboratory of Biophysical Chemistry, NHLBI, NIH. Her expertise originates from her research on structural/functional studies of enzymes involved in one-carbon metabolism using biophysical and biochemical approaches and later mass spectrometry-based quantitative analysis of complex proteomes by integrating genomics, proteomics, and bioinformatics. She holds a Ph.D. degree in biochemistry and molecular biology (1999) from the Medical College of Virginia.

Tara Hiltke, Ph.D., Program Manager, OCCPR

Dr. Hiltke provides leadership and oversight to the Monoclonal Antibody Characterization program and coordinates activities with Argonne National Laboratory. She also works in developing other methods of antigen generation and manages oversight of the U24 grant to University of California, San Francisco. Previously, she served as a senior scientist/project manager in assay development at both Wellstat Diagnostics and BioVeris Corporation, where she developed clinical assays for diagnostic markers using electrochemiluminescence platform and magnetic beads. She holds a Ph.D. degree (1999) in biology from the University of Buffalo.

Christopher Kinsinger, Ph.D., Program Manager, OCCPR

Dr. Kinsinger focuses on the expansion and coordination of open data access and programmatic goals involving mass spectrometry, informatics, and biospecimens. In this role he works with NCI staff and investigators to optimize proteomics technology, establish policies for sharing data and biospecimens, and generally improve the quality and reliability of proteomic measurements. He also manages oversight of the U24 grant to Purdue University. He completed postdoctoral training at NIST, where he researched fragmentation pathways of peptide ions in mass spectrometry. He holds a Ph.D. degree in chemistry (2004) from the University of Minnesota.

Mehdi Mesri, Ph.D., Program Manager, OCCPR

Dr. Mesri provides leadership in integrating emerging technologies for the development of protein diagnostics and therapeutics. He coordinates activities with NCI's SBIR Office and manages oversight to R21, R21/R33 investigator grants and also manages oversight of the U24 grant to Memorial Sloan-Kettering Cancer Center. Prior to the NCI, he served as a principal scientist/projects manager in the Department of Protein Therapeutics at Celera. There, he used mass spectrometry technologies to discover and validate biologic antibody targets in oncology, including prostate cancer, lung and angiogenesis. He holds a M.Med.Sci. degree in clinical pathology (1991) from the University of Sheffield and a Ph.D. degree in immunology (1995) from the University of Aberdeen.

Amir Rahbar, Ph.D., M.B.A., Program Manager, OCCPR

Dr. Rahbar oversees the development, implementation, and assessment of proteomic technology platforms for cancer research and industrial relations. He also coordinates outreach activities with industry and activities with NCI's SBIR Office and manages oversight of the U24 grant to Vanderbilt University. His experience in protein science stems from research focused on chemoresistance in cancer, pathogen detection/analysis, and the study of membrane proteins and other drug targets at the U.S. Naval Research Laboratory's Systems Biology Group, and was previously a Senior Science Market Analyst at BioInformatics, LLC. He holds a Ph.D. degree in biochemistry (2004) from the University of Maryland and an M.B.A. degree (2008) from American University's Kogod School of Business.

Robert Rivers, Ph.D., AAAS Fellow, OCCPR

Dr. Rivers is an American Association for the Advancement of Science (AAAS) Science and Technology Policy Fellow and serves as liaison between OCCPR and the Center to Reduce Cancer Health Disparities in the development of diversity training opportunities in clinical proteomics. He also serves as a liaison between CPTC and the FDA in the area of multiplexed proteomic technologies. He holds a Ph.D. degree in chemistry (2008) from the University of Cambridge.

Appendix 2: Timeline of CPTC Development Process

Timeline of CPTC Development Process	
April 2002	Proteomics Planning Workshop (NCI/NHGRI/NIGMS)
April 2003	Proteomic Technologies for Early Cancer Detection Workshop
September 2003	NCAB – Commissions Working Group on Biomedical Technology
June 2004	Initial draft proposal for a Clinical Proteomic Technologies Initiative
September 2004	Clinical Proteomics and Biomarker Discovery in Cancer Research East Coast Workshop
November 2004	Clinical Proteomics and Biomarker Discovery in Cancer Research West Coast Workshop
	BSA – Proteomics Need for Early Detection presentation
January 2005	EC – approves Clinical Proteomic Technologies for Cancer biomarker concept
February 2005	Strategies to Integrate Biomarkers into Cancer Clinical Trials Workshop
	Proteomic Technologies Informatics Workshop
	NCAB – update presentations
March 2005	Proteomic Affinity/Capture Methods Workshop
June 2005	BSA – approves revised concept focused solely on Technology Assessment/ Improvement
October 2006	Clinical Proteomics Technology for Cancer initiative (CPTC) launched
November 2006	NCAB – update to Board
March 2007	BSA – CPTC update
February 2008	NCAB – CPTC update
August 2008	International Summit on Proteomics Data Release and Sharing Policy: Amsterdam Principles
Spring 2009	Independent Evaluation of the CPTC update
June 2009	BSA – CPTC update
September 2009	Reissuance Strategic Workshop – Implementation of a New Cancer Biomarker Development Pipeline
January 2010	EC – CPTC Phase II initiative approved
March 2010	BSA – approved CPTC reissuance
June 2010	CPTC Phase II RFA-CA-10-016 released
January 2011	Anticipated Council Review Date
April 2011	Anticipated Start Date

Appendix 3: Partnerships

National Institute of Standards and Technology (U.S. Department of Commerce)
Through an Interagency Agreement with OCCPR, develops mass spectrometry proteomic standard reference materials for CPTC investigators and the research community.

National Institute of Statistical Sciences
Provides expertise to the CPTAC teams in experimental study design, metrology, statistical analysis, and methodological approaches to applying proteomic technology platforms toward clinical measurement.

Argonne National Laboratories (U.S. Department of Energy)
Through an Interagency Agreement with OCCPR, produces cancer-related proteins that serve a dual purpose for CPTC investigators. Proteins are 15N-labeled for use as internal calibrants in mass spectrometry studies, while simultaneously being used to generate monoclonal antibodies. This produces reagents that can be utilized in mass spectrometry and affinity arrays.

Human Protein Atlas (Karolinska University)
Further characterizes monoclonal antibodies generated by CPTC in a large variety of normal human tissues, cancer cells, and cell lines with the aid of immunohistochemistry (IHC) images and immunofluorescence (IF) confocal microscopy images.

DNASU Plasmid Repository of the Biodesign
Serves as a public repository for CPTC's plasmid clone collections and distribution to the research community.

Virginia G. Piper Center for Personalized Diagnostics
Further characterizes monoclonal antibodies generated by CPTC using Nucleic Acid Programmable Protein Arrays (NAPPA) technology to measure off binding proteins.

Developmental Studies Hybridoma Bank at the University of Iowa
Created by the NIH as a national resource, collects, stores, grows, and distributes all hybridomas and monoclonal antibodies generated by CPTC.

Millipore
Distributes select monoclonal antibodies created and characterized by the CPTC Antibody Characterization Program.

U.S. Food and Drug Administration
Through a memorandum of understanding between the NCI (OCCPR) and the Food and Drug Administration, both agencies collaborate in proteomics areas involving standardization among technology platforms and assay standards development; instrument/technology validation; sample collection, preparation, storage, and processing; bioinformatics and data analysis; discovery and qualification of biomarkers; and surrogate biomarkers of cancer development and drug response.

American Association for Clinical Chemistry
Through a memorandum of understanding between the NCI (OCCPR) and the American Association for Clinical Chemistry, both organizations collaborate on promoting and educating the clinical chemistry community in the area of proteomic standards and technology advances.

Korea Institute of Science and Technology

Through a memorandum of understanding between the NCI (OCCPR) and the Korea Institute of Science and Technology, both organizations collaborate on promoting proteomic technology optimization and standards implementation in large-scale international programs.

European Bioinformatics Institute

Collaborates on establishing minimum information and community standards for the representation of protein affinity reagents.

NCI SBIR

CPTC integrates its efforts with the small business community via the NCI's SBIR program. Topics include proteomic technology commercialization and alternative affinity capture reagents.

NIH Activities

- *National Heart, Lung, and Blood Institute:* The Directors of NHLBI's Clinical Proteomics program and NCI's CPTC initiative will now participate on each other's governing bodies to accelerate collaborations and share technological development in each initiative.
- *National Human Genome Research Institute:* Collaborating on development of an MRM mass spectrometric database (MRM Atlas at the Institute for Systems Biology).
- *National Library of Medicine:* Collaborating on data release policies.

Appendix 4: Antigen Targets for Antibody Production

	Publicly available	In production
1	14-3-3 sigma	14-3-3 beta
2	26S proteasome non-ATPase regulatory subunit 4	14-3-3 eta
3	Aldo-keto Reductase Family 1 Member B1	14-3-3 protein epsilon (14-3-3E)
4	Aldo-keto reductase family 1 member C2	8-oxoguanine DNA glycosylase
5	Annexin A1 (Annexin I)	AKR1C1
6	BCL2-like 2	Alpha-1-antitrypsin
7	Calcyclin (Prolactin Receptor Associated Protein)	Annexin A2 (Annexin II)
8	Chloride Intracellular Channel 1	Annexin A4 (Annexin IV)
9	Chromogranin A	apolipoprotein A-I
10	Crystallin Alpha B	ATX1 antioxidant protein 1 homolog (yeast)
11	Ezrin (p81)	BASP-1/CAP-23/ NAP-22
12	Fascin	Calcineurin B homologous protein 2 (Hepatocellular carcinoma-associated antigen 520)
13	Fatty acid-binding protein, epidermal	Calgranulin B (Leukocyte L1 complex heavy chain)
14	Gelsolin	Calmodulin
15	Glucose phosphate isomerase	Carbonic anhydrase VIII [Homo sapiens]
16	Glutamate-Cysteine Ligase Regulatory Subunit	Casein kinase 2 alpha 1 polypeptide
17	Glutathione S Transferase Mu3	Cell division cycle 34 Ubiquitin-conjugating enzyme E2-32 kDa complementing
18	Glutathione S-Transferase Mu1	Chemokine (C-X-C motif) ligand 9 Small inducible cytokine B9(CXCL9)
19	Glutathione S-Transferase Mu2	CKB
20	Heat shock 27kDa protein 1	Cyclin-dependent kinase inhibitor 2D (p19 inhibits CDK4)
21	Interleukin 18	Cystatin A
22	Lactoylglutathione lyase	Cystatin B
23	Melanoma Antigen Family A, 4	Cytochrome c oxidase copper chaperone
24	Metastasin 100 calcium-binding protein A4 (Calvasculin)	DNA-(apurinic or apyrimidinic site) lyase, APEX
25	Methyl CpG Binding Protein 1	DNA-damage-inducible transcript 3
26	Mitogen-activated protein kinase 14	Dynactin 2 (p50)
27	Moesin	Eukaryotic translation initiation factor 4H
28	Nucleoside Diphosphate Kinase A (nm23-H1)	Eukaryotic translation initiation factor 5A
29	Nucleoside Diphosphate Kinase B	Fas (TNFRSF6) associated factor 1
30	Ornithine Decarboxylase 1	Fatty acid-binding protein, intestinal
31	Peroxiredoxin 4	FK506 binding protein 5
32	Phosphoserine Aminotransferase 1	Fructose-bisphosphate aldolase C
33	Protein Phosphatase 2A	Geminin
34	Ras-related C3 botulinum toxin substrate 1 (rho family small GTP binding protein Rac1)	Growth arrest and DNA-damage-inducible alpha
35	Spermidine or Spermine N1-Acetyltransferase 1	Growth arrest and DNA-damage-inducible gamma
36	Squamous cell carcinoma antigen 1	Heat shock 10kDa protein 1 (chaperonin 10)
37	synuclein-gamma	Indoleamine-pyrrole 2,3 dioxygenase [Homo sapiens]
38	tyrosine 3-monooxygenase or tryptophan 5-monooxygenase activation protein, epsilon polypeptide	Interleukin 6
39	Ubiquitin-conjugating enzyme E2C	Malate dehydrogenase 1 NAD (soluble)
40		NFKB2
41		Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha
42		Osteopontin andesnehsdvidsqelskc
43		Osteopontin cdsyetsqlddqsaethshk
44		Osteopontin GDSVVYGLR
45		Phosphatidylinositol-4-phosphate 5-kinase type II beta
46		Protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1
47		protein kinase, cAMP-dependent, catalytic, alpha
48		protein phosphatase 1G ARHGEF7/COOL-1/p85
49		PSPHL (From CCR - Troy Taylor / Tiffany Wallace)
50		Pyruvate kinase, isozymes M1/M2
51		RAD52 homolog (S. cerevisiae)
52		Receptor-binding cancer antigen expressed on SiSo cells (Cancer associated surface antigen RCAS1)
53		S100 calcium binding protein A2
54		Sulfotransferase family 1E, estrogen-preferring, member 1 [Homo sapiens]
55		Superoxide dismutase 1 soluble
56		Top1
57		Transgelin
58		Tropomyosin 1 ALPHA CHAIN (ALPHA-TROPOMYOSIN)
59		XMRV CA
60		XMRV MA
61		XMRV P12

Office of Biorepositories and Biospecimen Research (OBBR)

1. OBBR Background, Mission, Goals, and Vision

As early as 2002, access to high-quality, well-annotated biospecimens was identified in multi-sector national meetings as the main limitation to progress in cancer research, and in parallel a number of countries began to develop national biobanks. Since that time, experience through NCI funding has confirmed that the varied biobanking practices in place in NCI-supported biorepositories and across the biomedical research communities often failed to produce consistently high-quality biospecimens to support research using advanced technological platforms.

