ADVANCES IN CANCER NANOTECHNOLOGY: NCI ALLIANCE 2005-2010

The NCI Office of Cancer Nanotechnology Research/
Center for Strategic Scientific Initiatives

National Cancer Institute/ NIH

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Foreword

This text provides an overview of National Cancer Institute (NCI) Alliance for Nanotechnology in Cancer's (ANC) efforts over the last five years. The ANC was launched on the premise that nanotechnology based materials and devices can strongly benefit cancer research and clinical oncology. They can also contribute to new solutions in molecular imaging and early detection, *in vivo* imaging, and multifunctional therapeutics for effective treatment.

We, the Office of Cancer Nanotechnology Research Program team, have been privileged to work with more than 400 researchers involved in the ANC. It has been an incredible and enriching experience, and all of us are grateful to have been a part of this astonishing effort.

As we end Phase I of the ANC and begin Phase II, we look forward to advancing the program further and watching new solutions derived from its work arriving in the clinical setting.

We are confident that these efforts will ultimately change the life of cancer patients.

Office of Cancer Nanotechnology Research/ Center for Strategic Scientific Initiatives
National Cancer Institute/ NIH

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CHAPTER 1

About the Alliance for Nanotechnology in Cancer

Cancer is arguably the most complex genetic disease known today. The incomplete understanding of its root cause, together with its unique ability to spread metastatically to other organs, makes diagnosis and subsequent treatment of the disease a daunting task. Cancer nanotechnology, a discipline at the intersection of engineering, physical sciences, cancer biology, and clinical practice, has the potential to radically influence disease diagnosis, treatment, and management. As a result, nanotechnology is likely to have a profound impact on the outcome of the disease. The discovery and development of new materials is central to innovation in nanotechnology. The unique and diverse properties of these nanomaterials will aid and benefit oncology applications by enabling selective drug delivery to tumors; increasing the therapeutic index of drugs; and enhancing imaging sensitivity that can enable early tumor detection, intraoperative guidance of tumor resection, and real-time monitoring of therapeutic response. Similarly, nanotechnology devices are capable of simultaneously recognizing and monitoring minute quantities of several biomarkers in *in vitro* or *in vivo* environments, enabling highly sensitive and specific diagnosis and therapeutic monitoring.

The National Cancer Institute (NCI) at the National Institutes of Health (NIH) recognized the value of nanotechnology in oncology, early. In the late nineties, the NCI established the Unconventional Innovative Program (UIP) to work with academic groups and small companies to evaluate the potential of using nanotechnology in cancer applications. Building upon the solid foundation of the UIP program, the NCI subsequently established the Alliance for Nanotechnology in Cancer (ANC) in September 2004 and pledged $144 million to the five year initiative to further promote the development of nanotechnology for cancer-related applications. The ANC's role is to serve as a national resource linking physical scientists, engineers, and technologists working at the nanoscale with cancer biologists and oncologists specializing in the diagnosis, prevention, and treatment of cancer. The ANC program and its infrastructure are specifically designed to rapidly advance new nanotechnology discoveries and transform them into cancer-relevant applications with potential clinical utility.

In Phase I (funding period 2005 to 2010), the ANC operated as an integrated constellation comprised of eight Centers for Cancer Nanotechnology Excellence (CCNEs) and twelve smaller Cancer Nanotechnology Platform Partnerships (CNPPs). Additionally, Multidisciplinary Research Training Fellowships and the Nanotechnology Characterization Laboratory (NCL) were established to play an integral role in the program. The CCNEs form the foundation of the ANC and focus on integrated nanotechnology solutions with practical clinical applications and pursue the aggressive development of these solutions to the pre-clinical stage and provide a path to clinical translation. The CNPP's pursue smaller and more focused nanotechnology projects. The Multidisciplinary Research Training Fellowships serve to increase the pool of young investigators who have the multidisciplinary training needed to drive the field of cancer nanotechnology forward over the coming years. The NCL is an intramural resource to perform standardized characterizations of nanoscale materials

developed by researchers from academia, government, and industry. The NCL operates under a three-way agreement among the NCI, the National Institute of Standards and Technology (NIST), and the U.S. Food and Drug Administration (FDA). The NCL has developed an assay cascade that serves as the standard protocol for physicochemical, preclinical toxicological, pharmacological, and efficacy testing of nanoscale materials and devices.

Scientific Focus

Over the past five years, the ANC has applied nanotechnology to accelerate the discovery and development of research tools, diagnostic tools, and therapeutic agents. Specifically, ANC research was focused on six themes for which nanotechnology was thought to have great potential for both short- and long-term impacts, including:

Molecular imaging and early detection of cancer: Novel nanotechnologies offer the promise to complement and augment existing genomic and proteomic techniques to analyze variations across different tumor types, thus offering the potential to distinguish between normal and malignant cells. Sensitive biosensors constructed of nanoscale components (e.g., nanocantilevers, nanowires, and nanochannels) can recognize genetic and molecular events and have reporting capabilities, thereby offering the potential to detect rare molecular signals associated with malignancy. Such signals may then be collected for analysis by nanoscale harvesters that selectively isolate cancer-related molecules from tissues. Another area with near-term potential for early detection is the identification of mutations and genomic instability *in situ*.

***In vivo* imaging:** A pressing need in clinical oncology is for imaging methods that can identify tumors that are orders of magnitude smaller than those detected with current technology. When utilized in conjunction with powerful contrast agents, and coupled with nanoparticles such as dendrimers, these methods can improve targeting capability and increase signal intensity, thereby enabling the detection of very small tumor masses and minimal residual disease. In the future, implantable nanoscale biomolecular sensors may enable clinicians to more carefully monitor the disease status of patients who have undergone treatment or individuals susceptible to cancer because of various risk factors. Furthermore, imaging agents that target changes such as angiogenesis that take place in the environment surrounding a tumor will further augment methodologies and will be invaluable for obtaining the optimal benefit from therapeutics that target such specific cancer-related processes.

Reporters of therapeutic efficacy: Nanotechnology offers the potential to develop highly sensitive imaging-based devices and *ex vivo* imaging tools that can determine whether a therapeutic agent is reaching its intended target and whether that agent is killing malignant or support cells. Optical imaging devices that use nanoscale agents may also enable surgeons to more readily detect the margins of a tumor prior to resection or to detect micrometastases in lymph nodes or tissues distant from the primary tumor. Other potential applications of nanotechnologies as reporters of efficacy include nanoparticle-based systems to detect apoptosis or reactivation of the critical tumor suppressor systems, as well as targeted nanoparticles that can bind to a tumor and be re-released into the bloodstream as tumor cells undergo apoptosis following therapy.

Multifunctional therapeutics: Because of their multifunctional capabilities, nanoscale devices can contain both targeting agents and therapeutic payloads. Multifunctional nanoscale devices also offer the opportunity to utilize new approaches to therapy, such as localized heating or reactive oxygen generation, and to combine a diagnostic or imaging agent with a therapeutic and/or a reporter of therapeutic efficacy. "Smart" nanotherapeutics may provide clinicians with the ability to time the release of an anticancer drug or deliver multiple drugs

sequentially in a timed manner or at several locations in the body, potentially ushering in an era of sustained therapy for cancers that must be treated chronically. Such nanotherapeutics could also house engineered cellular "factories" that make and secrete proteins and other antigrowth factors that impact a tumor and/or its microenvironment. Many nanotherapeutics, such as nanoparticulate hydrogels, nanoparticles, and quantum dots can also double as imaging agents.

Prevention and control of cancer: Many of the advances that nanotechnology will enable in each of the four preceding areas will also find widespread applicability in efforts to prevent and control cancer. Once specific biomarkers of cancer susceptibility and precancerous lesions are identified, nanotechnology can enable devices that signal their presence and deliver targeted therapy. Nanoscale devices may also prove valuable for delivering or mimicking polyepitope cancer vaccines that engage the immune system or cancer-preventing nutraceuticals or other chemopreventive agents in a sustained, timed-release, and targeted manner.

Research enablers: Nanotechnology offers a wide range of tools for the research community, including chip-based nanolabs capable of monitoring and manipulating individual cells and nanoscale probes that can track the movements of cells and individual molecules as they move about in their environments. Use of such tools is enabling cancer biologists to study, monitor, and alter the multiple systems implicated in the cancer processes and to identify key biochemical and genetic targets for future molecular therapies. As such, nanotechnology is complementing other technology platforms, such as proteomics and bioinformatics.