As a first step in addressing this issue, the NCI collaborated with a number of cancer organizations to develop the first national biorepository plan to address biospecimen quality and availability. The *National Biospecimen Network Blueprint (NBN Blueprint)* defined the following goal:

> "to establish a national, pre-competitive, regulatory compliant and genetic privacy protected, standardized, inclusive, highest quality network of biological sample(s) banks; supported by and developed via novel financial and other partnerships with cancer survivors and advocates, the private sector and nonprofit organizations as appropriate; that is shared, readily accessible, and searchable using state-of-the-art informatics systems (e.g., amenable to molecular profiling capability)."

Carolyn Compton, M.D., Ph.D., was recruited to the NCI in 2006 to develop and implement the Office of Biorepositories and Biospecimen Research (OBBR). The mission of OBBR is to improve the quality of scientific data by developing data and guidance to specifically ensure the availability of consistently high-quality biospecimens to support cancer and biomedical research. OBBR achieves its mission through:

- Development and deployment of standards that represent the state of the science for biobanking;
- Improvement of standards by supporting the development of biobanking science;

- Improvement of the tools for biobanking and biospecimen acquisition, processing, storage, and analysis by fostering technology development;

- Implementation of standards and infrastructure to provide human biospecimens and biobanking services through a public resource to support NCI science and its translation to medical advances.

Developing Standards. The trans-divisional NCI Biorepository Coordinating Committee (BCC) was formed in 2005 to formulate guiding principles for the optimal collection, handling, processing, storage, annotation, and consenting of biospecimens and coordinate biobanking activities throughout the Institute. This group, under the leadership of OBBR, assembled available evidence—corroborated by expert input, recommendations from professional workshops, and public comment—and published a draft *First-Generation Guidelines* in the *Federal Register* in 2006. The following year, this state-of-the-science document was further refined to become the *NCI Best Practices for Biospecimen Resources (NCI Best Practices)*. These represented the first specific guidance generated by the NCI to harmonize processes and improve quality of operations throughout the NCI-supported biobanking enterprise. In late 2007 and early 2008, OBBR hosted public forums in Bethesda, Boston, Chicago, and Seattle to disseminate the NCI Best Practices and promote their adoption. In 2009-10, the *Best Practices* have been updated and are scheduled for re-release in fall 2010. It is OBBR's goal to ensure that the *NCI Best Practices* keep pace with scientific advances in biobanking and U.S. policy changes so that they always represent the state of the science in biobanking and stand as an authoritative reference document for the scientific community.

Improving Standards. The Biospecimen Research Network (BRN) was created in 2007 and, as directed by the NCI Executive Committee, employs a contract mechanism. The BRN sponsors research that systematically addresses the impact of the wide range of variables that impact human biospecimens (pre- and post-removal from patients) on downstream molecular analysis data generated by advanced technologies. The BRN extramural research program, Biospecimen Research for Molecular Medicine, also initiated an annual symposium on biospecimen science in 2008, which attracts scientists, biobankers, regulators, and other stakeholders to exchange current ideas, issues, and data and creates a nexus for collaborative activities.

In 2008, the Innovative Molecular Analysis Technologies (IMAT) program, established in 1998 and specifically designed to foster high-risk but potentially transformative inventions, was administratively relocated to the OBBR. The IMAT program has helped shepherd dozens of breakthrough technologies from concept to commercialization and widespread scientific use. Examples of such technologies include RNALater®, Affymetrix GeneChip CustomSeq® Resequencing Arrays, MELT® technology, MudPIT®, COLD-PCR®, RainDance®, ICAT®, Illumina SentrixBeadChip® and BeadArray® technologies, and others. Under OBBR's leadership, IMAT has been expanding its biospecimen science and biobanking applications to address critical technological needs in those areas.

Implementing Standards. In formulating the NCI Best Practices and the BRN, OBBR noted that biospecimen science does not have a central venue for information exchange. The BRN symposia series rectifies this situation for professional interaction and new data exchange with the creation of the Biospecimen Research Database (BRD) to address the issue for print media. The BRD was designed in collaboration with the NCI Center for Biomedical Informatics and Information Technology as a publicly available, searchable online database of curated peer-reviewed articles concerning how various methods of biospecimen collection, processing, and storage affect the biology of the biospecimen and molecular research results. With more than 300 curated articles to date from over 100 journals, this resource serves as a central evidence base for biospecimen science. Curation of 900 additional publications is under way. The BRD also will serve as a dissemination tool for data emanating from the BRN and will house data-driven standard operating procedures, linkable to the evidence base, for biospecimen-specific, analyte-specific, and analysis type-specific uses.

The main recommendation of the *NBN Blueprint* (National Biospecimen Network) was to establish a national resource of high-quality, well-annotated biospecimens. OBBR took on this challenge in 2009 when it began planning for the cancer Human Biobank (caHUB), a unique, nonprofit public resource that will ensure the adequate and continuous

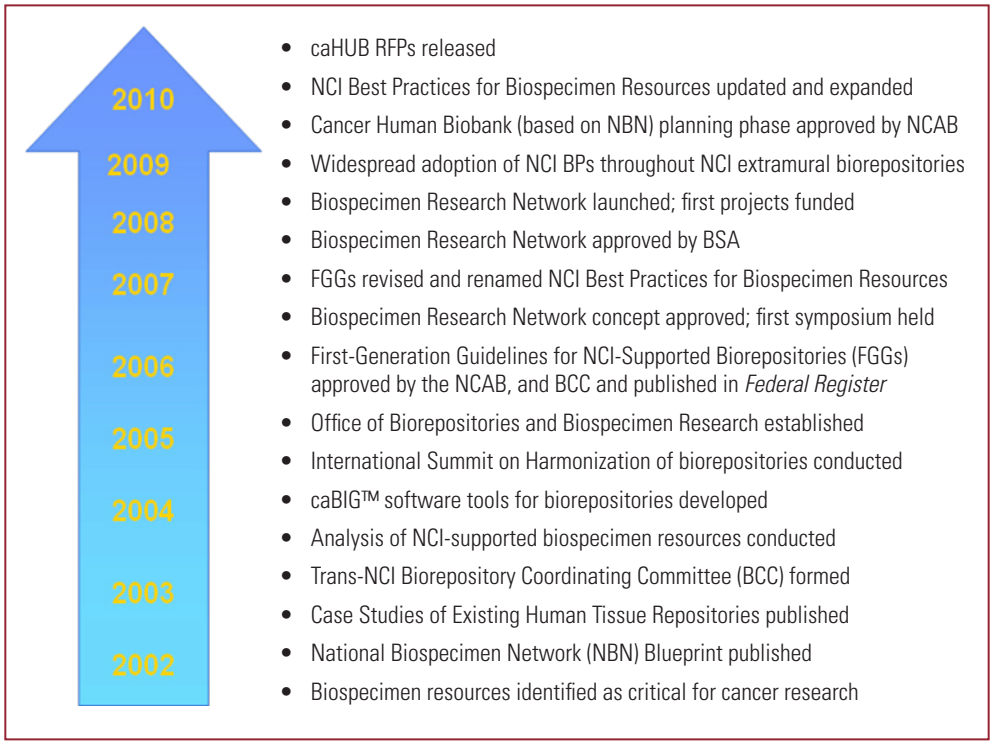
supply of human biospecimens and associated data of defined, high quality acquired within an ethical framework. Based on lessons learned from other countries in which national biobanks have been built based on networked, decentralized models that afforded little control over quality and greatly increased the costs of operation, the caHUB will operate using a centralized model rather than a networked operational plan. The caHUB was founded on the basis of the following key concepts:

- Centralized source of normal human specimens;
- Scientifically designed collection strategies (including rare diseases);
- Standardized, annotated collection and processing of all specimens;
- Centralized quality control and pathology analysis of every specimen;
- Rich, standardized data profile for each sample;
- Multiple aliquots of every specimen;
- Deposition of research (molecular analysis) results to inform studies on matched specimens; and
- Provision of tools, resources, and training for U.S. biospecimen resources.

Planning and market research for caHUB began in 2009 following approval of the concept by the National Cancer Advisory Board (NCAB). With funding from the American Recovery and Reinvestment Act in late 2009, implementation and planning proceeded simultaneously, with the decision by the NCI to engage the support of SAIC Frederick to manage the project. The caHUB staff (OBBR staff and SAIC staff engaged in caHUB management and execution activities on NCI's behalf) relocated to Rockville in April 2010 and have been engaged in implementing the recommendations of more than 200 stakeholders from the dozen working groups that participated in caHUB planning, awarding multiple contracts from eight overarching requests for proposals. caHUB operations will begin officially early in 2011.

The caHUB will generate collections of biospecimens and data that meet the stated unmet needs of the broad scientific community (academia, industry, advocacy, other government) and serve as a source of molecular analytes of verifiable quality. caHUB will also serve as a resource for national biospecimen research standards, and its experience will inform further validation and refinement of biospecimen best practices. As a pioneer model resource, caHUB is poised to become an international leader in biospecimen science, develop tools and protocols that will define the state of the science, and—through consultant services, training, and education—provide significant benefits to NCI and NIH, other government operations, academia, advocacy groups, and industry.

OBBR Summary Timeline: Key events in the history of OBBR, including approval and launch of the NCI Best Practices, the BRN, and the caHUB.



Vision

In 5 years, OBBR has become a leader in the field of biobanking and the driving force behind the new field of biospecimen science. It encompasses a multifaceted system of resources addressing the most pressing problem facing 21st century molecular medical research: limited availability of carefully collected and controlled, high-quality human biospecimens. The 2010 Consensus Report from the AACR-FDA-NCI Biomarkers Collaborative (Advancing the Use of Biomarkers in Cancer Drug Development. Khleif SN, Doroshow JH, Hait WN. *Clin Cancer Res* 2010;16:3299-3318) emphasized that biospecimens constitute the raw materials for biomarker discovery and that biospecimens with intact, accessible biomolecules as well as appropriate, informative annotation are required for the development and accurate detection of biomarkers using state-of-the-science techniques. Additionally, it was pointed out that a critical component of a national strategy to accelerate biomarker research and development is the access to reference standards that will enable methodological standardization and increase the confidence with which quality control data are interpreted, and all proficiency monitoring of personnel. Improvement in national infrastructure, biospecimen reference standards, strengthening biospecimen science, and the development of a publicly available resource for biospecimens were specifically cited as necessities for enhancing progress in discovery and development of biomarkers for clinical use.

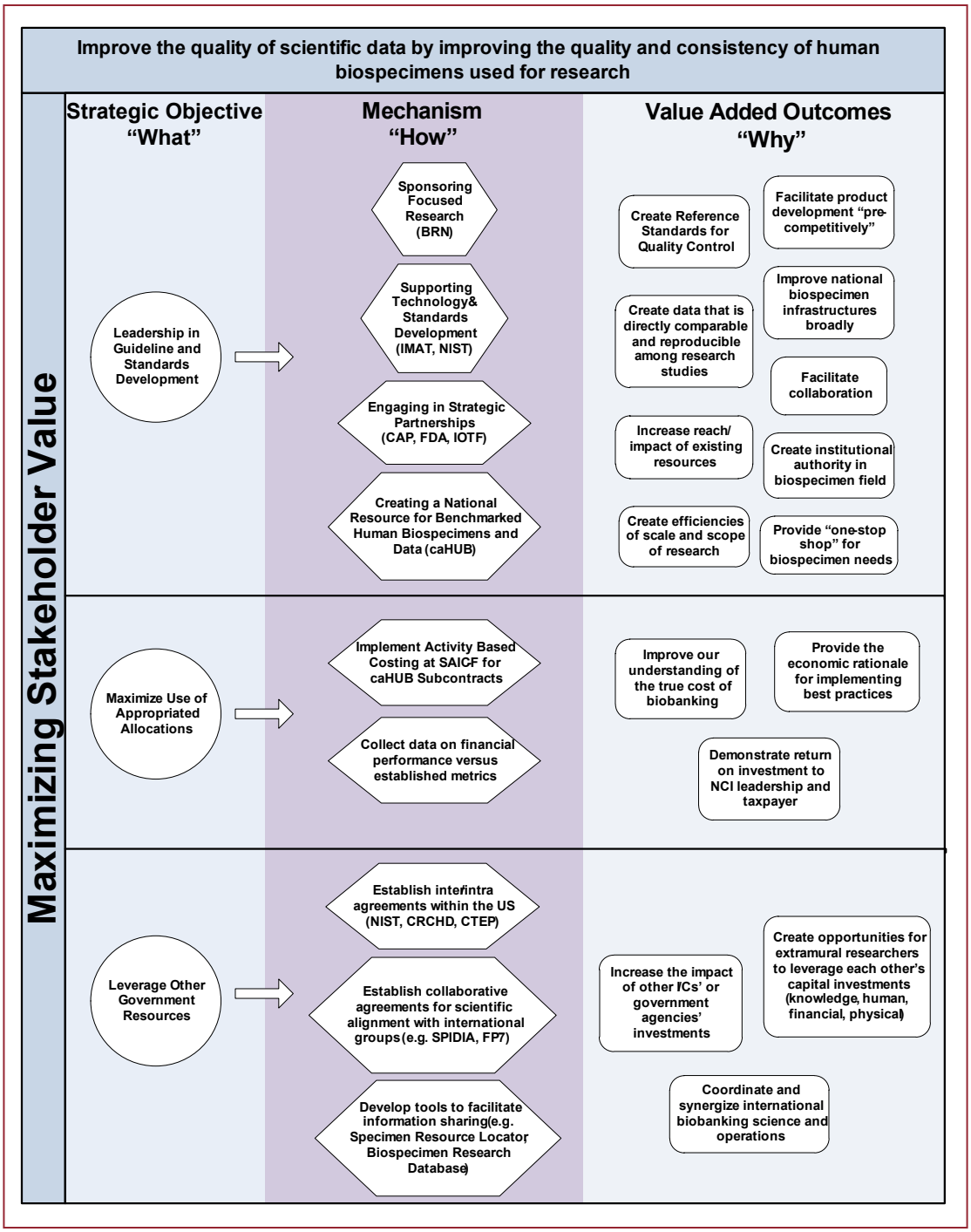
It is envisioned that OBBR's efforts in the development and implementation of evidence-based, standardized procedures for human biospecimens used for molecular analysis and for the harmonization of approaches to ethical, legal, and policy issues will not only improve the efficiency, quality, accuracy and applicability of research but also lay the foundation for the standards of clinical practice for molecular medicine. Regulatory approval of molecular therapeutics and companion molecular assays and their analytical performance will be facilitated. Technology development also will be enabled through the availability of standardized biospecimens and derivative analytes of verified quality to facilitate head-to-head comparisons of performance. Biospecimens of known quality will enable researchers to take full advantage of

the newest, most stringent molecular platforms, have greater confidence in the quality of the generated data based on greater confidence in the analyte quality, and move the findings more efficiently toward clinical application.

Ultimately, the overriding and guiding vision of OBBR is to improve outcomes for cancer patients.

OBBR Strategy Map

Below is a graphical representation of the OBBR mission and how OBBR initiatives transform established objectives into benefits for stakeholders, which includes NCI/NIH research programs, extramural researchers, biobanking initiatives in academic medical centers and community hospitals, patient populations and patient advocacy groups, and the taxpayer.



2. Programs

2.1 Guidelines and Standards Development

Best-Practices Development for Biospecimen Resources

The lack of standardized, high-quality human biospecimens is widely recognized as one of the most significant impediments to both cancer research and cancer product development. One of OBBR's first objectives, driven by systematic evaluation of NCI-funded biospecimen resources, was to develop and disseminate guidelines for biobanking operations. The *First-Generation Guidelines for NCI-Supported Biorepositories* were formulated and then revised to become the *NCI Best Practices*. After release of the *NCI Best Practices*, OBBR conducted educational outreach programs targeting investigators, industry representatives, hospital administrators, patient advocates, and the general public with nationwide forums. The *NCI Best Practices* document does not mandate detailed laboratory procedures; rather, it comprises guiding principles for state-of-the-science biospecimen-resource practices, quality control of biospecimens and associated data, and adherence to ethical and legal requirements.

Best-Practices Development for Biospecimen Resources	
Goals on Inception (2005)	• Demonstrate the consequences of research conducted using poor biospecimen practices • Identify and publish technical, operational, and policy biospecimen best practices • Identify the cost drivers for implementing best practices • Develop policies guiding ethical and legal practices where none exist • Promote widespread adoption of biospecimen best practices
Accomplishments	• 2005: Trans-NCI Biorepository Coordinating Committee formed • 2005: International Harmonization of Biorepository Practices Summit • 2006: First-Generation Guidelines for NCI-Supported Biorepositories published • 2006: Biospecimen Research Network (BRN) established • 2007: *NCI Best Practices for Biospecimen Resources (Best Practices)* published • 2007: Custodianship and Ownership Issues workshop hosted • 2007-2008: 4 nationwide NCI Best Practices for Biospecimen Resources Forums held • 2008: The Ethical Use of Pediatric Biospecimens in Research workshop hosted • 2008: Biospecimen Economics workshop hosted • 2009: Dedicated *Best Practices* Web site launched • 2009-2010: Revised *Best Practices* prepared • 2010: Release of Results to Research Participants workshop hosted
Future Goals	• Continue to update NCI *Best Practices* with evidence-based, state-of-the-science information • Expand Best Practices to reflect needs expressed in the community • Develop a Best Practices self-assessment tool for biospecimen resources • Leverage biospecimen science initiatives, such as those funded by the BRN

Biospecimen Research Network

Biospecimens are essential to the biomedical research enterprise but can be compromised or degraded by preanalytical factors, i.e., the environmental and biological stresses introduced by the collection, processing, storage, and transport that biospecimens undergo before they are used in a research assay. These variables may transform the molecular profile of the biospecimen and, when not properly controlled for and understood, can be misinterpreted as disease-related or even disease-specific findings. Workshops convened in 2005 to prepare for the BRN program launch established three areas on which to focus:

- Bridge the gap between clinical practice and the needs of emerging molecular technologies.

- Identify the variables most likely to affect the viability of prospectively collected biospecimens.
- Develop evidence-based, platform-specific biospecimen quality indicators.

The BRN was established with the mission of improving the quality of human biospecimen-based research by sponsoring, collaborating in, and disseminating studies that assess the effects of preanalytical variables on different biospecimen types—such as blood, urine, or tumor tissue—and on DNA, RNA, protein, and other molecular analytes as detected and measured on different platforms.

The extramural research program "Biospecimen Research for Molecular Medicine" is funding RFPs to develop innovative approaches to the control, monitoring, and assessment of biospecimen quality. Funded projects include the following:

"Research Studies on the Effects of Intraoperative Ischemia Time on Gene and Protein Expression Patterns in Liver and Colon Tissue" (Hartmut Juhl, Indivumed)

- Preliminary data indicate that intra-operative ischemia time has a dramatic effect on gene expression patterns; additional experiments will examine ischemia-dependent changes in immunohistochemical targets, and assay for ischemia dependent proteins HIF1-α and HSP27 and key phosphoproteins in cell lysates.

"Investigations Into the Effects of Blood Specimen Handling Procedures on Protein Integrity" (Chris Becker, Caprion)

- Highly annotated biospecimens are being collected from breast cancer and prostate cancer patients using controlled preanalytical variation.
- Multiplexed, highly reproducible protein assays for cancer-related proteins are being developed and tested on biological samples in preparation for testing the supported collection; preliminary LC-MS studies are being performed to assess proteolysis and post-translational modifications resulting from specific biospecimen preanalytical variables.

"Credentialing Plasma and Serum Biospecimen Banks for Proteomics Analyses" (Katy Williams and Susan Fisher, University of California, San Francisco)

- Blood collection from 50 donors, highly annotated and process controlled, has been completed for the project.
- Several analytical approaches are under way to examine the effects of preanalytical variables on the proteolysis-driven changes in plasma and serum proteins: ultrafiltration to examine LMW peptides for degradation; oxidation assays; iTRAQ labeling for relative quantitation of peptides to discover a signature combination of peptide quantities that indicates degradation.

"Intrinsic and Extrinsic Controls for FFPE tissue" (David Rimm, Yale)

- Tissue microarrays have been generated from several existing collections of highly annotated breast cancer biospecimens with known postoperative ischemic time intervals.
- A robust antibody validation process has been developed and two potential predictors of tissue integrity have been identified (Beta-Actin and Histone H4); additional studies are planned to assess the effects of post-operative ischemic time on clinically relevant markers (ER, PR, HER2, Ki67, P53, Cytokeratin) as well as phosphor-signaling molecules.

"Effects of Biospecimen Integrity, Intratumoral Heterogeneity, and Analytical Variance on Microarray-Based Pharmacogenomics Tests of Breast Cancer" (W. Fraser Symmans, M.D. Anderson, and Christos Hatzis, Nuvera Biosciences)

- Manuscript in preparation reports that a moderate extension of postoperative ischemic time (up to 3.5 hours) does not significantly alter expression levels of key breast cancer genes or multi-gene signatures of breast cancer.
- Preliminary data indicate that intratumoral heterogeneity is not much greater than analytical variability; preliminary studies show that eight clinical needle biopsies from liver metastases of breast cancer had an estimated <10% liver RNA content.

New research contracts:

"Effects of Pre-analytic Variables on Circulating microRNAs" (Hua Zhao, Roswell Park)

"Rapid Methods for the Assessment of Tissue Quality" (Charles Saller, ABS)

"Cancer and Normal Tissue Pre-analytical Variables" (Mary Kay Washington, Vanderbilt, and Therese Bocklage, University of New Mexico)

Biospecimen Research Network	
Goals on Inception (2006)	• Provide an evidence-based scientific foundation for research involving biospecimens • Provide a forum for research on how preanalytical variables affect molecular analyses • Promote the generation of new biospecimen-science research data • Collaborate internationally to facilitate biospecimen science and evidence-based practices
Accomplishments	• Three "Advancing Cancer Research through Biospecimen Science" annual symposia held • Online Biospecimen Research Database developed and launched (see below) • Two RFPs involving six extramural "Research on Human Biospecimen Molecular Integrity" projects funded • "Innovative and Applied Emerging Technologies in Biospecimen Science" RFA released • Small Business Innovation Research topics developed • NCI's Clinical Proteomics Technologies Initiative collaboration established • The Cancer Genome Atlas collaboration established • NIH Office of Rare Disease Research collaboration established • NIH Biospecimen Interest Group collaboration established • College of American Pathologists collaboration and MOU established • Collaborations established with EU partners • FDA and NIST collaborations under development • *Biospecimen Reporting for Improved Study Quality* guidelines developed
Future Goals	• Continue to build the scientific knowledge base for biospecimen science to enable better, more reproducible cancer research • Continue to sponsor and communicate new research through RFPs, publications, meetings, workshops, and the Biospecimen Research Database • Foster existing and establish new collaborations to integrate BRN results into the national and international R&D agendas • Provide an evidence-based scientific foundation for appropriate biospecimen collection, processing, and storage procedures • Establish a BRN R01 grant program

Innovative Molecular Analysis Technologies

In 1998 NCI established the Innovative Molecular Analysis Technologies (IMAT) program to identify and support creative, cutting-edge ideas that are high risk but, if successful, could prove truly transformative to cancer research by stimulating next-generation analytical methods, tools, and technologies. At the time, no other program at NIH was taking this revolutionary—as contrasted with the incremental or evolutionary—approach to the challenge of

translating basic research discoveries to patient care applications. From its inception, IMAT support of high-risk concepts has contributed to successfully commercialized products such as RNALater®, Affymetrix gene chips, Illumina bead platforms, and quantum dot labeling technology. Since 2008, leadership and management for the IMAT program have been provided by OBBR, and solicitations for technological solutions to biospecimen challenges have been highlighted. Significant challenges for biomedical research remain—including a need to rapidly assess all epigenetic changes in single cells, to directly measure the role of the microenvironment on cancer metastasis, and to collect rare cells from the blood of patients with recurrent disease—and will benefit from the creative thinking and risk-taking that IMAT identifies and supports.

Innovative Molecular Analysis Technologies	
Goals on Inception (2005)	• Solicit and support cutting-edge ideas that could be transformative to cancer research • Stimulate next-generation analytical methods, tools, and technologies • Enable rapid dissemination of transformative technology through commercialization • Take risks to substantiate the utility and value of innovative technology development • Stimulate biologists and clinicians to partner with individuals from other sectors who face similar technical challenges
Accomplishments	• Cold PCR Technology • Microfluidic Genetic Analysis (MGA) Technology • RainDance RDT-1000 Droplet Technology • Two IMAT investigators received trans-NIH TR01 awards to continue product development • IMAT-related awards cited in 343 publications in academic and industrial peer-reviewed journals • IMAT-related awards cited in 71 new patent submissions • IMAT-related awards cited in 27 new patent approvals
Future Goals	• Continue to pursue original program goals • Expand solicitation of biospecimen science-related technology development applications • Anticipate new directions and paradigm shifts in cancer research

Standards and Technology Development

Applying currently available molecular techniques to biospecimens is challenging because clinically derived samples are often of unknown quality and limited availability and/or utility. Additional complications arise when samples are subjected to preanalytical variations, which can make analysis unreliable or subject to pitfalls with the potential to confound results. To address these issues—and the lack of a universal standard by which to adequately and quantitatively assess the quality and integrity of biospecimens—OBBR has recently initiated a collaboration with the National Institute of Standards and Technology (NIST). Preliminary discussions with Dr. Elizabeth Mansfield at the U.S. Food and Drug Administration (FDA) have begun to explore including FDA scientists in this collaborative effort to ensure alignment with FDA issues and goals for regulatory science. The intention of the collaboration is the development of specific and quantitative quality standards and metrics that can be used to ensure the appropriate, consistent, and well-controlled sample quality necessary for effective biomedical research and clinical use. Such standards will permit cross-laboratory, cross-platform, and cross-analytical biospecimen comparisons.