Another near-term application of nanotechnology to accelerate basic research is to use molecular-sized nanoparticles, such as quantum dots, that have a wide range of optical properties to track individual molecules or cells as they move through local environments, thereby monitoring multiplexed cellular and molecular events in real time. When combined with mouse models that reproduce the genetic, biochemical, and physiological properties of human cancers, these nanolabels will be useful for integrative, systems biology research. Finally, nanoscale devices that enable simultaneous biochemical measurements (*e.g.*, time, size, and dynamic events) on multiple cells, particularly those grown in such a way as to mimic tissue development *in vivo*, will open new dimensions to basic cancer research.

CHAPTER 2

Program Operation and Structure

The 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 CCNEs as pillars of the ANC, 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 CNPPs pursue circumscribed nanotechnology projects with transformative potential for basic and/or preclinical development.

Program Operation

The ANC is governed by the Coordinating and Governance Committee (CGC). CGC membership includes at least one member from each CCNE, an OCNR Program Scientist, and a public advocacy group representative. The CGC identifies new research opportunities, establishes scientific and translational priorities, and considers policy recommendations in the program. In 2005 and 2006, CGC co-chairs were Chad Mirkin, principal investigator of the Northwestern University CCNE, and Jonathan Simons, co-principal investigator of the Emory-Georgia Tech CCNE. Chairs rotated every 18 months; in June 2007, Sanjiv Sam Gambhir, principal investigator of the Stanford University CCNE became the chair. In 2009, Joseph DeSimone, principal investigator of the University of North Carolina CCNE took over co-chairmanship. Reflecting the intended dynamic nature of the ANC funded programs, the CGC meets three times per year to assess scientific progress, identify new research opportunities, establish priorities, consider policy recommendations, and discuss strategies for increasing collaboration among ANC investigators and both public and private sector investigators outside the ANC structure.

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 (IAC) that help develop commercialization strategies. The Principal Investigator (PI) meeting, held annually in autumn, is the main venue for ANC investigators to meet in person, exchange ideas and experiences, and develop new collaborations with fellow investigators. Furthermore, Program Staff promote and monitors inter-ANC collaborations among different centers. The NCI program management occurs in conjunction with the operation of the CGC.

The review of a CCNE operation occurs at multiple levels and is performed by several bodies. Typically, Steering Committees meet and review a Center's operation annually. Similarly, NCI Program Staff conduct site visits to each CCNE on an annual basis. The site visit is a combination of technical update presentations, meetings with faculty and students, and laboratory tours. The visits are usually held in spring – March and April of each year.

Each Center also submits two progress reports per year; these reports detail scientific progress and provide an update on translational efforts

ongoing at each Center. The reports also provide updates on leveraged funding, publications, patents, and collaborations, which are detailed in the next section of this report.

Office of Cancer Nanotechnology Research

In addition to external governance, the ANC operations are managed by the NCI Office of Cancer Nanotechnology Research (OCNR), led by Program Director, Dr. Piotr Grodzinski and assisted by current staff members (Drs. Dorothy Farrell, Sara Hook, Nicolas Panaro and Krzysztof Ptak) and past (Travis Earles, and Drs. Jerry Lee, Linda Molnar, and Larry Nagahara) who have developed several tools and have coordinated numerous activities to help facilitate progress of ANC projects and promote inter-ANC collaborations. These include topic-oriented working groups, informatics, annual meetings, communications and outreach activities, all of which are discussed further in the following sections.

Program Infrastructure

During the last five years, the ANC funded a constellation of eight CCNEs and twelve CNPPs, together with Multidisciplinary Research Training and Team Development awards (eleven awardees) and the NCL. A description of each Award is provided below.

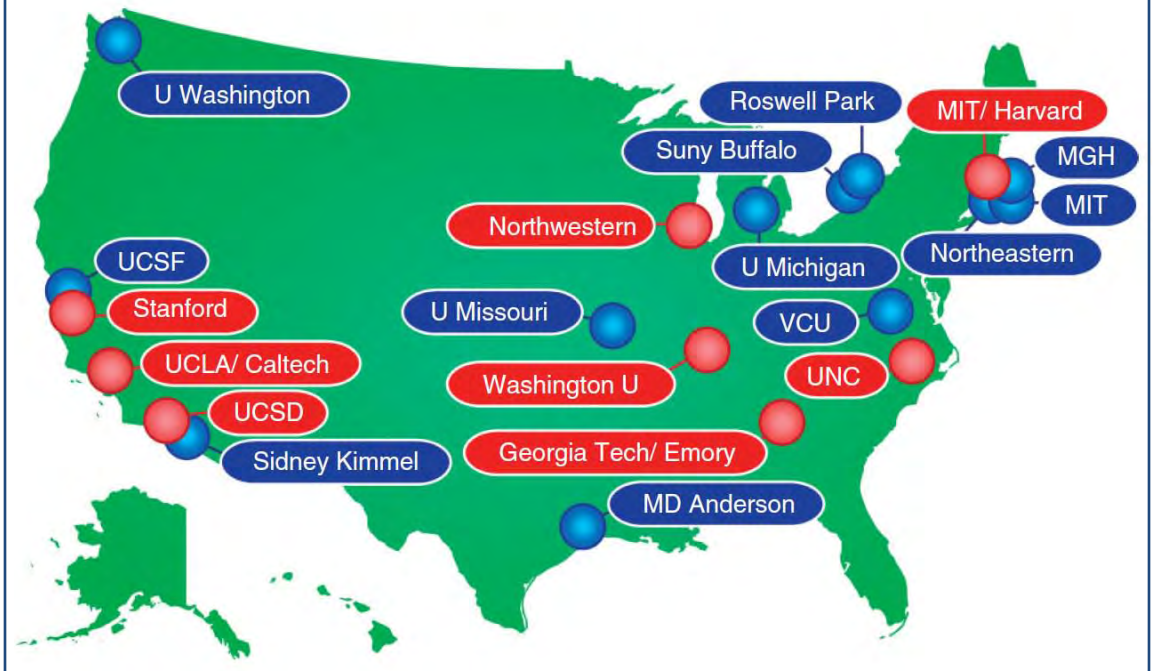

Figure 1: Map of the NCI Alliance for Nanotechnology in Cancer projects funded between 2005 and 2010. CCNE are in red, CNPP are in blue.

Centers of Cancer Nanotechnology Excellence

The primary goal of the CCNEs, which are funded through the U54 grant mechanism, is to integrate nanotechnology development into basic and applied cancer research and oncology and develop novel techniques that can ultimately lead to practical clinical solutions. Each Center is affiliated with an NCI Cancer Center and engages engineering and physical science departments of the university. Highlighted below are the ANC Phase I (2005-2010) CCNE awards (in alphabetical order) and their Centers' areas of focus:

Carolina Center of Cancer Nanotechnology Excellence

University of North Carolina, Chapel Hill, North Carolina

Principal Investigators: Rudolph Juliano, Ph.D., and Joseph DeSimone, Ph.D. (both from the University of North Carolina).

This Center, based on collaboration between the University of North Carolina and the Lineberger Comprehensive Cancer Center, was to design and fabricate innovative, multifunctional nanodevices and test their *in vivo* performance using sophisticated mouse models of human cancer. In addition, this Center was to use its breakthrough nanodevice fabrication technologies to develop nanoscale tools for research and detection applications.

This CCNE focused on:

- Smart nanoparticles for cancer therapy and imaging
- Carbon nanotube x-ray devices for *in vivo* cancer detection and treatment
- Targeted magnetic nanoparticles for brain tumor imaging and therapy
- Chemically patterned nanoscale surfaces for capturing tumor cells
- Nanofluidic devices for rapid, single-cell analysis of tumor cell signaling and migration

Center for Cancer Nanotechnology Excellence Focused on Therapy Response

Stanford University, Palo Alto, California

Principal Investigator: Sanjiv Sam Gambhir, M.D., Ph.D. (Stanford University).

This Center brought together scientists and physicians from Stanford University, the University of California Los Angeles (UCLA), Cedars Sinai Medical Center, the Fred Hutchinson Cancer Center, and the University of Texas at Austin. This Center's goal was to develop nanotechnology-enabled *ex vivo* and *in vivo* diagnostic tools that could be used in conjunction to advance both cancer detection and disease management.

The projects of this CCNE included:

- Magneto-nanotechnology and nanotube/ nanowire based technologies for *ex vivo* protein detection
- Biologically targeted quantum dots for molecular imaging of living subjects
- Use of mouse models for integrating *ex vivo* tissue/serum protein patterns and *in vivo* molecular imaging to predict response to anticancer therapy

Center of Nanotechnology for Treatment, Understanding, and Monitoring of Cancer

University of California, San Diego, California

Principal Investigator: Sadik Esener, Ph.D. (University of California San Diego).

This Center was a collaborative effort involving the University of California San Diego (UCSD), the Moores UCSD Cancer Center, the University of California Santa Barbara, the University of California Riverside, the Burnham Institute, and market research organization NanoBioNexus. The focus of this Center was to develop smart multifunctional nanoplatforms capable of targeting tumors and delivering large payloads of therapeutics and nanosensors to the tumor environment.

Specific projects of this CCNE included development of:

- Tumor-homing peptide-nanoparticle complexes

- Porous nanoparticles for drug and sensor delivery
- Computational methods for monitoring tumor progression and response using data from nanoparticle-delivered sensors
- Enzyme-sensitive nanoparticle coatings to increase the tumor-targeting capabilities of smart nanoparticle platforms

Emory-Georgia Tech Nanotechnology Center for Personalized and Predictive Oncology

Emory University & Georgia Institute of Technology, Atlanta, Georgia

Principal Investigator: Shuming Nie, Ph.D. (Emory University and Georgia Institute of Technology).

This collaboration between Emory University's Winship Cancer Institute, the Georgia Institute of Technology, and Nanoplex Technologies was focused on the development of bioconjugated nanoparticles for cancer molecular imaging, molecular profiling, and personalized cancer therapy. This CCNE, together with partners at the American Cancer Society and the U.S. Centers for Disease Control and Prevention (CDC) and with additional funding from the Georgia Research Alliance and Georgia Cancer Coalition, tackled projects that included the development of:

- Tumor-targeted infrared quantum dots with both optical and magnetic imaging capabilities
- Smart nanoparticle probes for intracellular drug delivery and gene expression imaging
- Antibody-conjugated quantum dots to detect and quantify human breast cancer biomarkers
- Nanoparticle tags for tracking multiple biomarkers in biological specimens using surface-enhanced Raman spectroscopy
- Nanoparticles for delivering therapeutics directly to bone metastases

MIT-Harvard Center of Cancer Nanotechnology Excellence

MIT & Harvard Cambridge, Massachusetts

Principal Investigators**:** Robert Langer, Ph.D. (MIT) and Ralph Weissleder, M.D., Ph.D. (Harvard and the Massachusetts General Hospital).

This CCNE involved collaboration among investigators from MIT, Harvard University, Harvard Medical School, Massachusetts General Hospital, and the Brigham and Women's Hospital. It was focused on developing a diversified portfolio of nanoscale devices for targeted delivery of cancer therapies, diagnostics, non-invasive imaging, and molecular sensing. In addition, this CCNE conducted toxicology and *in vivo* testing using its partner institutions' extensive collection of mouse models of cancer.

Examples of projects that this CCNE undertook included the development of:

- Targeted nanoparticles for treating prostate cancer
- Polymer nanoparticles and quantum dots for siRNA delivery
- Next-generation magnetic nanoparticles for multimodal, non-invasive tumor imaging
- Implantable, biodegradable microelectromechanical systems (MEMS), also known as lab-on-a-chip devices, for *in vivo* molecular sensing of tumor-associated biomolecules
- Low-toxicity nanocrystal quantum dots for biomedical sensing

Nanomaterials for Cancer Diagnostics and Therapeutics

Northwestern University, Evanston, Illinois

Principal Investigator: Chad Mirkin, Ph.D. (Northwestern University).

This CCNE consisted of a collaboration of investigators from Northwestern University's International Institute for Nanotechnology, the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, the University of Chicago, the University of Illinois at Urbana-Champaign, and Yonsei University in South Korea. Investigators from these institutions developed a range of nanotechnology-enabled tools for translation into the clinic.

This CCNE focused on the development of:

- Bio-barcodes to detect ovarian cancer and prostate markers
- A new class of drugs to inhibit or reduce metastasis
- Bioactivated nanoprobes for molecular imaging of cancer
- Targeted, multifunctional nanoparticles for drug and radiopharmaceutical delivery
- Nanocomposites for imaging prostate cancer cells and treatment of advanced prostate cancer
- Self-assembling supramolecular nanostructures that deliver chemotherapy agents directly to breast and other cancer tumors

Nanosystems Biology Cancer Center

California Institute of Technology, Pasadena, California

Principal Investigator: James Heath, Ph.D. (California Institute of Technology).

This CCNE established a collaborative team comprising investigators from the California Institute of Technology (Caltech), the Institute for Systems Biology, UCLA's Geffen School of Medicine, and the Jonsson Comprehensive Cancer Center. The focus of this effort was to develop and validate tools for the early detection and stratification of cancer through rapid and quantitative measurements of panels of serum and tissue-based biomarkers, and then to use those tools to evaluate the efficacy of various cancer therapies.

This CCNE conducted research on:

- Molecular imaging and targeting probes using "click" chemistry
- Integrated Nanoelectronics/microfluidics chips for multi-parameter diagnostic and measurement tools capable of detecting and quantifying trace biomolecules involved in cancer
- Chip-based tools for isolating rare circulating immune system cells as a means of evaluating the efficacy of immune-based cancer therapies
- Identification of organ-specific serum-based biomarkers for the detection and stratification of various cancers through blood analysis
- Methods for manufacturing low-cost nanofluidic diagnostic chip-based devices

The Washington University Center of Cancer Nanotechnology Excellence

Washington University, St. Louis, Missouri

Principal Investigator: Samuel Wickline, M.D. (Washington University in St. Louis).

This collaboration between Washington University in St. Louis, the University of Illinois at Urbana-Champaign, and the Alvin Washington University Cancer Center concentrated on developing nanoparticles for *in vivo* imaging and drug delivery as well as new imaging tools for characterizing the interactions of nanoscale materials with living cells.

Projects conducted by this Center's collaborators included the development of:

- Magnetic nanoparticles that can target multiple tumors for early detection and therapy of cancer
- Nanoparticle-based contrast agent for ultrasound imaging and therapy of tumors
- Bioinformatics tools to create a database for modeling the behavior of targeted nanoparticles in the body
- Novel nanoscale sensors for rapidly screening potential anticancer drugs in single cells

Cancer Nanotechnology Platform Partnerships

CNPPs were awarded through the R01 mechanism to 12 individual investigators. The CNPPs pursue circumscribed nanotechnology projects with transformative potential for basic and/or preclinical development.

An overview of the Phase I CNPP Awards is given below:

Nanotherapeutic Strategy for Multidrug Resistant Tumors

Northeastern University, Boston, Massachusetts

Principal Investigator: Mansoor Amiji, Ph.D.

Dr. Amiji and his collaborators developed and tested *in vivo* a nanoparticle-based strategy to overcome multiple drug resistance (MDR) that relies on a multifunctional construct to optimize delivery of proapoptotic drugs to the tumor mass and increase the intracellular concentrations of those drugs. At the same time, the multifunctional nanoparticle helps reverse cellular resistance to the proapoptotic drug by modulating levels of intracellular ceramide, a molecule normally involved in triggering apoptosis but whose activity is suppressed in malignant cells.

The goals of this CNPP were to:

- Develop, characterize, and optimize long-circulating, biodegradable polymeric nanocarriers with encapsulated paclitaxel, ceramide, and tamoxifen, either alone or in combination
- Evaluate the uptake, distribution, cytotoxicity; intracellular concentrations; and apoptotic activity of paclitaxel, ceramide, and tamoxifen in cultures of sensitive and resistant tumor cells
- Examine the biodistribution and pharmacokinetic profiles of drugs administered in the control and nanocarrier formulations in sensitive and resistant xenograft tumor models established in nude mice
- Determine the antitumor efficacy of single and combination therapy in PENs in sensitive and resistant xenograft models
- Use mathematical modeling to improve the design of nanocarriers for tumor-targeted delivery of single and combination drug therapy

DNA-linked Dendrimer Nanoparticle Systems for Cancer Diagnosis and Treatment

University of Michigan, Ann Arbor, Michigan

Principal Investigator: James Baker, Jr., M.D.