Standards and Technology Development	
Goals on Inception (2010)	• Determine the reliability of current biomarkers to standardize clinical analysis technologies • Develop specific, quantitative quality standards for circulating DNA in blood biospecimens • Determine the stability of circulating-DNA standards in various standard-of-care practices • Develop definitions, standards, and evidence-based best practices for alternatives to conventional metrics and benchmarks
Accomplishments	• OBBR-NIST leadership meeting held
Future Goals	• Establish an inter-agency agreement providing for each of the inception goals • Develop a panel of quality standards for blood biospecimens stored for various times • Assist in the development of standards to determine the viability of stored biospecimens • Assist in the development of standards to determine biospecimens' fit-for-purpose • Assist the FDA and other agencies with new therapeutics and device submissions

The Cancer Human Biobank (caHUB)

As early as 2002, the National Dialogue on Cancer identified access to appropriately collected and annotated tissues as critical to accelerating progress against cancer by capitalizing on new genomic and proteomic technologies. Several subsequent reports[1] have corroborated this conclusion and called for the creation of a national resource that provides standards and benchmarked specimens to the research community. The NCI Executive Committee and Board of Scientific Advisors in December of 2008 approved planning of the caHUB initiative by OBBR. This began with a large-scale survey and several focus group research projects, which provided insight into the types and quantities of biospecimens and data most needed by the research community and helped to identify the challenges and opportunities OBBR would face in launching this endeavor. At the same time OBBR established a series of expert working groups to consider the critical areas that would need to be addressed, including strategic planning; ethical, legal, and social issues; partnership development; informatics; cost modeling and cost recovery; and biospecimen collection and processing standard operating procedures. A business and operational model for caHUB was developed by OBBR with the aid of expert consultants. Congressional interest in the project led to three invitations to brief Congressman Christopher Van Hollen, staffers for Senators Edward Kennedy and Kay Bailey Hutchison, and Senator Jon Tester and staff in 2009.

In the summer of 2009, OBBR was awarded $60 million through the American Recovery and Reinvestment Act (Recovery Act) to establish the caHUB and implement the pilot phase, which will be carried out by FFRDC, SAIC-F. OBBR has been working closely with the FFRDC, SAIC-F contractor to assemble the project team, create the strategic and project plans, and issue a series of RFPs to subcontract with organizations capable of performing the core functions of the caHUB enterprise. These functions include collecting biospecimens and data, performing comprehensive pathology review and standardized biospecimen processing and analysis, creating the overarching informatics architecture, and conducting research on biospecimen handling variability to inform evidence-based standard operating procedures. When these awards are in place, toward the end of 2010, the caHUB pilot project will officially be launched.

1 Genomics and Personalized Medicine Act of 2007; Institute of Medicine Report: Cancer Biomarkers 2007; Department of Health and Human Services Personalized Health Care Report Sept. 2007; President's Council of Advisors on Science and Technology: Priorities for Personalized Medicine Sept. 2008; President's Cancer Panel Report Maximizing Our Nation's Investment in Cancer Sept. 2008; Kennedy-Hutchison Cancer Bill (ALERT Bill: "War on Cancer, Part II") 2009; The NCI Bypass Budget FY2010.

The Cancer Human Biobank	
Goals on Inception (2009)	• Meet the need for standardized, well-characterized human biospecimens and clinical data • Provide standardized benchmark materials and standards • Create a program of guidelines, policies, and procedures that can be widely used • Establish an infrastructure to track and share biospecimens and data • Create a network of partners to provide resources and financial support • Create programs of biospecimen research, standards development, and technology development
Accomplishments	• Market research completed • caHUB working groups established and shepherded • Biospecimen User-Group workshop held • Working-group analyses completed and recommendations provided • Strategic framework formulated and charter created • Key members of the project team recruited • Material transfer agreement template formulated • Five RFPs developed: two released, three pending release • Standard operating procedures and process workflow in development • Tissue and data access policies in development • Intellectual property policy in development
Future Goals	• Publish market research findings, economic analyses, and cost-recovery modeling • Make public recommendations from the expert working groups • Finalize biospecimen-collection strategy for pilot benchmark collection • Design and conduct prospective biospecimen collection in cooperation with end-users • Provide samples and data to end-users • Perform formal program evaluation and adjust as needed to ensure sustainability and economic viability

2.2 Education and Outreach

OBBR Communications and Outreach

Education and outreach to all potential biospecimen-enterprise contributors and customers are essential for OBBR to realize its strategic goals and vision. However, biospecimen issues cross professional and lay boundaries in medicine and science and thus have diverse target audiences for education about relevant issues, requiring a multidimensional education and outreach approach. During OBBR's first 5 years, a number of platforms and strategies have been developed to sharpen communication with specific groups and audiences. For example, in collaboration with OBBR, the NCI Office of Communications and Education developed effective communications plans for such projects as the *NCI Best Practices* and caHUB to reach stakeholder communities and to enhance OBBR's position as an authoritative source of information and guidance. Content, materials, and events were developed to keep researchers, clinicians, and the general public informed of ongoing activities.

OBBR Communications and Outreach	
Goals on Inception (2006)	• Convey to patients the importance of biospecimens in medical research • Encourage patients to contribute specimens to the research enterprise • Provide expert information about the risks, benefits, and details of biospecimen donation • Serve as NCI's expert resource on biospecimen and biorepository issues • Enhance OBBR's authority on technical, legal, economic, and ethical biospecimen issues • Create and maintain a responsive, dynamic, and appealing Web site • Create presentations and other communication materials to promote *NCI Best Practices* • Inform and solicit input from patient advocates concerning OBBR activities • Promote interaction and collaboration with industrial and academic developers of new biospecimen technologies
Accomplishments	• OBBR program Web site established and maintained • *NCI Best Practices* interactive Web site established and maintained • caHUB Web site established and maintained • IMAT Web site established and maintained • Numerous informational publications produced • Numerous peer-reviewed publications produced and coauthored • Multimedia educational content produced • Numerous seminars, workshops, and conferences hosted • Technical information concerning biospecimens and biobanking provided to Congress • Interviews and statements provided to the popular press • Numerous presentations offered by OBBR staff at major national and international meetings
Future Goals	• Seek novel, effective ways to pursue inception goals • Develop a quarterly online newsletter • Develop caHUB podcast and webinar series • Develop "drop-in" content for professional society publications

Biobanking Economics Research

Although major economic issues face biobanking researchers, stakeholders, and the biomedical community, no overarching framework is in place to address them. While the scientific value of high-quality, well-annotated biospecimens is incontrovertible, the economic value is less well defined or understood. Fledgling efforts to perfect a national system of biospecimen collection and storage are driven by the specter of the economic consequences to society when insufficient quality biospecimens shackle the progress of medical science and medicine itself in the new molecular era. Understanding the value of that biobanking system—and the economic issues associated with building and sustaining it—is critical for public and private support, yet that too is not at all clear. Economic models are required to frame the discussion and pursue solutions. Foremost is the need to define and measure the value and quality of biospecimens for molecular analysis. Once that currency is established, the resources and organizations that house these specimens will need an inventory of sound business plans, including cost-accounting and cost-recovery systems, to ensure they can survive and prosper.

Biobanking Economics Research	
Goals on Inception (2008)	• Improve understanding of economics of developing and managing biospecimen resources • Understand and educate biobankers about the economic value of adopting best practices • Evaluate the value proposition and total life cycle cost of ownership for caHUB • Develop a cost recovery system for caHUB
Accomplishments	• 2007-2008: Economics discussions held at *NCI Best Practices* Forums • 2008: Economic Considerations for Implementing the NCI Best Practices for Biospecimen Resources workshop hosted • Economists and other experts consulted regarding biospecimen value and cost recovery • Manuscripts for *Journal of the National Cancer Institute* monograph prepared
Future Goals	• Collect caHUB cost-recovery data • Continue to publish manuscripts as discussions and activities coalesce into action • Develop case studies showing the economic benefits of biospecimen best practices

OBBR Publications and Presentations

Since its inception, OBBR has effectively educated researchers and other stakeholders about the critical roles played by biospecimens and biobanking in research via presentations at professional meetings worldwide and numerous peer-reviewed journal articles, book chapters, and other publications. Increasing numbers of speaking invitations and manuscript requests are received each year as the recognition spreads that advancing biomedical research depends on obtaining, storing, and sharing high-quality, richly annotated biospecimens.

The Biospecimen Research Database

The Biospecimen Research Database (https://brd.nci.nih.gov/) is designed to serve the entire community of researchers who utilize human biospecimens in their research. Developed in collaboration with NCI's Center for Biomedical Informatics and Information Technology (CBIIT), this interactive, freely available, online database provides a searchable library of difficult-to-find, scientifically curated publications that describe how different methods of biospecimen collection, processing, and storage affect the biology of the biospecimen.

Biospecimen Research Database	
Goals on Inception (2008)	• Create an online searchable library of scientifically curated publications describing how different methods of biospecimen collection, processing, and storage affect the biology of the biospecimen
Accomplishments	• Prototype database developed • Approaches for finding and curating appropriate literature into the database identified • Web-based application for curation and for end-user browsing developed • Curation operations migrated from prototype to Web application • System search capabilities and terminology expanded • More than 300 papers from more than 119 scientific journals curated
Future Goals	• Over 900 papers in the queue undergoing review • Continue to fortify scientific knowledge base for biospecimens • Continue to communicate new research through a variety of media • Foster existing and establish new collaborations to integrate BRN results into the national and international research and development agendas • Provide an evidence-based scientific foundation for research involving biospecimens • Expand BRD functionality to include libraries of data-driven SOPs

The Specimen Resource Locator and Common Biorepository Model

To provide cancer Biomedical Informatics Grid (caBIG®)-compatible solutions, the Center for Biomedical Informatics and Information Technology (CBIIT) is working with OBBR and the vendor community to establish a Common Biorepository Model (CBM) as a way for biorepositories to advertise their holdings to the research community. The CBM will work as a federated back-end database to support query tools, such as the OBBR's Specimen Resource Locator (SRL). The Specimen Resource Locator (SRL) is an online application launched in 2002, which pools summary level information on biospecimen collections from approximately 30 biobanks and repositories. This tool was created to overcome the siloed nature of repositories across the United States by enabling researchers to find biobanks that have the biospecimens in one publicly available location. An analysis of the SRL's usage statistics was conducted in October 2009, revealing an annual average of 1,830 unique queries, predominantly stemming from nonprofit and academic institutions throughout the United States and in 19 other countries. These results emphasized the importance of such a tool to the research community while highlighting the need for specific improvements and updates. Consequently, concrete steps are being taken to revise and update the SRL and the data therein.

The Common Biorepository Model and Specimen Resource Locator	
Goals on Inception (2008)	• To create an online public/private resource that allows biobanks to share summary-level information about biospecimen collections.
Accomplishments	• Project Inception and Statement of Work documents created • Conceptual functional architectural specification created • Project estimate and project plan created • Locator usage report completed • Stakeholder workflow questionnaire developed and results compiled • Data update plan for existing and future Specimen Resource Locator created • Memorandum of Understanding to enable partnership among multiple NIH groups drafted • Summary-level, de-identified Common Biorepository Model generated
Future Goals	• Revise and update Specimen Resource Locator using automated, standardized infrastructure from the Common Biorepository Model • Integrate the updated SRL to the Customer service function of caHUB

2.3 OBBR Support to Other Programs

Two projects involving more than one NIH IC are highlighted below; a brief compendium of additional projects suported by OBBR follows.

Tissue Acquisition for The Cancer Genome Atlas (TCGA)

TCGA is a comprehensive and coordinated effort to accelerate understanding of the molecular basis of cancer through genome-analysis technologies. In September 2006, the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) launched TCGA as a 3-year pilot to assess the technical feasibility and clinical relevance of conducting a comprehensive analysis of the entire spectrum of genomic changes in human cancer. A suite of analysis platforms was applied to the interrogation of a common set of molecular analytes obtained from clinically annotated, high-quality tumor biospecimens and matched control tissue or blood biospecimens. Data were generated on the characterization of DNA copy number changes, gene transcription profiling, epigenetic modifications, and sequence variation. When tissue-sample shortages became a significant impediment to TCGA progress, OBBR was asked to lead the pilot's tissue-acquisition. OBBR was responsible for identifying new sources of tissue and coordinating all tissue source activities for the pilot phase, increasing the flow of biospecimens into TCGA's analysis pipeline. OBBR served an educational role as well and developed a suite of educational print materials and SOPs, conducted a series of webinars, and made numerous presentations at tissue source sites and TCGA meetings to educate tissue providers

about the specimen requirements (pathological, molecular, consent-related, MTA-related) for the project. In 2010, TCGA transitioned from pilot to program, thus ending OBBR's involvement in specimen procurement.

Feedback received from Biospecimen Source Sites, including Emory University, Boston University, M.D. Anderson, University of Kentucky, groups at Memorial Sloan-Kettering Cancer Center, Cedars-Sinai, Cureline, Asterand, ABS Bio, indicate that they have modified their biospecimen collection protocols to accommodate the stringent quality requirements imposed by TCGA project. Many other sites have reported that they plan to modify their protocols as they initiate prospective collection for the project.

The Cancer Genome Atlas	
Goals on Inception (2008)	• Increase and accelerate the flow of biospecimens into TCGA analysis pipeline • Develop a strategy to improve the rate of new tissue source site (TSS) recruitment • Identify and recruit potential TSSs • Develop educational and training materials for operational and scientific approaches • Ensure compliance with contract milestones, timelines, and deliverables • Improve communications between the TSSs, SAIC-Frederick, and TCGA project management team.
Accomplishments	• Site-enrollment binder, CD-ROM, and at-a-glance cards developed and put into use • Webinar presentation for the clinical tissue-collection staff developed and put into use • Web-accessible tissue-tracking database developed and put into use • "Biospecimen Access Working Groups" held to facilitate enrollment of new TSSs • Process to review informed consent documents at TSSs developed and put into use • Materials to assist with IRB review developed and put into use • Project workflows and checklists developed and put into use • TSS specimen-flow reporting and cost-tracking for contract deliverables improved • Contributing sites increased from 24 to 53 • Number of cases collected increased from 400 to 1,800
Future Goals	• Continue to consult with TCGA team on biospecimen collecting and processing issues • Collaborate with TCGA to provide matched samples from TCGA to other programs

Genotype-Tissue Expression (GTEx) Project

Genome-wide association studies (GWAS) have shown great promise in identifying genetic loci associated with common human diseases. Despite this progress, the majority of the single nucleotide polymorphisms (SNPs) and other genetic changes significantly associated with disease phenotypes lie outside the protein-coding regions of genes and often even outside the genes themselves. This makes it difficult to discern which genes underlie the association signals and by what mechanism. The Genotype-Tissue Expression (GTEx) project, an NIH Common Fund Initiative, aims to provide a resource to the scientific community with which to study the relationship between genetic variation and regulation of gene expression on a tissue-specific basis by collecting and analyzing multiple human tissues. By treating global RNA expression levels as quantitative traits, loci with polymorphisms that are highly correlated with variation in expression will be identified as expression quantitative trait loci. OBBR will perform the biospecimen acquisition, quality control, and long-term management for the project in conjunction with SAIC-Frederick. Comprehensive biospecimen management activities, including tissue acquisition, biorepository operations, pathology review, data coordination, and program management, will be organized under the caHUB.