Dr. Baker's team developed a set of multifunctional nanoparticles by creating single-function dendrimer nanoparticles that can be hooked together by a chemical linker. This approach allows targeting, imaging, and therapeutic dendrimers to be combined into multifunctional therapeutics simply by heating mixtures of these agents to a temperature to activate the linkage.

The goals of this CNPP were to:

- Design and synthesize linkers that can be used to form multifunctional nanoparticles from single-function dendrimers
- Characterize the self-assembled nanodevices using a variety of physiochemical techniques
- Test the linked nanodevices for binding and internalization *in vitro*
- Employ animal models to assess the effectiveness of the dendrimer-linked therapeutics to treat tumors *in vivo*

Metallofullerene Nanoplatform for Imaging and Treating Infiltrative Tumor

Virginia Commonwealth University, Richmond, Virginia

Principal Investigator: Panos Fatouros, Ph.D.

Dr. Fatouros and his team developed techniques for creating water-soluble, targeted metallofullerene nanoparticles for use in imaging and treating glioblastomas. The versatility of their synthetic methods allowed the investigators to construct nanoparticles with a variety of metals that can serve as magnetic resonance imaging probes, fluorescent labels for interoperative imaging, or radiotherapy agents.

The goals of this CNPP were to:

- Develop, modify, and characterize metallofullerenes as a nanotechnology platform capable of greatly improving brain tumor imaging
- Deliver fluorescent labeling and radiation therapy to tumor cells

Detecting Cancer Early With Targeted Nanoprobes for Vascular Signatures

University of California, San Francisco, California

Principal Investigators: Douglas Hanahan, Ph.D., Henry VanBrocklin, Ph.D., and Erkki Ruoslahti, M.D., Ph.D.

This project developed targeted nanoprobes for molecular imaging to enable noninvasive, early detection of incipient cancer and yield substantive improvements in sensitivity and selectivity. It brought together three research groups with complementary expertise in angiogenesis and mouse models of cancer, vascular profiling, and in clinical and experimental molecular imaging. The collaborators in this CNPP discovered peptides that bind specifically to angiogenic blood vessels of high-grade tumors and invasive carcinomas. The researchers found that these peptides can distinguish cancerous lesions from normal tissues. Subsequently, the investigators created a novel synthetic peptide that not only targets tumor vasculature, but also promotes rapid diffusion of nanoparticles deep into tumors.

The goals of this CNPP were to:

- Develop imaging nanoprobes for detecting the blood and lymphatic neo-vasculature
- Discover and characterize a repertoire of new signature-finding peptides for blood and lymphatic vasculature of cervical and pancreatic ductal cancerous and precancerous lesions

Photodestruction of Ovarian Cancer: ErbB3 Targeted Aptamer-Nanoparticle Conjugate

Massachusetts General Hospital, Boston, Massachusetts

Principal Investigator: Tayyaba Hasan, Ph.D.

Dr. Hasan and her collaborators developed aptamer-targeted nanoparticles for delivering photosensitizers to tumors to enable light-activated therapy for ovarian tumors. The investigators created an aptamer that targets ErbB3, a receptor that is overexpressed by ovarian tumors and demonstrated using mouse models of human ovarian cancer that photosensitizer-loaded nanoparticles that target this tumor protein can deliver therapeutic levels of the photosensitizer to tumors. Light activation then destroys the tumors.

The goals of this CNPP were to:

- Synthesize and stabilize photosensitizer nanoparticle aptamer conjugates
- Demonstrate the selective phototoxicity of photosensitizer nanoparticle aptamer conjugates
- Develop optical imaging techniques with quantum dots to serve as reporters of therapeutic efficacy and of dosimetry parameters

Hybrid Nanoparticles in Imaging and Therapy of Prostate Cancer

University of Missouri, Columbia, Missouri

Principal Investigator: Kattesh Katti, Ph.D.

The project headed by Dr. Katti was the culmination of longstanding interdisciplinary partnerships in departments within the School of Medicine, the College of Arts and Sciences, the College of Veterinary Medicine, the Ellis Fischel Cancer Center, the Missouri University Research Reactor, and the College of Engineering at the University of Missouri, Columbia. Dr. Katti and his collaborators developed hybrid gold nanoparticle-based molecular imaging agents and targeted therapeutic agents for use in diagnosing and treating prostate cancer. This team also developed targeted nanoparticles that could be imaged with very high sensitivity using photoacoustic detection techniques.

The goals of this CNPP were to:

- Synthesize a library of gold nanoparticles (AuNPs) for conjugation with prostate tumor-specific bombesin peptides and investigate the photophysical properties, dispersity, and size of these gold nanoparticles
- Investigate biolocalization, pharmacokinetics, and *in vivo* profiles of AuNPs stabilized with starch, agarose, and arabinogalactan protein (gum arabic) in

pigs, and optimize the necessary analytical protocols
- Develop new theoretical models, computations, and simulations for the interaction of AuNPs with cells
- Investigate the utility of AuNPs and bombesin-conjugated hybrid AuNPs as image enhancers in computer tomographic (CT) and ultrasound imaging of prostate tumors in mouse models of cancer
- Optimize production of gamma-emitting nanoparticulate Au-198/199 and develop tumor-specific Au-198/199-nanoparticle-labeled bombesin peptides for prostate tumor therapy

Near-Infrared Fluorescence Nanoparticles for Targeted Optical Imaging,

University of Texas M.D. Anderson Cancer Center, Houston, Texas

Principal Investigator: Chun Li, Ph.D.

Dr. Li's team worked with investigators from the Eastman Kodak Company to develop novel nanoparticles for optical molecular imaging applications. Dr. Li's group fabricated dye-loaded nanoparticles and used a variety of targeting agents to create tumor-targeted near-infrared imaging agents.

The goals of this CNPP were to:

- Synthesize and characterize polymer-coated silica nanoparticles and cross-linked polyethylene glycol (PEG) nanoparticles suitable for near infrared fluorescent (NIRF) imaging
- Establish the effect of particle characteristics on the pharmacokinetics, biodistribution, clearance, extravasation, and intratumoral distribution of NIRF nanoparticles
- Establish the stability and signal intensity of NIRF nanoparticles *in vivo* and the specificity of their retention in tumors
- Construct NIRF nanoparticles targeted to angiogenic blood vessels and to tumor cell-associated surface receptors
- Develop smart, activatable NIRF nanoparticles and combine homing ligand and molecular beacon designs in a single nanoparticulate system

Integrated System for Cancer Biomarker Detection

Massachusetts Institute of Technology, Cambridge, Massachusetts

Principal Investigator: Scott Manalis, Ph.D.

Dr. Manalis' group developed a general approach for improving the performance of immunoassays that uses a nano/microfluidic device capable of preconcentrating protein biomarkers in serum in combination with a nanoscale cantilever that can make single molecule mass measurements. Dr. Manalis' team created their devices so that they can be made using conventional microprocessor techniques in order to enable widespread and low-cost distribution for point-of-care applications.

The goals of this CNPP were to:

- Integrate the nanofluidic concentrator with on-chip detection, demonstrate closed-loop concentration/detection, and validate with prostate specific antigen (PSA)
- Implement parallel detection for multiple biomarkers

Novel Cancer Nanotechnology Platforms for Photodynamic Therapy and Imaging

Roswell Park Cancer Institute, Buffalo, New York

Principal Investigator: Ravindra K. Pandey, Ph.D.

Dr. Pandey's team, comprising investigators from the Roswell Park Cancer Institute; the Institute for Lasers, Photonics, and Biophotonics at the State University of New York at Buffalo; and the Department of Chemistry at the University of Michigan, developed, characterized, and validated in preclinical studies a multifunctional nanoparticle platform that delivers tumor-avid, therapeutic photosensitizers that are active and toxic only when illuminated by light. The nanoparticles that this group developed also carry a payload of one or more imaging agents, enabling both multimodal diagnosis and image-guided therapy.

The goals of this CNPP were to:

- Prepare multifunctional organically modified silica and polymeric nanovectors capable of both tumor therapy and imaging that contain tumor-avid photosensitizing molecules (PSs) with or without additional targeting moieties, as well as optical, PET, and/or MRI imaging agents
- Characterize the different nanovectors in solution and *in vitro* and iteratively utilize the data to select and optimize formulations
- Examine selected nanovectors in animal tumor systems and iteratively apply the information to further refine the platform development

Multifunctional Nanoparticles in Diagnosis and Therapy of Pancreatic Cancer

State University of New York, Buffalo, New York

Principal Investigator: Paras Prasad, Ph.D.

Dr. Prasad's group developed multifunctional hybrid ceramic-polymeric nanoparticles, specifically indium phosphide quantum dots (InP Q-DOTS) and organically modified silica (Ormosil) nanoparticles, for comprehensive preclinical evaluation in pancreatic cancer models. This group has demonstrated that a multifunctional nanoparticle containing quantum dots and the anticancer agent doxorubicin is capable of imaging tumors and delivering their therapeutic levels of drug over the course of six days.

The goals of this CNPP were to:

- Synthesize long-circulating (PEGylated), surface-functionalized quantum dots and dye-doped Ormosil nanoparticles incorporating PET probes for improved imaging of early and metastatic pancreatic cancer *in vivo*
- Synthesize long-circulating, surface-functionalized Ormosil nanoparticles encapsulating the small-molecule inhibitor rapamycin (nanorapamycin) for systemic drug delivery to pancreatic cancer

Nanotechnology Platform for Targeting Solid Tumors

The Sidney Kimmel Cancer Center, San Diego, California

Principal Investigator: Jan Schnitzer, M.D.

Dr. Schnitzer and his collaborators used a systems biology approach coupled with nanotechnology-based tissue fractionation and subfractionation proteomics to enable the rapid identification of and validation of new cancer targets. They then used these targets to home nanoparticles to solid tumors *in vivo*. In particular, this group developed agents that target the caveolae on tumor surfaces and enhance tissue/tumor penetration by facilitating transport across the endothelium for direct access to underlying tissue tumor cells.

The goals of this CNPP were to:

- Generate and characterize various new nanoparticles that specifically bind select lung- and tumor-induced endothelial cell surface proteins in caveolae
- Define cell-surface dynamics and intracellular trafficking pathways of nanoparticles, specifically targeting caveolae in endothelial cells grown in culture
- Investigate tissue/tumor targeting and endothelial cell processing of antibody-conjugated nanoparticles *in vivo* after intravenous administration
- Test the ability of tumor-targeting nanoparticles to deliver drugs specifically in rat tumor models by assessing their bioefficacy *in vivo*

Nanotechnology Platform for Pediatric Brain Cancer Imaging and Therapy

University of Washington, Seattle, Washington

Principal Investigator: Miqin Zhang, Ph.D.

Dr. Zhang's team focused on developing a tumor-targeting dual magnetic resonance/optical nanoparticulate contrast agent that will enable presurgical planning and intraoperative delineation of tumor margins and then deliver therapeutic agent to brain tumors. In addition, Dr. Zhang's group demonstrated

that linking nanoparticles to chlorotoxin, a component of scorpion venom, is capable of providing specific targeting of the nanoparticles to brain tumors and enhancing uptake of the nanoparticles by the targeted malignancies.

The goal of this CNPP was to:

- Develop a tumor-targeting dual magnetic resonance/optical nanoparticulate contrast agent that will enable presurgical planning and intraoperative delineation of tumor margins
- Evaluate pharmacokinetics, the serum half-life, the biodistribution, and toxicity of developed nanomaterials
- Determine the feasibility of scaling up nanoparticles synthesis and purification to generate Good Manufacturing Process (GMP)-grade nanoparticles
- Develop a tumor-targeted dual magnetic resonance/optical nanoparticulate imaging agent carrying a chemotherapeutic payload. Following synthesis of the multifunctional nanoparticles, targeting specificity, contrast dose, timing of scanning, image contrast, and therapeutic efficacy will be optimized progressively in *in vitro* and then *in vivo* flank xenograft and spontaneous intracranial mouse models of pediatric brain cancer.

Multidisciplinary Research Training and Team Development

Multidisciplinary Research Training and Team Development fellowship awards were granted to postdoctoral trainees for multidisciplinary training. The awards, in alphabetical order by trainee, were granted to:

- **Nanoparticle-Bioconjugates as Cancer-Treating Agents,** Texas A&M University, College Station, Texas**.** Trainee: Sofi Bin-Salamon, Ph.D.
- **Nanoscale Mechanisms of Hsp90 and Its Co-chaperones,** Yale University, New Haven, Connecticut**.** Trainee: Ivo P. Doudevski, Ph.D.
- **Targeted Delivery Via Protein-Carbohydrate Interactions,** Liquidia, Inc., Research Triangle Park, North Carolina. Trainee: Ashley L. Galloway, Ph.D.
- **Liposomal Delivery of High LET Emitters to Cell Nuclei,** Johns Hopkins University, Baltimore, Maryland. Trainee: Yah-El Har-El, Ph.D.
- **Geldanamycin-Mediated Uptake of Nanoparticle Probes,** Purdue University, West Lafayette, Indiana. Trainee: Giselle M. Knudsen, Ph.D.
- **Nanolabels of Active Proteases for Cancer Detection,** University of California, San Francisco, California. Trainee: Mark D. Lim, Ph.D.
- **Single Walled Carbon Nanotube Based Tumor Vaccines,** Memorial Sloan-Kettering Institute for Cancer Research, New York, New York. Trainee: Rena J. May, Ph.D.
- **Short-Interfering RNA-Gold Nanoparticle Bioconjugates: A New Cancer Therapy,** Northwestern University, Evanston, Illinois. Trainee: Adam B. Braunschweig, Ph.D.
- **Design of Affinity Capture Agents for Akt1 Using *in situ* Click Chemistry,** California Institute of Technology, Pasadena, CA. Trainee: Steven W. Millward, Ph.D.
- **Targeted Photoactivated Nanoparticles for the Treatment of Ovarian Cancer,** Massachusetts General Hospital, Boston, MA. Trainee: Daniel Neuman, Ph.D.
- **Nanoprobes and Integrated Nanodevices for Cancer Detection and Treatment,** University of Colorado Health Services, Superior, CO. Trainee: Wounjhang Park, Ph.D.

The NCI also collaborated with the National Science Foundation (NSF) to fund four **Integrative Training and Team Development Awards** for U.S. science and engineering doctoral students to focus on interdisciplinary nanoscience and technology training programs. The funded programs were:

- **Integrative Nanoscience and Microsystems, University of New Mexico,** Albuquerque, New Mexico, a collaboration between the University of New Mexico's Center for High Technology Materials within the School of Engineering, College of Arts and Sciences, and Cancer Research and Treatment Center. Principal Investigator: Diana Huffaker, Ph.D.
- **NanoPharmaceutical Engineering and Science, Rutgers University,** New Brunswick, New Jersey, a collaboration between Rutgers, New Jersey Institute of Technology, and University of Puerto Rico. Principal Investigator: Fernando Muzzio, Ph.D.
- **Nanomedical Science and Technology, Northeastern University,** Boston, Massachusetts, a collaboration between the Dana-Farber Cancer Institute and Massachusetts General Hospital. Principal Investigator: Srinivas Sridhar, Ph.D.
- **Building Leadership for the Nanotechnology Workforce of Tomorrow,** University of Washington, Seattle, Washington. This joint institute for nanotechnology involves the University of Washington, the Pacific Northwest National Laboratory, and the Fred Hutchinson Cancer Research Center. Principal Investigator: Marjorie Olmstead, Ph.D.

Nanotechnology Characterization Laboratory

The NCL, the only intramural component of the ANC, operates at the NCI's Frederick, MD research facility and performs and standardizes the preclinical characterization of nanomaterials developed by researchers from academia, government, and industry. The NCL serves as a national resource and knowledge base for cancer researchers and will facilitate accelerated regulatory review and translation of nanomaterials and devices into the clinical realm. The NCL works in concert with NIST and the FDA. Additional details on the role the NCL plays in translation of nanotechnologies toward the clinic can be found on page 55 of this report.