Genotype-Tissue Expression Project	
Goals on Inception (2009)	• Normal biospecimen acquisition, quality control, storage • Clinical data collection and management for the project • Long-term management of tissue and specimen-associated data for the project • Assess project feasibility, including expansion to full project goals of 1,000 donors • Enrollment of 160 deceased donors and 160 surgery patients, at the rate of at least 10 per month • Analyze gene expression in at least 50 unique tissues per donor • RNA integrity numbers of at least 6 in 70% of the 12 highest priority tissue samples • Evaluate and optimize informed-consent process • Identify cis-expression quantitative trait loci for at least 4% of expressed transcripts
Accomplishments	• Request for proposals developed and released • Sites reviewed and visited; Basic Ordering Agreements and GTEx Task Orders issued • Best Practices for Post-Mortem Tissue Recovery document developed
Future Goals	• GTEx specimen IT requirements defined and IT Working Group initiated • Tissue procurement initiated in August 2010 • Active pilot data collection initiated in April 2011 • Preliminary statistical analysis and evaluation of the pilot completed by October 2011

Other Programs OBBR Supports

Other Programs OBBR Supports	
Clinical Proteomics Technologies for Cancer (CPTC)	caHUB is currently working to establish a relationship with CPTC to provide tissue biospecimens in support of their newly issued RFA for Proteome Characterization Centers. OBBR has contributed substantially to the CPTAC Biospecimens Working Group and is preparing a joint publication for the evidence-based standard operating procedure developed for use across the CPTAC consortium. In addition, the BRN collaborates with the CPTAC program on BRN research programs investigating the effects of biospecimen preanalytical variables on proteomic analyses.
Clinical Proteomics Technologies Assessment for Cancer (CPTAC)	OBBR has contributed substantially to the CPTAC Biospecimens Working Group and is preparing a joint publication for the evidence-based standard operating procedure developed for use across the CPTAC consortium. In addition, the BRN collaborates with the CPTAC program on its research programs investigating the effects of biospecimen preanalytical variables on proteomic analyses. CPTAC and OBBR are in discussions about how caHUB biospecimen collections might serve the next phase of the CPTAC program.
Clinical Therapy Evaluation Program (CTEP)	caHUB is currently establishing a Memorandum of Understanding with CTEP to perform a collaborative pilot project in which caHUB will provide biospecimen acquisition, processing, storage, and distribution services for CTEP correlative study projects.
Genotype-Tissue Expression Roadmap Initiative (GTEx)	See OBBR Strategic Initiatives III. OBBR Support to Other Programs
Investigational Drug Steering Committee, Biomarker Task Force (IDSC)	OBBR has a special membership on this task force to provide expertise on issues related to the use of biospecimens in biomarker and assay development research.

Other Programs OBBR Supports	
National Community Cancer Centers Program (NCCCP)	The NCCCP was a 3-year pilot designed to establish a baseline for status of operations at 16 community hospitals around the country and to assess the requirements to align these centers with NCI-designated Cancer Centers. The post-pilot phase is a 5-year extension of the original project and seeks to implement the recommendations that resulted from the baseline assessment. OBBR assisted with the review and selection of NCCCP pilot sites, participated on the project management committee and biospecimen committee, established the assessment checklists for biobanking operations at each participating institution, and continues to work with Phase II sites to assist in the implementation of operational improvements.
National Institute of Child Health and Human Development (NICHD) National Children's Study	OBBR assisted NICHD leadership in establishing specifications for NCS biospecimen collection and processing and in reviewing proposals to establish the central biorepository for the NCS.
National Institute of Dental and Craniofacial Research (NIDCR)	OBBR staff served on a steering committee in support of the NIDCR Salivary Gland Tumor Network.
NCI Applied Molecular Pathology Laboratory	OBBR representative serves on the steering committee of the AMP Laboratory to provide input and expertise on issues related to the acquisition and utilization of biospecimens.
NCI Patient Characterization Center (PCC)	caHUB is working with the PCC and the closely tied Clinical Assay Development Center (CADC) to develop a plan by which caHUB will provide these initiatives with the biospecimens and related data needed to achieve their missions.
NCI SBIR Development Center	OBBR has worked with the SBIR Development Center for the past 2 years to establish, issue, and award contracts in support of small businesses performing research and product development in the area of alternative biospecimen storage and stabilization methods. Members of OBBR serve annually on the Development Center's internal Technical Advisory Group.
The Cancer Genome Atlas (TCGA)	See OBBR Strategic Initiatives III. OBBR Support to Other Programs
NCI Office of Physical Sciences-Oncology (OPSO)	OBBR has assisted the Office of Physical Sciences-Oncology (OPSO) in its development of a PS-OC Bioresource Core Facility (PBCF), a centralized biorepository and biodistributor to support network-wide activities within the Physical Sciences-Oncology Centers (PS-OC). In collaboration with OBBR and caHUB, the PBCF services will eventually expand to be a biorepository of human biological specimens, acquiring and authenticating tissues from PS-OC Network investigators in accordance with the NCI Best Practices for Biospecimen Resources guidelines.

Other Programs OBBR Supports	
International Cancer Genome Consortium (ICGC)	The ICGC is a international network of cancer genome projects, which includes TCGA, and has as its goal to obtain a comprehensive description of genomic, transcriptomic, and epigenomic changes in 50 different tumor types and/or subtypes which are of clinical and societal importance across the globe. At the inception of this project, OBBR provided all educational materials, policies, and guidances developed for biospecimens in TCGA to the ICGC leadership and participants. OBBR shared all lessons learned and served in a consultancy capacity for biospecimen issues as the ICGC project got under way.
Office of Latin American Cancer Program Development (OLACPD)	OBBR supports the development of OLACPD's US-Latin America Cancer Research Network by advising program collaborators in Argentina, Brazil, Chile, Mexico, and Uruguay on biospecimen resource infrastructure and standardization of the network's specimen collection, processing, and storage efforts.
FNIH Biomarkers Consortium	OBBR provides overall guidance on biospecimen-related issues relevant to the projects identified by the group as meritorious and direct biospecimen-specific input into project plan development and implementation.

Appendix 1: Staffing

Carolyn C. Compton, M.D., Ph.D.
Director, Office of Biorepositories and Biospecimen Research (OBBR)

Dr. Compton is Director of the NCI Office of Biorepositories and Biospecimen Research. She received her M.D. degree from Harvard Medical School and Ph.D. degree from the Harvard Graduate School of Arts and Sciences and trained in anatomic pathology and clinical pathology at Brigham and Women's Hospital. She came to the NCI from McGill University in Montreal, where she was Strathcona Professor and Chair of Pathology and Pathologist-in-Chief of McGill University Health Center. Before this, she was Professor of Pathology at Harvard Medical School and Director of Gastrointestinal Pathology at Massachusetts General Hospital. Currently, she is an Adjunct Professor of Pathology at the Johns Hopkins Medical School. In addition to human biospecimen science, her research interests include translational studies in colon cancer, pancreatic cancer, and wound healing. Dr. Compton is currently Chair of the American Joint Committee on Cancer and holds several other national and international leadership positions in professional organizations such as the College of American Pathologists, Cancer and Leukemia Group B (CALGB), International Union Against Cancer, Commission on Cancer of the American College of Surgeons, and American Society of Clinical Oncology. She is currently a member of the editorial boards of *Cancer*, *Biopreservation and Biobanking*, and *Clinical Proteomics*. She has published more than 350 original papers, reports, review articles, books, and abstracts.

Jim Vaught, Ph.D.
Deputy Director, OBBR

Dr. Vaught is Deputy Director of the NCI OBBR. After initially working as a laboratory scientist specializing in the mechanisms of chemical carcinogenesis, Dr. Vaught has been working in the field of biorepository and biospecimen science for over 15 years. In 1999 he was a founding member of the International Society for Biological and Environmental Repositories (ISBER), and was its second president. He participated in the development of the first edition of ISBER's Best Practices for Repositories. In 2005 Dr. Vaught joined the OBBR and has participated in the development of NCI's Best Practices for Biospecimen Resources and the Office's other strategic initiatives.

Dr. Vaught actively participates in a number of international biobanking initiatives. Since 2005 he has served as one of NIH's representatives to the Interagency Working Group on Scientific Collections, which was created by the Office of Science and Technology Policy. In addition to ISBER, Dr. Vaught is a member of the American Association for Cancer Research (AACR), Association for Laboratory Automation, American Society for Pharmacology and Experimental Therapeutics, and American Association for Clinical Chemistry. He is Senior Editor for Biorepository and Biospecimen Science for the AACR journal *Cancer Epidemiology, Biomarkers, & Prevention* and a member of the editorial board of *Biopreservation and Biobanking*, the official journal of ISBER.

Helen M. Moore, Ph.D.
Director, Biospecimen Research Network, OBBR

Dr. Moore directs the NCI Biospecimen Research Network (BRN). Under Dr. Moore's leadership, the BRN has grown from concept stage to a multidimensional program encompassing intramural and extramural research programs, a Web-based biospecimen literature database, and community outreach activities, including the current Advancing Cancer Research Through Biospecimen Science symposium. Dr. Moore has a broad background in research and product development. She joined the NCI from Celera Genomics, where she led and managed cross-functional teams to develop bioinformatics products focused on comparative genomics and data visualization; developed new drug targets for complex diseases using multiple approaches, including genetic analysis of disease association study data, biological pathways analysis, literature mining, and genomic analysis; and contributed to the assembly and annotation of the human genome map. Dr. Moore earned her B.A. degree at Wellesley College and her Ph.D. degree at Cornell University. Her research experience includes work on human genomics and bioinformatics, fruit fly signaling, plant molecular biology, Alzheimer's disease, and synthetic skin.

Sherilyn J. Sawyer, Ph.D.
Biospecimen Technology Program Manager, OBBR

Dr. Sawyer joined the OBBR staff in 2009 as a Biospecimen Technology Program Manager with primary responsibilities in the planning and management of research projects in biospecimen science funded by OBBR's Biospecimen Research Network. Dr. Sawyer has a broad interest in science and technology innovation in cancer research. Dr. Sawyer first joined the NCI as a Presidential Management Fellow (PMF) in 2007. As a PMF she completed assignments with NCI's Specialized Programs of Research Excellence (SPOREs), the Small Business Innovation and Research Development Center (SBIR), and the Division of Extramural Activities Review Branch. Dr. Sawyer earned her Ph.D. degree in molecular biology, cell biology, and biochemistry from Boston University and a B.S. degree in molecular biology from the University of Nevada-Reno. Her dissertation research focused on the field of molecular endocrinology, specifically on the role of select nuclear receptors in normal neural development and as targets and mediators of environmental endocrine disruption.

Joyce Rogers, M.B.A., PMP
Scientific Program Manager, OBBR

Ms. Rogers has served as program staff in the OBBR since September 2006. In this capacity, she contributes to strategic planning, finance, and project management across OBBR's initiatives. Before coming to the NCI, Ms. Rogers served as Administrator for the Department of Pathology at McGill University in Montreal, Canada. She has many years of experience in coordinating academic medical research programs. In addition to holding an M.B.A degree with a concentration in management, she is a certified Project Management Professional.

Kimberly Myers, Ph.D.
Biospecimen Technology Program Manager, OBBR

Dr. Myers joined the OBBR in 2008 and works on issues related to biobanking in support of personalized medicine. Her work has included strategic planning for a national biospecimen resource, defining scientific rationales for the development of national initiatives, and strategically partnering with non-government stakeholders, such as industry and patient advocacy organizations, to achieve stated scientific goals. Dr. Myers has broad interests in science policy and scientific strategic planning. She first joined the NCI as a Presidential Management Fellow (PMF) in 2006. While completing details as a PMF, Dr. Myers worked in NCI's Office of Science Planning and Assessment and Small Business Innovation Research (SBIR) Development Center. She also completed a detail in the Legislative Office of the Federation of American Societies for Experimental Biology (FASEB). Dr. Myers earned her B.S. degree in microbiology from Middle Tennessee State University and her Ph.D. degree from Harvard University's Program in Virology in the Division of Medical Sciences. Her dissertation research focused on viral entry mechanisms employed by nonenveloped virions, with a focus on viral protein structure-function relationships.

Nicole Lockhart, Ph.D.
Biospecimen Technology Program Manager, OBBR

Dr. Lockhart joined the OBBR in 2006 as an AAAS Policy Fellow. She has since served as a Biospecimen Technology Program Manager working on ethical, legal, and policy issues related to biospecimen research. Primary issues include informed consent for donation and use of biospecimens, maintenance of privacy, biospecimen ownership, and custodianship, and the ethical implications of using pediatric biospecimens for research. Dr. Lockhart received her Ph.D. degree in molecular and integrative physiology from the University of Michigan and holds a B.S. degree in biology from Brown University.