CHAPTER 3

Scientific Achievements

The initial experiences in establishing this program in 2005 were challenging yet educational as common goals and processes were being established. Each CCNE consists of approximately 40 researchers - senior academics, young faculty, post-doctoral fellows, and students - representing a variety of disciplines. These researchers have established a common language and set of goals and demonstrated that research and development performed in such multidisciplinary environments can be highly productive and creative. A steady flow of innovation has opened new opportunities to deepen understanding in cancer biology and to enable novel clinical applications. Several principal scientists within the program come from disciplines that are not usually part of 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 have subsequently directed their research toward the most relevant and pressing oncology issues. They have also introduced new research approaches, with a focus on developing platform technologies. The CCNEs have evolved into research organisms having distinct areas of technical excellence and core resources (*e.g.*, fabrication and materials development, diagnostic assays, toxicology, *in vivo* technology validation, informatics).

To date, the ANC has generated very strong scientific output, including over 1250 peer-reviewed publications. The average impact factor of these publications was 7.4. Without doubt, this prolific output has helped in establishing the field of cancer nanotechnology.

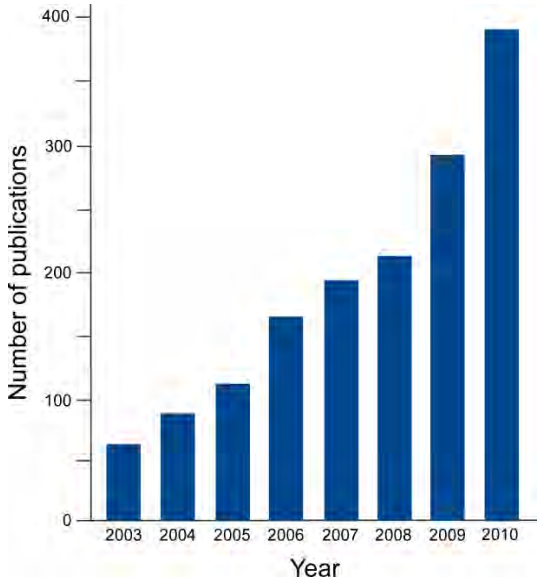

Figure 2: Research Articles in Cancer Nanotechnology from 2002 to 2010. The information was obtained from MEDLINE/ PUBMED indexed articles using U.S. National Library of Medicine's Medical Subject Headings (MESH) terminology related to "cancer" and "nanotechnology" in title or abstract.

Recognition Awards

ANC investigators are prominent scientists who have won many accolades and are members of prestigious societies and academies. The list below highlights some awards that ANC investigators have received:

Nobel Prize – the annual international awards bestowed by Scandinavian committees in

recognition of scientific advances – was given to five investigators who are participating in the ANC Program. In 1975, Dr. David Baltimore (Caltech CCNE) shared the Nobel Prize in Medicine for his or their discoveries concerning the interaction between tumor viruses and the genetic material of the cell. In 1993, Dr. Philip Sharp (MIT-Harvard CCNE) received the Nobel Prize in Medicine for his discoveries of split genes. Dr. Robert Grubbs (Caltech CCNE) shared the 2005 Nobel Prize in Chemistry for the development of the metathesis method in organic synthesis. Dr. Roger Tsien from UCSD CCNE shared the 2007 Nobel Prize in Chemistry for his contribution in discovering and development of the green fluorescent protein (GFP). In 2010, Dr. Jack Szostak (MIT-Harvard CCNE) along with two other scientists was awarded the Nobel Prize in Medicine for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase.

Lasker Foundation Awards recognize the contributions of scientists, physicians, and public servants who have made major advances in the understanding, diagnosis, treatment, cure, or prevention of human disease. Three ANC researchers are recipients of this recognition award. In 1987, Dr. Leroy Hood (Caltech CCNE) was recognized for his prolific and imaginative studies of somatic recombination in the immune system, detailing in molecular terms the genetics of antibody diversity. In 1988 Dr. Philip Sharp (MIT-Harvard CCNE) received a Lasker Award for his series of revelations regarding the ability of RNA processing to convert DNA's massive store of genetic data to biological. And in 2006, Jack Szostak (MIT-Harvard CCNE) was honored for the prediction and discovery of telomerase, an RNA-containing enzyme that synthesizes the ends of chromosomes, protecting them and maintaining the integrity of the genome.

Millennium Technology Award is given by the Technology Academy of Finland to tribute life-enhancing technological innovations. The prize has been established to steer the course of technological development to a more humane direction. In 2008, Dr. Robert Langer (MIT-Harvard CCNE) became the laureate of this award for discovering and developing many advanced drug delivery systems that have had a significant impact on fighting cancer, heart disease, mental health illnesses and numerous other diseases.

US National Medal of Science is an honor bestowed by the President of the United States to individuals in science and engineering who have made important contributions to the advancement of knowledge. The Medal has been given to: Dr. David Baltimore in 1999 (Caltech CCNE); Dr. Mostafa El-Sayed in 2007 (Emory-Georgia Tech CCNE); and Dr. Robert Langer and Dr. Phil Sharp, both in 2007 (MIT-Harvard CCNE)

NIH Pioneer Awards are designed to support individual scientists of exceptional creativity who propose pioneering and possibly transforming approaches to major challenges in biomedical and behavioral research. A few ANC investigators have received NIH Pioneer Awards, including Drs. Joseph DeSimone (UNC CCNE) and Chad Mirkin (Northwestern CCNE).

Kyoto Prize in Advanced Technology is a recognition award given by the Inamori Foundation. It recognizes outstanding works in the fields of philosophy, arts, science, and technology. In 2002, Dr. Leroy Hood from Caltech CCNE became a laureate of the Kyoto Prize for his contributions to life sciences through the automation of protein and DNA sequencing and synthesis.

The **Japan Prize** is awarded to honor the achievements of people throughout the world, who have contributed to the progress of science and technology and the advancement of world peace and prosperity. In 2005, Dr. Erkki Ruoslahti (UCSD CCNE) shared this award for his contribution in elucidating the molecular mechanisms of cell adhesion.

Lemelson-MIT Prize honors outstanding mid-career inventors dedicated to improving our world through technological invention and innovation. Four ANC investigators received this award: Dr. Robert Langer in 1998(MIT-Harvard CCNE), Dr. Leroy Hood in 2003 (Caltech CCNE), Dr. Joseph DeSimone in 2008 (UNC CCNE), and Dr. Chad Mirkin in 2009 (Northwestern CCNE).

Foresight Nanotech Institute Feynman Prize in Nanotechnology is an award for excellence in theory or experiment to the researchers whose

recent work has most advanced the state of molecular manufacturing, defined as the construction of atomically-precise products through the use of molecular machine systems. The award has been given to Drs. James Heath (Caltech CCNE), Chad Mirkin, and Fraser Stoddart (both from the Northwestern CCNE).

Canada Gairdner International Award honors outstanding biomedical scientists who have made original contributions to medicine with the ultimate goal of contributing through research to the conquest of disease and relief of human suffering. Several ANC investigators are recipients of the award: Dr. David Baltimore in 1974 (Caltech CCNE), Dr. Philip Sharp in 1986 (MIT-Harvard CCNE), Roger Tsien in 1995 (UCSD CCNE), Dr. Robert Langer in 1996 (MIT-Harvard CCNE), Dr. Erkki Ruoslahti in 1997 (UCSD CCNE).

In addition to recognition awards, some ANC researchers have been selected to be members of prominent scientific and engineering societies, or have become members of the US President's Council of Advisors on Science and Technology. It includes elected members of:

National Academy of Sciences (Drs. David Baltimore, Moungi Bawendi, Mark Davis, Douglas Hanahan, Leroy Hood, Robert Langer, Chad Mirkin, Michael Phelps, Erkki Ruoslahti, Philip Sharp, Roger Tsien, Richard Van Duyne, Owen Witte)

Institute of Medicine of the National Academies (Drs. David Baltimore, Sanjiv Sam Gambhir, Robert Langer, Chad Mirkin, Michael Phelps, Erkki Ruoslahti, Philip Sharp and Owen Witte)

National Academy of Engineering (Dr. Mark Davis, Joseph DeSimone, Robert Langer, and Chad Mirkin)

Two ANC investigators, Drs. Robert Langer and Chad Mirkin are members of all three Academies.

The US President's Council of Advisors on Science and Technology (Drs. James Baker Jr. and Chad Mirkin)

Scientific Highlights

The ANC has demonstrated that a multidisciplinary approach to research can catalyze scientific developments and contribute to successful clinical translation efforts. With nearly 1250 peer-reviewed scientific papers published during the last five years, the ANC advanced diagnostic technology, using both *in vitro* assays and novel imaging methods, and offered improved therapies and therapeutic efficacy measures.

The following section discusses a few exemplary scientific advances in the six major challenge areas described on 4 that were achieved by ANC investigators. For additional highlights, please see the News and Highlights section at the ANC website: nano.cancer.gov/action/news.

Molecular Imaging and the Early Detection of Cancer

Magneto-Nano Protein Chip and Multiplex Sorter for Monitoring Tumors Antigens

Medical decision making is increasingly based on molecular testing; quantitative detection of disease-specific proteins in serum and other bodily fluids forms the foundation of many diagnostic tests to direct therapy in diverse areas of clinical medicine. Current methods for protein detection are often limited by their sensitivity, multiplexing capacity or, most importantly, uncontrollable response to the composition of complex biological samples. Dr. Shan Wang's group from the Stanford CCNE has developed a protein detection comprising two main elements: a magneto-nano sensor chip and a nanoparticle-based magnetic sorter. This device is capable of rapid, multiplexed protein detection with resolution down to attomolar concentrations and an extensive linear dynamic range. The magneto-nano sensors (64 giant magnetoresistive sensors in an 8 × 8 array)

function by exhibiting significant resistance changes in response to external magnetic fields; the magnetic sensors are insensitive to solution conditions such as buffers, pH or ionic strength (Fig. 3). Biological sensing is accomplished by affinity labeling both the sensor surface and magnetic nanoparticles tags to simultaneously attach to distinct domains of the target biological molecules. The magneto-nano sensor then detects the attachment of the biomolecules through the external magnetic field induced by the magnetic nanoparticles. For sufficiently small sensors and appropriate magnetic nanoparticle tags, affinity bonding resulting from a single, specific molecule can be detected as a simple change in the sensor electrical resistance, so expensive excitation sources or remote sensors are not required. The magnetic sorter rapidly segregates biomolecules based upon the tunable magnetic properties of the magnetic nanoparticles that bind them by causing them to deflect at different speeds under a given magnetic field and gradient. The matrix insensitivity of this platform to various media demonstrated that the magnetic nanosensor technology can be directly applied to a variety of settings such as molecular biology or clinical diagnostics. Recently, this technology was used to detect serum biomarkers such as carcinoembryonic antigen (CEA) down to 50 attomolar (0.01 pg/ ml) levels and allow multiplexed detection of eight serum biomarkers with negligible cross reactivity.

To commercialize this system, Dr. Wang and his collaborator Dr. Nadar Pourmand cofounded the startup company MagArray. To read more about MagArray, please go to page 69 of this book.

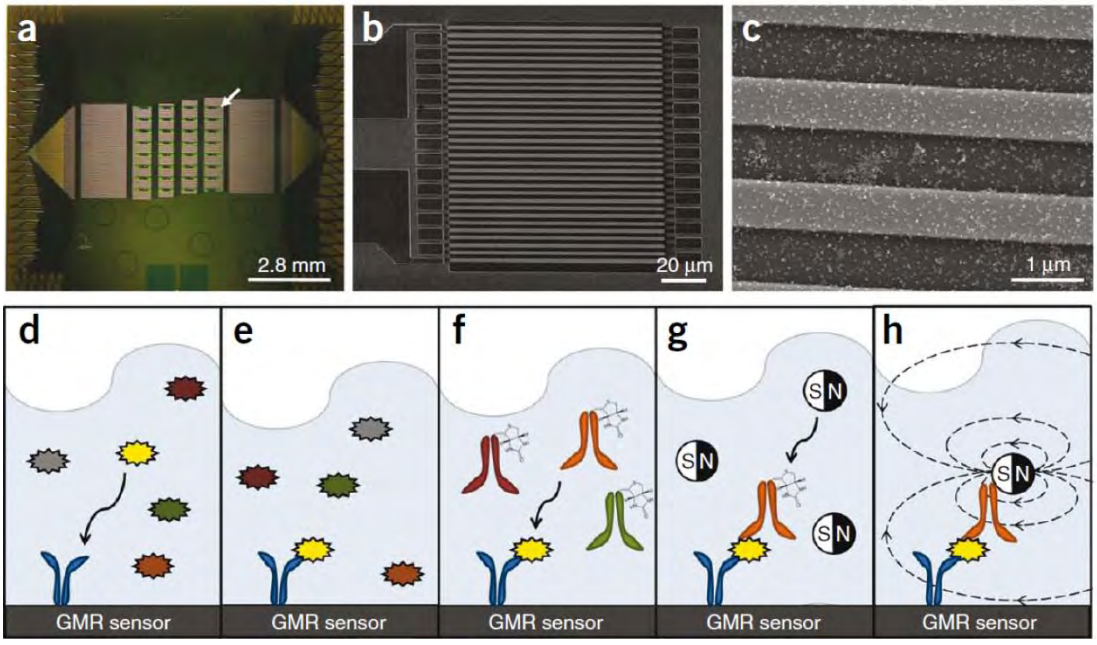

Figure 3: Sensor architecture and assay. (a) Image of the Stanford magnetonanosensor chip containing 64 sensors in an 8 ×8 array. Each green square is a uniquely addressable GMR sensor (white arrow). The horizontal lines leaving the sensors are leads connecting each sensor to a unique bond pad. (b) Scanning electron microscope (SEM) image of the sensor's serpentine architecture at 800×. (c) SEM image at 50,000× showing the sensor (light gray stripes) with magnetic nanoparticle tags (white dots). (d–h) A schematic of the sandwich assay. (d) Capture antibodies (blue) that are complementary to a chosen antigen (yellow) are immobilized onto the surface of each sensor. (e) The noncomplementary antigens are subsequently washed away. (f) After adding a cocktail of detection antibodies, the biotinylated detection antibody (orange) complementary to the antigen of interest binds in a sandwich structure, and the noncomplementary antibodies are washed away. (g) Finally, a streptavidin-labeled magnetic nanoparticle tag is added to the solution, and it binds the biotinylated detection antibody. (h) As the magnetic tags diffuse to the GMR sensor surface and bind the detection antibody, the magnetic fields from the magnetic nanoparticles can be detected by the underlying GMR sensor in real-time in the presence of a small external modulation magnetic field. (Reprinted with permission from Gaster RS et al. Matrix-insensitive protein assays push the limits of biosensors in medicine. Nat Med (2009) 15: 1327-1332. Copyright © 2009 Nature Publishing Group).

Suspended Microchannel Resonators for Cancer Biomarkers Detection

Improved immunoassays for quantifying biomarkers in blood are necessary for earlier detection and characterization of cancer, with the ultimate goal of improving treatment. Immunoassays such as ELISA are well established for biomarker detections, but the fidelity of the assay is governed by the dissociation constant K_d of the antibody-antigen complex. If the antigen concentration is significantly below K_d, then the binding kinetics are slow, and readout precision of the antigen-antibody complex can be degraded by non-specific background noise. By increasing the dynamic range of the immunoassay, more stringent filtering can be used to remove abundant background proteins, and low-affinity capture agents such as peptides can be used if high-affinity antibodies do not exist.

Label-free approaches are based on direct readout of target presence and offer a simple one-step assay that conserves reagents and minimizes fluidic handling. The ideal label-free sensor would have high sensitivity (comparable to or better than ELISA), require little or no sample preparation, enable multiplexed measurements, and be inexpensive to manufacture and use. Dr. Scott Manalis' team from the MIT CNPP has developed suspended microchannel resonators (SMRs), which are vacuum-packaged silicon microcantilevers with embedded microchannels of picoliter-scale volume. Adsorption of biomolecules to microchannel surfaces displaces an equivalent volume of running solution. The increased density of the biomolecules relative to the displaced solution (typically, protein density averages 1.35 g/mL) results in a net addition of mass, equivalent to the buoyant mass of the bound biomolecules. This changes the microcantilever resonant frequency in proportion to the amount of bound biomolecules. SMRs are batch-fabricated in a commercial MEMS foundry at approximately 200 devices per six in. silicon wafer (Fig. 4). Suitable scale-up could make the SMR a platform for routine, inexpensive monitoring of cancer biomarkers with extremely low sample volume and preparation requirements.