Tony Dickherber, Ph.D.
AAAS Science and Technology Policy Fellow, OBBR

Dr. Dickherber received his B.S. (1999) and M.S. (2003) degrees in electrical engineering from the Georgia Institute of Technology, where he specialized in telecommunications and signal processing. He spent 4 years as a Research Engineer at the Georgia Tech Research Institute working on classified telecommunications projects. He earned his Ph.D. degree at the Georgia Institute of Technology (2008), focusing on development of cancer protein microarray biosensors based on microelectronic acoustic device technology. He served as a Sam Nunn Security Program Fellow (2006-2007) and Director of the Biotechnology Policy Forum (2006-2008) in Atlanta. Before joining the National Cancer Institute as an AAAS Science and Technology Policy Fellow, he worked on designing arrayable ion-trapping structures for quantum-bit computing at the Nanotechnology Research Center. Dr. Dickherber currently works in the NCI OBBR assisting in planning the cancer Human Biobank (caHUB), a unique institution and the first U.S. national biobank.

Joanne Peter Demchok, M.S. (ASCP)
Medical Technologist, OBBR

Ms. Demchok recently joined OBBR, where she is developing a quality management plan for tissue accrual. These quality control monitors will be the foundation for the collection, processing, and storage of biospecimens for the caHUB. Ms. Demchok received a master of science degree from the University of Maryland, School of Medical and Research Technology. Her research focused on developing in vitro antifungal susceptibility testing and correlation with in vivo response. Ms. Demchok has extensive research experience from her tenure at the Pediatric Oncology Branch, specializing in infectious disease of immunocompromised patients. She has authored and coauthored several publications studying various lipid formulations of amphotericin B, neutrophil function, and antifungal agent-induced renal cell cytotoxicity. She has taught laboratory techniques and quality control to numerous medical, graduate, and high school students.

Richard Aragon, Ph.D.
Program Director, IMAT, OBBR

Dr. Aragon is the current Program Director of the NCI's Innovative Molecular Analysis Technologies (IMAT) program, an OBBR-affiliated initiative directed at innovative technology development for cancer detection, treatment, and diagnosis. The IMAT program is a trans-divisional, multimillion dollar program aimed at the inception, development, maturation, and commercialization of cross-cutting and research-enabling molecular and cellular analytical technologies. Dr. Aragon received his bachelor's degree in neurobiology from the University of California, Santa Cruz, and his doctoral degree in biochemistry and molecular biology from The George Washington University Medical Center. His dissertation research was done in the Laboratory of Neurogenetics at the National Institute on Alcohol Abuse and Alcoholism and his postdoctoral work in molecular and cellular oncology at the Georgetown University Medical Center. He has been affiliated with the National Institutes of Health for over 12 years.

Appendix 2: OBBR Statistics

OBBR Statistics			
Current Federal Employees		9	
Presentations in 2009-2010	9 abstracts/posters		102 talks
Publications Since 2005	21 Primary	24 Coauthored	20 in Preparation
OBBR-Sponsored Meetings	Year		Number of Meetings
	2005		3
	2006		1
	2007		2
	2008		4
	2009		4
	2010		8
OBBR Web Site	Year		Number of Unique Visitors
	2006		9,255
	2007		11,413
	2008		15,525
	2009		20,956
	2010 (first 6 months)		17,185

Appendix 3. OBBR Manuscripts and Publications

Primary OBBR Publications

Compton C. Getting to personalized cancer medicine: Taking out the garbage. *Cancer*. 2007;110(8):1641-3.

Compton CC. Optimal pathologic staging: defining stage II disease. *Clin Cancer Res*. 2007;13(22 Pt 2):6862s-70s.

Compton CC. The surgical specimen is the personalized part of personalized cancer medicine. *Ann Surg Oncol*. 2009;16(8):2079-80.

Compton CC. Making economic sense of cancer biospecimen banks. *Clin Transl Sci*. 2009;2(3):172-4.

Lim MD. (2009, Fall). Catalyzing innovation by supporting risk-taking: The National Cancer Institute's program for Innovative Molecular Analysis Technologies. NIH Public-Private Partnerships Program Advisor, 1(4):2.

Lim MD. (2009, Fall). Do you have an innovative idea that can revolutionize cancer research? Opportunities and Resources for Innovative Cancer Technologies from the National Cancer Institute. American Chemical Society—Division of Analytical Chemistry Newsletter.

Lim, M.D. (2009) Strengthening the Pipeline for Innovation in Cancer Research: The National Cancer Institute's Program for Innovative Molecular Analysis Technologies (pp 1-6). In SE Cozzens and P Catalán (Eds.) Proceedings of the 2009 Atlanta Conference on Science and Innovation Policy. Piscataway, NJ: Wiley-IEEE Press.

Lim MD, Compton CC. (2010) Resources from the National Cancer Institute to Support Your Innovative Analytical Technologies for Cancer. In DL Farkas, DV Nicolau, and RC Leif (Eds.) Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues VIII: Proceedings of SPIE Volume 7568. Bellingham, WA: SPIE Press.

Moore HM, Compton CC, Lim MD, Vaught J, Christiansen KN, Alper J. 2009 Biospecimen research network symposium: Advancing cancer research through biospecimen science. *Cancer Res*. 2009; 69(17):6770-2.

NCI Best Practices for Biospecimen Resources. National Cancer Institute, NIH, 2007.

Robb JA, Moore HM, Compton CC. Documenting biospecimen conditions in reports of studies. *JAMA*. 2008;300(6): 650-1.

Vaught JB. Biorepository and biospecimen science: A new focus for CEBP. *Cancer Epidemiol Biomarkers Prev*. 2006; 15(9):1572-3.

Vaught JB. Blood collection, shipment, processing, and storage. *Cancer Epidemiol Biomarkers Prev*. 2006;15(9):1582-4.

Vaught JB. Approaches to improving biospecimen quality through research. *Cell Preserv Tech*. 2007;5(4):178-9.

Vaught JB, Lockhart N, Thiel KS, Schneider JA. Ethical, legal, and policy issues: Dominating the biospecimen discussion. *Cancer Epidemiol Biomarkers Prev*. 2007;16(12):2521-3.

Vaught JB, Caboux E, Hainaut P. International efforts to develop biospecimen best practices. *Cancer Epidemiol Biomarkers Prev*. 2010;19(4):912-5.

Vaught JB, Hsing AW. Methodologic data: important foundation for molecular and biomarker studies. *Cancer Epidemiol Biomarkers Prev*. 2010;19(4):901-2.

Vaught JB, Kelly AB, Hewitt R. A Review of International Biobanks & Networks: Success Factors and Key Benchmarks. *Biopreservation and Biobanking*. 2010;7(3):143-50.

Yassin R, Lockhart N, González del Riego M, Pitt K, Thomas JW, Weiss L, Compton C. Custodianship as an ethical framework for biospecimen-based research. *Cancer Epidemiol Biomarkers Prev*. 2010;19(4):1012-5.

Collaborative OBBR Publications

Bertagnolli MM, Niedzwiecki D, Compton CC, Hahn HP, Hall M, Damas B, Jewell SD, Mayer RJ, Goldberg RM, Saltz LB, Warren RS, Redston M. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. *J Clin Oncol*. 2009; 27(11):1814-21.

Bertagnolli MM, Warren RS, Niedzwiecki D, Mueller E, Compton CC, Redston M, Hall M, Hahn HP, Jewell SD, Mayer RJ, Goldberg RM, Saltz LB, Loda M. p27Kip1 in stage III colon cancer: Implications for outcome following adjuvant chemotherapy in Cancer and Leukemia Group B Protocol 89803. *Clin Cancer Res*. 2009;15(6):2116-22.

Betsou F, Luzergues A, Carter A, Geary P, Riegman P, Clark B, Morente M, Vaught J, Dhir R, et al. Towards norms for accreditation of biobanks for human health and medical research: Compilation of existing guidelines into an ISO certification/accreditation norm-compatible format. *Quality Assurance Journal*. 2007;11:221-94.

Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008;455(7216):1061-8.

Edge SB, Compton CC. The American Joint Committee on Cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*. 2010;17(6):1471-4.

International Cancer Genome Consortium. International network of cancer genome projects. *Nature*. 2010;464(7291): 993-8.

Lemrow SM, Colditz GA, Vaught JB, Hartge P. Key elements of access policies for biorepositories associated with population science research. *Cancer Epidemiol Biomarkers Prev*. 2007;16(8):1533-5.

Misdraji J, Oliva E, Goldblum JR, Lauwers GY, Compton CC; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with invasive carcinomas of the appendix. *Arch Pathol Lab Med*. 2006;130(10):1433-9.

Riegman PH, Morente MM, Betsou F, de Blasio P, Geary P; Marble Arch International Working Group on Biobanking for Biomedical Research. Biobanking for better healthcare. *Mol Oncol*. 2008;2(3):213-22.

Rosen LS, Bilchik AJ, Beart RW Jr, Benson AB 3rd, Chang KJ, Compton CC, Grothey A, Haller DG, Ko CY, Lynch PM, Nelson H, Stamos MJ, Turner RR, Willett CG. New approaches to assessing and treating early-stage colon and rectal cancer: Summary statement from 2007 Santa Monica Conference. *Clin Cancer Res*. 2007;13(22 Pt 2):6853s-6s.

Rubinstein YR, Groft SC, Bartek R, Brown K, Christensen RA, Collier E, Farber A, Farmer J, Ferguson JH, Forrest CB, Lockhart NC, McCurdy KR, Moore H, Pollen GB, Richesson R, Miller VR, Hull S, Vaught J. Creating a Global Rare Disease Patient Registry linked to a Rare Diseases Biorepository Database: Rare Disease-HUB (RD-HUB). *Contemp Clin Trials*. 2010 Jul 8. [Epub ahead of print]

Signoretti S, Bratslavsky G, Waldman FM, Reuter VE, Haaga J, Merino M, Thomas GV, Pins MR, Libermann T, Gillespie J, Tomaszewski JE, Compton CC, Hruszkewycz A, Linehan WM, Atkins MB. Tissue-based research in kidney cancer: Current challenges and future directions. *Clin Cancer Res*. 2008;14(12):3699-705.

Turner RR, Li C, Compton CC. Newer pathologic assessment techniques for colorectal carcinoma. *Clin Cancer Res*. 2007; 13(22 Pt 2):6871s-6s. Review.

Washington MK, Berlin J, Branton PA, Burgart LJ, Carter DK, Fitzgibbons PL, Frankel WL, Jessup JM, Kakar S, Minsky B, Nakhleh RE, Compton CC; Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with primary carcinomas of the colon and rectum. *Arch Pathol Lab Med*. 2008 Jul;132(7):1182-93. Erratum in: *Arch Pathol Lab Med*. 2008;132(9):1384.

Washington MK, Berlin J, Branton P, Burgart LJ, Carter DK, Fitzgibbons PL, Halling K, Frankel W, Jessup J, Kakar S, Minsky B, Nakhleh R, Compton CC; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with primary carcinoma of the colon and rectum. *Arch Pathol Lab Med*. 2009;133(10):1539-51.

Washington MK, Berlin J, Branton PA, Burgart LJ, Carter DK, Compton CC, Fitzgibbons PL, Frankel WL, Jessup JM, Kakar S, Minsky B, Nakhleh RE, Vauthey JN; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with carcinoma of the distal extrahepatic bile ducts. *Arch Pathol Lab Med*. 2010;134(4):e8-13.

Washington MK, Berlin J, Branton PA, Burgart LJ, Carter DK, Compton CC, Frankel WL, Jessup JM, Kakar S, Minsky B, Nakhleh RE, Vauthey JN; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with carcinoma of the intrahepatic bile ducts. *Arch Pathol Lab Med*. 2010; 134(4):e14-8.

Washington MK, Berlin J, Branton PA, Burgart LJ, Carter DK, Compton CC, Fitzgibbons PL, Frankel WL, Jessup JM, Kakar S, Minsky B, Nakhleh RE, Vauthey JN; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with carcinoma of the perihilar bile ducts. *Arch Pathol Lab Med*. 2010;134(4):e19-24.

Washington MK, Tang LH, Berlin J, Branton PA, Burgart LJ, Carter DK, Compton CC, Fitzgibbons PL, Frankel WL, Jessup JM, Kakar S, Minsky B, Nakhleh RE; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with neuroendocrine tumors (carcinoid tumors) of the appendix. *Arch Pathol Lab Med*. 2010;134(2):171-5.

Washington MK, Tang LH, Berlin J, Branton PA, Burgart LJ, Carter DK, Compton CC, Fitzgibbons PL, Frankel WL, Jessup JM, Kakar S, Minsky B, Nakhleh RE; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with neuroendocrine tumors (carcinoid tumors) of the colon and rectum. *Arch Pathol Lab Med*. 2010;134(2):176-80.

Washington MK, Tang LH, Berlin J, Branton PA, Burgart LJ, Carter DK, Compton CC, Fitzgibbons PL, Frankel WL, Jessup JM, Kakar S, Minsky B, Nakhleh RE; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with neuroendocrine tumors (carcinoid tumors) of the small intestine and ampulla. *Arch Pathol Lab Med*. 2010;134(2):181-6.

Washington MK, Tang LH, Berlin J, Branton PA, Burgart LJ, Carter DK, Compton CC, Fitzgibbons PL, Frankel WL, Jessup JM, Kakar S, Minsky B, Nakhleh RE; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with neuroendocrine tumors (carcinoid tumors) of the stomach. *Arch Pathol Lab Med*. 2010 Feb;134(2):187-91.

Zlobec I, Vuong T, Compton CC, Lugli A, Michel RP, Hayashi S, Jass JR. Combined analysis of VEGF and EGFR predicts complete tumour response in rectal cancer treated with preoperative radiotherapy. *Br J Cancer*. 2008;98(2):450-6.

OBBR Manuscripts in Progress

Lim MD, Dickherber A, Compton CC, Before You Analyze a Human Specimen—Think Quality, Variability and Bias. Anal Chem. In review.

Alper J, Moore HM. BRN International Priorities for Biospecimen Research. In preparation.

Dickherber A, Vaught JB. Future Directions in Biospecimen Repositories and the Role of the NCI. In S Jewell and D Hansel (Eds.) Developing and Organizing an Institutional Biorepository. College of American Pathologists Press. In preparation.

Compton C. Biospecimen Banking in the Post Genome Era. In G Ginsberg and H Willard (Eds.) Genomic and Personalized Medicine, 2nd Edition. Elsevier. In preparation.

Development of a National Cancer Human Biobank (caHUB) by NCI. JNCI Monograph. In preparation.

Monograph includes a series of invited commentaries representing viewpoints from industry, advocates, academia, etc., along with the following articles:

Massett HA, Atkinson NL, Weber D, Myles R, Ryan C, Grady M, Compton C. Assessing the Need for a Standardized Cancer Human Biobank (caHUB): Findings from a National Survey with Cancer Researchers.

Myles R, Massett HA, Seigler C, Comey G, Aslop D, Rogers J, Compton C. Focus Group Findings on Biospecimen Needs and Reactions from Stakeholders to the Development of a National Cancer Human Biobank (caHUB) by NCI.

Rogers J, Carolin T, Vaught J, Compton C. A Taxonomy for Evaluating the Economic Benefits that Biobanks Contribute to Research and to the Public.

Vaught J, Rogers J, Carolin T, Compton C. Biobankonomics: Developing a Sustainable Business Model Approach for the Formation of a Human Tissue Biobank.

Vaught J, Rogers J, Myers K, David Lim MD, Lockhart N, Moore HM, Sawyer S, Furman J, Compton C. An NCI Perspective on Creating Sustainable Biospecimen Resources.

Engel KB, Moore HM, Vaught J. Publication Venues for Biospecimen Science. In preparation.

Engel KB, Moore HM. Review: Effects of Pre-analytical Variables on the Detection of Proteins by Immunohistochemistry in Formalin Fixed Paraffin Embedded Tissues. In preparation.

Jewell SD, Kelly AB, McShane LM, Clark D, Greenspan R, Hayes DF, Hainaut P, Kim P, Mansfield E, Potapova O, Riegman P, Rubinstein Y, Seijo E, Somiari S, Watson P, Weier H-U, Zhu C, Moore HM, Vaught J. Biospecimen Reporting for Improved Study Quality (BRISQ). In preparation.

Lim MD, McShane LM, Speed TP, Moore HM, Goldthwaite CA, Compton CC. Designing Experiments to Investigate the Effects of Variability on Biospecimen Molecular Profiles. In preparation.

Moore HM, Compton CC, Sawyer S, Vaught J, Alper J. 2010 Biospecimen Research Network Symposium: Advancing Cancer Research Through Biospecimen Science. In preparation.

Moore HM. Clinical Proteomic Technologies for Cancer Biospecimen Working Group: Devising an Evidence-Based Protocol. Molecular and Cellular Proteomics. In preparation.

Moore HM, Engel KB, Fore I, Breychak A. The Biospecimen Research Database. Clinical Chemistry. In preparation.

Moore HM. The Importance of Standard Operating Procedures in Biospecimen Preservation. Biotechnic & Histochemistry. In preparation.

Myers KS and Lockhart NC Relations with Outside Institutions and Industry. In S Jewell & D Hansel (Eds.) Developing and Organizing an Institutional Biorepository. College of American Pathologists Press. In preparation.

NCI Best Practices for Biospecimen Resources, Revised. National Cancer Institute, NIH, 2010. In preparation.

Appendix 4. Highlights From Recent BRN Symposia

"Pitfalls and Gaps in Research Using Human Biospecimens," Patrick O. Brown, M.D., Ph.D., Stanford

"The Role of Biospecimens in Biomarker Research: Challenges and Opportunities," David F. Ransohoff, M.D., University of North Carolina at Chapel Hill

"HER2/neu: Lessons Learned From Paired Diagnostics and Therapeutics," Paul Waring, M.D., Ph.D., Genentech, Daniel F. Hayes, M.D., University of Michigan Comprehensive Cancer Center, Dennis J. Slamon, M.D., Ph.D., University of California, Los Angeles

"Locking in Pre-Analytical and Post-Analytical Performance: Being Pound Wise, Not Penny Foolish," Steven Gutman, M.D., U.S. Food and Drug Administration

"CAP: Preparing Pathologists," M. Elizabeth H. Hammond, M.D., FCAP, University of Utah School of Medicine

"Harmonizing Biospecimen Best Practices Across the Prostate SPORES," Angelo M. De Marzo, M.D., Ph.D., Johns Hopkins University

"Overcoming Challenges to Tissue-Based Studies," Christopher J. Logothetis, M.D., M. D. Anderson Cancer Center

"Pre-Analytical Variables in Validation of Methylated Biomarkers in Blood Plasma," Theo deVos, Ph.D., M.S.P.H., Epigenomics, Inc.

"Levels of Evidence for Retrospective Analyses of Banked Samples," Scott D. Patterson, Ph.D., Amgen Inc.

"Preanalytical Variables Affecting FNA and Their Influence on Clinical Diagnosis," Douglas P. Clark, M.D., Johns Hopkins School of Medicine

"Impact of Ischemia and Tissue Procurement Conditions on Gene Expression in Renal Cell Carcinoma," Gennady Bratslavsky, M.D., NCI, NIH

"Design and Analysis of Experiments Exploring the Main Effects of Preanalytical Variables on Molecular Research," Terry Speed, Ph.D., University of California, Berkeley

"The Power of the "Right" Biospecimens in Clinical Research and Care," David B. Agus, M.D., Cedars-Sinai Medical Center

"Banking AIDS-Related Malignancies in Sub-Saharan Africa," Leona W. Ayers, M.D., Ohio State University

"Development and Real-World Use of a System for Tracking Biospecimens and Biospecimen Data," Mark A. Watson, M.D., Ph.D., Washington University School of Medicine

"How to Improve Intraoperability of Biobanks: The Role of Standardization of Preanalytical Variables," Kurt Zatloukal, M.D., Medical University of Graz, Austria

"Assessing Biospecimen Quality," Andrew I. Brooks, Ph.D., Robert Wood Johnson Medical School

Appendix 5. OBBR-Sponsored Meetings

Title	Purpose	Event Date
Best Practices for Biorepositories That Support Cancer Research	The purpose of this workshop was to identify and recommend best practices for the establishment and maintenance of human biospecimen and associated clinical-data repositories designed to broadly support cancer research and development. These best practices will address biorepository operational, infrastructural, and informatics requirements, as well as the procedural boundaries that ensure compliance with bioethical, legal, biosafety, quality assurance, and quality control guidelines.	2005
Biospecimen Ethical, Legal, and Policy Issues	This workshop brought together leaders from the national and international academic communities, private sector, and government to provide recommendations for ethical policy guidelines that will govern the collection and use of human biological specimens and associated data in NCI-sponsored resources/repositories. The goal is to facilitate the collection and future use of resource/repository specimens and associated data while protecting the subjects from whom the specimens and data are obtained.	2005
International Harmonization of Biorepository Practices	This workshop convened a group of more than 80 biorepository experts from 15 countries in North America, Europe, and Asia to share information about international biorepository efforts. Through a series of plenary and roundtable sessions, the participants addressed a range of scientific, technical, ethical, legal and policy issues affecting biorepositories.	2005
Biospecimen Custodianship Workshop	This was a 1½ day workshop to define the parameters of custodianship that would allow biospecimen resources to operate in a culture of transparency, fairness, and accountability to all stakeholders. Topics addressed included (1) considerations for research participants, investigators, and institutions; (2) financial conflicts of interest; (3) intellectual property; and (4) access to products and benefits.	2007
NCI Best Practices for Biospecimen Resources Public Outreach Meetings	OBBR hosted four public meetings (Bethesda, Boston, Chicago, and Seattle) with the purpose of educating the biobanking and research communities about the benefits of implementing the NCI Best Practices, as well as providing an opportunity for the NCI to address questions and concerns regarding the guidelines and their application.	2007
BioEconomics Workshop: Economic Considerations for Implementing the NCI Best Practices for Biospecimen Resources	This was a 1-day workshop to address the long-term economic strategies for establishing and maintaining a biospecimen resource, including strategies for defining the scientific and economic impact of biospecimen resources from both case-study and operational points of view.	2008
The Ethical Use of Pediatric Biospecimens in Research	This was a 1-day workshop to convene experts in the field and appropriate stakeholder groups to discuss the ethical issues involved in storage and use of pediatric biospecimens in research.	2008

Title	Purpose	Event Date
caHUB Informational Public Meetings (2 half-day meetings: 1 on NIH campus and 1 in Los Angeles)	The purpose of this meeting was to inform the general public of the caHUB planning process; address the need for transparency with all Recovery Act-funded projects; provide a context for the subprojects and current RFPs by explaining the overall mission and vision of caHUB; and bring together and inform potential offerors.	2009
Ethical and Legal Considerations in the Return of Research Results	The purpose of this workshop was to (1) define circumstances for when return of research results to individual participants is appropriate, (2) define mechanisms and IT solutions for return of aggregate results to research participants, and (3) define common approaches to the discovery of a discrepancy in diagnosis. Outcomes of this workshop will allow expansion of the NCI Best Practices and will be essential in crafting policies for large NCI projects, such as caHUB.	2010
BRN Annual Symposium: Advancing Cancer Research Through Biospecimen Science	This annual symposium provides a public forum for presentation and discussion of biospecimen research results. The symposium encourages participation from all stakeholders to improve the quality of biospecimen-based cancer research and fuel advances in personalized medicine.	2008 2009 2010
Biospecimen Reporting to Improve Study Quality (BRISQ)	This was a face-to-face roundtable discussion of a group currently meeting by teleconference to develop biospecimen reporting guidelines for authors of publications that utilize human biospecimens. Journal editors were included to thoroughly vet proposed guidelines and plan dissemination of such.	2010
International Biospecimen Research Meeting	This was a face-to-face meeting of BRN program, SPIDIA (European BRN), SBIR, IMAT, FDA, and others, to share information on separate biospecimen research programs and develop joint strategies for prioritizing the most urgent biospecimen research questions for program support.	2010
Annual Principal Investigators Meeting of the Innovative Molecular Analysis Technologies Program	This annual meeting convenes all currently supported principal investigators of the IMAT program; attendance is required in their grant award.	2008 2009 2010
Developing Benchmarked Biospecimen Standards: An OBBR-NIST Partnership	The purpose of this workshop is to bring together biomedical scientists, measurements/standards/metrology scientists, experimental-design and research-methods experts, and regulatory scientists to delineate the current state of the science, identify unmet needs, and provide a framework of projects that aim to develop fit-for-purpose biospecimen standards.	Planned - Fall 2010
caHUB Pilot Project Launch Meeting	The purpose of this 2-day meeting is for the OBBR to provide an overview of the scientific and operational goals of the pilot project to team members, including subcontractors; explain the organizational structure; discuss project goals and deliverables; and allow team members to meet and make presentations on their respective roles within the larger project framework. Also, this meeting will provide training and education regarding the caHUB standard operating procedures and quality control metrics.	Planned - Fall 2010

Appendix 6. caHUB Working Groups Executive Summary

SAIC-Frederick, Inc.
Clinical Monitoring Research Program in Support of the
National Cancer Institute
Office of Biorepositories and Biospecimen Research

cancer Human Biobank Subgroup Work Products

Executive Summary

In response to the need for high-quality human biospecimens and data, the National Cancer Institute (NCI) Office of Biorepositories and Biospecimen Research (OBBR) is developing the cancer Human Biobank (caHUB). The OBBR initiated the planning phase for the caHUB in July 2009 with the establishment of the Administration Working Group (AWG), which comprises a wide range of experts and opinion leaders. The AWG subsequently created a series of strategic and operations subgroups and charged each one with the production of a deliverable(s) relevant to the establishment of the caHUB. Over approximately 9 months, the subgroups responded to their charges, culminating in the production of a group of recommendations, standard operating procedures (SOPs), best practices, research findings, and issues for consideration. Documents containing each subgroup product are compiled within this report for consideration by the OBBR.

This executive summary provides an overview of the products, or suite of products, developed by each subgroup as well as a report on economic considerations for the caHUB prepared by a consulting team at Booz Allen Hamilton and a draft caHUB Communications Plan created by the NCI Office of Communications & Education.

Strategic Planning and Organizational Structure Subgroup

This subgroup was charged with defining the caHUB's mission, objectives, and scope of operations as well as its organizational structure. The subgroup responded to its charge by generating an eight-component strategic plan document. The first and key component of the strategic plan is a vision statement, which the subgroup recommends that the OBBR endorse and adopt. The corresponding Mission Statements and Implementation Milestones and Success Factors establish an operational framework for the caHUB and provide a high-level work plan by which the caHUB's development and achievements toward realizing this vision can be measured and tracked. Some activities with regard to defining the scope of the caHUB in Phases I and II remain outstanding, e.g., developing a client profile and conducting a gap analysis for clients' needs. The Implementation Milestones and Success Factors document identifies many of the activities that are yet to be accomplished and provides a suggested timeline for completion. In terms of the caHUB's organization and function, the strategic plan provides recommended organizational structures for Phase I and Phase II as well as a section that outlines a diverse portfolio of market-driven service competencies that caHUB may consider providing to strengthen its financial foundation. Finally, the strategic plan provides charters for two expert groups recommended for establishment in Phase I caHUB—the Tactical Discussion Group and the External Strategic Scientific Group—as well as for a number of Professional Resource Groups comprising existing subgroups that will continue in perpetuity throughout the life of the caHUB or new groups yet to be established. It is expected that these groups will serve as a resource to the OBBR in refining and implementing the strategic plan.

Biospecimens Subgroup

This subgroup was charged with defining caHUB business and operating plans related to the prioritization, collection, processing, and storage of biospecimens. In response, the subgroup produced 10 deliverables that include a tissue prioritization matrix, tissue collection SOPs, tissue morphologic and molecular qualification SOP, fixation SOPs, blood collection and processing SOPs, and a preliminary draft of quality monitors to serve as the basis for the caHUB Total Quality Management plan. One of the major products developed by this subgroup is the tissue prioritization quantitative

matrix, which is based on the NCI Surveillance Epidemiology and End Results program's malignant neoplasm categories. Each cancer is scored using a value-ranking system, as described in the tool, to provide an overall score for each specimen type that creates a priority of biospecimen collection for research. The other major product is a set of 42 SOPs, based on input from nationally recognized pathologists and surgeons, that offer guidance for procuring cancerous and "normal" tissues. Also produced was a fresh-frozen and paraffin-embedded tissue qualification criteria set. Collectively, the documents described above will serve as the basis for caHUB's collection strategy and tissue banking operations.

Acquisition of Normal Tissues Subgroup

This subgroup was charged with defining "rapid autopsy" parameters and the range of normal ("nondiseased") sample and data requirements to meet the needs of the Genotype-Tissue Expression project and other identified market needs. The subgroup's primary deliverable, which is in draft form, is a manuscript titled "Best Practices for Postmortem Recovery of Normal Human Tissue for Research" that aims to define ideal "best" technical, operational, and ethical practices for biospecimen resources and tissue banks, recovery organizations, and scientists working with postmortem tissues. These best practices, which supplement the NCI Best Practices for Biospecimen Resources, offer guidance on donor identification and screening, tissue recovery, coordination of biospecimen collection, the preparation, storage, and processing of biospecimens and associated clinical data, and quality concerns related to interpreting advanced analytical research methodologies. In addition, this document includes a draft set of case studies of normal postmortem research tissue collection projects that highlights methods of implementation, real-world outcomes, and useful lessons learned. In addition to serving as a resource for current practitioners, this document is intended to support the harmonization of postmortem research tissue collection efforts to align with preferred biobanking practices. The document is currently being shared with collaborators and, pending the receipt of feedback from the entire subgroup and the OBBR, the document will be submitted for publication to a high-impact journal. Still to be completed is a draft program checklist/evaluation criteria for postmortem tissue recovery programs.

Ethical, Legal, and Social Issues (ELSI) Subgroup

The ELSI Subgroup was charged with defining the ethical, legal, and social issues that surround creation of the caHUB. The first major product of this subgroup is a document titled "Preliminary Ethical, Legal, and Social Considerations for the caHUB." It was prepared based on the deliberations of the ELSI Subgroup and contains their preliminary recommendations in the areas of governance, privacy, access to data and biospecimens, data sharing, custodianship and intellectual property, return of research results, informed consent, and conflicts of interest. Special issues related to research participation by children and collection of biospecimens through rapid autopsy are also addressed. Where issues remain unresolved or require further consideration, the subgroup has provided recommendations on how the caHUB should proceed in the near term. The second product of the ELSI Subgroup is an informed consent document (ICD) template for collection of diseased tissues at the biospecimen source sites for the caHUB. The ICD template was developed by the subgroup after careful review of relevant consent forms created by other NCI programs and academic research institutions. The ELSI Subgroup did not develop an ICD template for the collection of normal tissues; however, the Preliminary Considerations document contains recommendations and issues for further consideration relevant to collection of biospecimens through normal autopsy that can serve as the foundation for development of a normal tissue collection ICD template.

Facilities Subgroup

This subgroup was tasked with defining the caHUB business and operating plans; more specifically, with bridging the requirements/needs of caHUB processes with repository facility attributes. In response, the subgroup began by developing a set of biospecimen shipping and storage flow charts. These documents identify appropriate shipping containers for specific temperature requirements and also identify the proposed storage temperature for the quantity and type of material that the caHUB needs to store/manage, which links into facility design. The flow charts document tier 2 processes that are contingent upon tier 1 processes in the OBBR's "caHUB Case Flow and Sample Quantities Diagram"; thus, modifications to either set of processes should be reflected in the other. In addition, the Facilities Subgroup has provided a facilities plan that covers the topics of total collection targets, storage requirements, staffing

numbers, and space needs. The plan is accompanied by a comprehensive set of facilities planning recommendations based on the expertise within the subgroup and that of a group of external biorepository facility experts who were convened to review and provide input on the facilities plan. The OBBR is encouraged to consult these recommendations in the buildout of the Phase II caHUB facility.

Informatics Subgroup

This subgroup was charged with four tasks: (1) To establish an architectural framework that comprehensively illustrates interoperable components of the caHUB, (2) to establish high-level use cases that illustrate the informatics vision for the caHUB, (3) to address and compile subject matter vocabularies, and (4) to provide operational informatics input to the other caHUB subgroups. The subgroup's response to the first charge was development of the caHUB National Informatics Architecture (NIA) diagram based on the information systems requirements described in the National Biospecimen Network Blueprint, a concept that forms the basis of the caHUB vision. The NIA diagram provides a high-level view of caHUB's Pathology Resource Center and the organizations and systems that will connect to it. Accompanying the diagram is a list of data categories (groupings of data that are necessary to manage the caHUB), informatics components required to support the caHUB and the categories of data stored, and a description of data flows within the caHUB enterprise. Ultimately, these products will inform development of use cases for caHUB's Comprehensive Data Resource (CDR), interoperability specifications, and an actual informatics architecture. Development of high-level interoperability use cases and defined lists of vocabularies, including semantic infrastructure to handle establishment and change control, is ongoing.

Partnerships Subgroup

The Partnerships Subgroup was formed with the charge to define partnering objectives and potential targets for the caHUB. The deliberations of the subgroup focused on whether the caHUB should transition from a Government entity to a public-private partnership (PPP) at the end of Phase I and, if so, how this transition might be accomplished. The subgroup's conclusions are reflected in its deliverable, a document titled "Recommendations of the caHUB Partnerships Subgroup: Making Phase II Possible." Its contents include key principles on which a caHUB PPP should be based, and pros and cons of maintaining the caHUB as a Government entity versus a PPP along several domains: Control, intellectual property, the role of partners, public trust and benefit, and human subjects/ownership issues. The document also outlines key factors for success of a biobanking partnership based on case studies of a diverse group of national and international biobanking models. It concludes with the recommendation that the caHUB form a PPP under the auspices of the Foundation for the NIH (FNIH) and further recommends five activities that should occur during Phase I of caHUB to ensure a smooth transition to a PPP model in Phase II. The subgroup did not address its second task of identifying and/or profiling potential caHUB partners; it is expected that this work will occur under the auspices of the PPP formed with the FNIH during Phase I of the caHUB.

Economic Considerations

The consultant team at Booz Allen Hamilton created a document, "Economic Considerations for the Formation of a National Cancer Human Biobank (caHUB)," that provides a comprehensive economic analysis and business case for the formation of the caHUB initiative. It features development of a caHUB biobanking value chain methodology; a total life cycle cost of ownership (TLCO) approach for establishing, maintaining, and sustaining the caHUB operation; research results on the industry financial landscape and pricing considerations; analysis of potential cost recovery models and recommended approach; and justification of the investment through a quantified benefits analysis. Each section includes the teams' recommended action items for the OBBR. Notably, the TLCO approach allowed the team to generate an estimated life cycle cost for the caHUB for the years 2011 to 2027, which amounts to $941.6 million. The report breaks down costs by value stream and estimates the financial burden for the NCI over the life of the caHUB to amount to $91.73 million in facilities costs. The remainder $850.14 million would be funded through caHUB or potentially through a PPP.

Communications Plan

In response to a request from the OBBR to define communications strategies for the caHUB, the NCI Office of Communications and Education developed a draft communications plan for the caHUB that outlines six communication goals and three strategies, with the relevant strategies for achieving those goals as follows: (1) Develop multiple communication tools for dissemination to key stakeholders that address a broad set of messages, (2) raise general awareness about the caHUB and disseminate new tools, and (3) engage in patient/doctor/pathologist communication on the importance of biospecimen donation and processing. The plan also offers a priorities timeline for the near term with suggested completion dates.

Appendix 7. Strategic Partnerships

Strategic Partnerships	
American Association for Cancer Research (AACR)-FDA-NCI Cancer Biomarkers Collaborative (CBC)	AACR-FDA-NCI CBC was formed to advance the Critical Path Initiative CPI, a high-priority FDA project intended to change the way clinical research is being conducted from early discovery and translational research through marketing. The AACR-FDA-NCI CBC addresses two of the CPI's six foci: biomarkers and clinical trials.
Center for Biomedical Informatics and Information Technology (CBIIT)	OBBR works closely with the NCI Center for Biomedical Informatics and Information Technology in developing the Biospecimen Research Database and in planning caHUB.
Center to Reduce Cancer Health Disparities (CRCHD) programs	The IMAT program has partnered with the CRCHD to provide training opportunities to historically underrepresented groups in areas of cross-cutting, research-enabling emerging technologies. Through diversity supplements capable of supporting up to two student trainees per laboratory, and an upcoming R25 application, the program hopes to foster members of the next generation of technology-savvy researchers and clinicians and contribute to the diversity of the next wave of biomedical scientists.
College of American Pathologists (CAP)	OBBR and the College of American Pathologists collaborate under an NCI Letter of Intent with the goal of facilitating the transfer of information related to OBBR products including updates to the NCI Best Practices and new data from the BRN to support evidence-based biospecimen protocols.
U.S. Food and Drug Administration (FDA)	OBBR has worked closely with the FDA in making presentations at FDA and OBBR public meetings and serving on joint committees and workshops.
Foundation for the NIH (FNIH)	The OBBR is working with the FNIH to launch the cancer Human Biobank (caHUB) as a robust public-private partnership, with broad community support and input. This relationship will allow NCI to engage industry, academia, advocacy, and other private partners in the creation of a state-of-the-science infrastructure to support cancer research. The benefits of caHUB becoming a public-private partnership are multifaceted and include ensuring the public trust, sharing risks, leveraging resources from the public and private sectors, and creating greater potential to generate commercial uses that will translate discoveries to the marketplace as drugs or diagnostics.
Interagency Oncology Task Force (IOTF)-FDA-NCI	The IOTF-FDA-NCI collaboration was established to bring together representatives from various government agencies to identify ways to collectively facilitate cancer research. OBBR actively participated in the development of recommendations for the standardization and harmonization of biobanking practices for research in the diagnostic and therapeutic development arenas.
National Institute of Standards and Technology (NIST)	The OBBR has recently established a collaboration with NIST to develop specific and quantitative quality standards and metrics that can be used to ensure the appropriate, consistent, and well-controlled sample quality necessary for effective biomedical research and clinical use. The development and subsequent utilization of such standards will not only allow for cross-laboratory, cross-platform, and cross-analytical comparisons between biospecimens used in the biomedical research and development enterprise, but also address a clear need for such standards.

Strategic Partnerships	
NIH Biospecimens Interest Group (BIG)	OBBR and the NIH Office of Rare Diseases Research co-chair a new NIH Biospecimens Interest Group which organizes seminars on various issues in biobanking and biospecimen science. These seminars are open to the public and live webcast.
Office of Advocacy Relations (OAR)	The OBBR works closely with the Office of Advocacy Relations to educate and receive input from the patient-advocacy community on key biobanking and biospecimen research issues.
Office of Government and Congressional Relations (OGCR)	The OBBR works closely with OGCR to monitor legislation impacting biobanking and biospecimen research. Additionally, through OGCR, the OBBR routinely receives requests to comment on proposed legislation and/or conduct educational visits with Capitol Hill staffers.
Standardization and Improvement of Generic Pre-analytical Tools and Procedures for In Vitro Diagnostics (SPIDIA)	OBBR, working with Fogarty International, is developing a Letter of Intent for the European Union-funded initiative SPIDIA to facilitate collaboration and research harmonization.
Trans-NIH Bioethics Committee Data and Specimen Committee (TNBC DSC)	The TNBC DSC includes representatives from all NIH Institutes and Centers with an interest in biospecimen or data repositories. OBBR staff members serve as the official NCI representatives to the Committee and provide regular updates on NCI and OBBR initiatives such as the caHUB and the NCI Best Practices to gather input and coordinate efforts across NIH. The TNBC DSC is currently in the process of finalizing NIH Guidelines related to the stewardship of biospecimens; these guidelines are being carefully coordinated with the most current version of the NCI Best Practices.

NIH Publication No. 11-7776
Printed May 2011