Dr. Manalis' team has demonstrated the detection of immuno-based protein binding within the SMR and improved label-free surface binding assay for the SMR that enables picomolar detection of a protein target in serum. These improvements were made possible by using a superlow fouling surface based on zwitterionic polymers and a reference microcantilever. Similar polymers have been used in SPR systems to improve specificity of biomolecular detection in undiluted human serum and plasma. The group has demonstrated that these surfaces in SMRs can be used to enable detection of activated leukocyte cell adhesion molecule (ALCAM) in undiluted serum with a limit of detection of 10 ng/mL. ALCAM is a 105 kDa glycoprotein identified as a potential biomarker for various carcinomas; it is typically found in blood serum at concentrations of ≈84 ng/mL, with levels over 100 ng/mL potentially indicating pancreatic carcinoma.

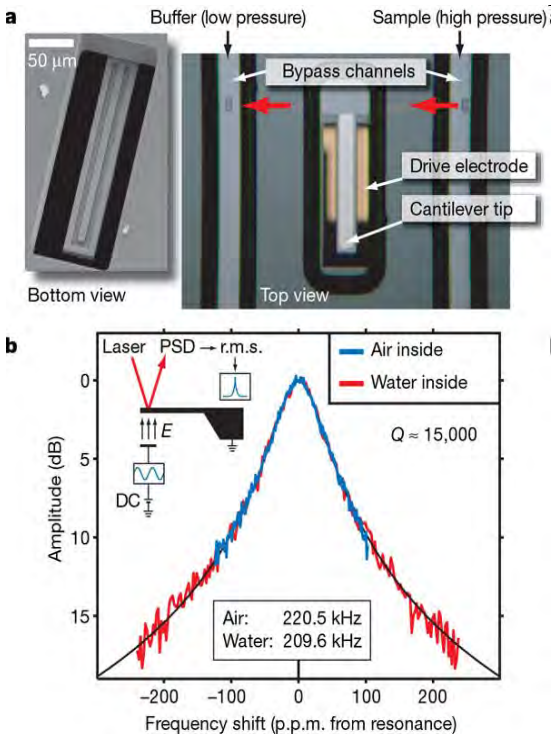

Figure 4: Micrographs and frequency response of a suspended microchannel resonator. a, The 200 × 33 × 7 μm (length × width × thickness) microcantilever containing a 3 × 8 μm (height × width) channel is suspended in a vacuum cavity (optical micrograph, right). Microfluidic bypass channels (30 × 100 μm, height × width) are connected to the inlet and the outlet of the suspended channel, and enable the quick exchange of samples by pressure driven flow (red arrows). The electron micrograph (left) shows a bottom view of a cantilever that has been intentionally etched open to visualize the fluidic conduit inside. b, Frequency response plots of a cantilever before (blue) and after (red) filling with water reveal different resonance frequencies but indistinguishable quality factors. To measure the frequency response, we monitored the vibration amplitude with a laser and a position sensitive photodetector (PSD) while the cantilever was being driven electrostatically at different frequencies (inset; E denotes the electric field, and 'DC' represents a bias voltage of ~60 V). (Reprinted with permission from Burg TP et al. Weighing of biomolecules, single cells and single nanoparticles in fluid. Nature (2007) 446, 1066-1069. Copyright© 2009 Nature Publishing Group).

Biobarcode Assay for Measuring Undetectable Levels of Prostate Specific Antigen

Although several diagnostic procedures for the detection and measurement of biological analytes exist, the major methods of choice for quantifying proteins are typically ELISA or immunoblots. These assays, however, are limited with respect to sensitivity and multiplexing capabilities and furthermore do not compare with the sensitivity that PCR affords in the nucleic acid field. Recent advances in nanomaterials-based assays have resulted in higher sensitivity diagnostic systems. Certain nanostructured materials are ideal for high sensitivity multiplexed analyte detection because their physical properties can be systematically varied to produce materials with specific emissive, absorptive and light scattering properties that offer advantages over conventional molecular probe technology. Some of these technologies have been developed into relatively rapid ways of identifying both protein and nucleic acid targets. Dr. Chad Mirkin from the Northwestern University CCNE has developed an ultra-sensitive bio-barcode assay, which is used for the detection of Prostate Specific Antigen (PSA). This assay uses antibody-oligonucleotide Au nanoparticle (NP) conjugates for PSA detection and Au NP initiated gold or silver deposition for signal amplification and assay readout. Signal readout and quantification is done by measuring Au NP-mediated light scattering (Fig. 5).

This test is currently clinically validated for monitoring PSA levels in patients following radical prostatectomy and assesses response to adjuvant and salvage therapy. Specifically, Dr. Mirkin together with the group of Dr. C. Shad Thaxton, also from the Northwestern CCNE were able to reliably and accurately quantify PSA values at less than 0.1 nanograms per milliliter, the clinical limit of detection for commercial assays. The lower limit of detection for PSA using the bio-barcode assay is approximately 300 times lower than the lower limit of detection for commercial tests. The PSA measurements were used to classify the patients as either having no evidence of disease or having a relapse of disease. The Northwestern CCNE team, and in collaboration with Dr. William Catalona, M.D. and Nanosphere, Inc. is now conducting a similar retrospective study of over 400 patients and eventually plans to do a large prospective study.

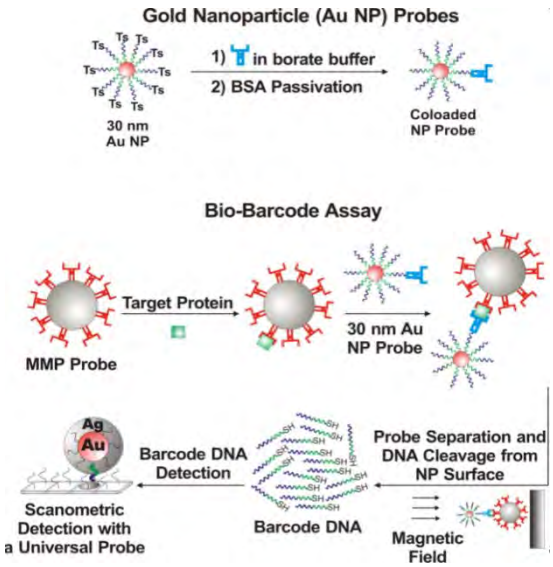

Figure 5: Schematic representation of the PSA Au-NP probes (Upper) and the PSA bio-barcode assay (Lower). (Upper) Barcode DNA-functionalized Au-NPs (30 nm) are conjugated to PSA-specific antibodies through barcode terminal tosyl (Ts) modification to generate the coloaded PSA Au-NP probes. In a second step, the PSA Au-NP probes are passivated with BSA. (Lower) The bio-barcode assay is a sandwich immunoassay. First, MMPs surface-functionalized 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 ½ of the barcode DNA sequence (green) is complementary to the "universal" scanometric Au-NP probe DNA, and the other ½ (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 the permission from Thaxton CS et al. Nanoparticle-based bio-barcode assay redefines "undetectable" PSA and biochemical recurrence after radical prostatectomy PNAS (2009) 106 (44): 18437-18442. Copyright© 2009 National Academy of Sciences USA).

Recently, a novel Scanometric immunoassay was reported by the Mirkin group and may represent the next generation of ultra-sensitive nanoparticle-based protein detection assays. Specifically, an antibody microarray was fabricated by spotting monoclonal capture antibodies to the surface of N-hydroxysuccinimide-activated glass slides. The slides were then passivated with amine-terminated poly(ethylene glycol). The assay nanoparticle probes were prepared by modifying 13 nm diameter Au NPs with 3′-propylthiol and 5′-decanoic acid modified oligonucleotides. After isolation of the oligonucleotide Au NP conjugate from excess oligonucleotide strands, detection antibodies were covalently bonded to the surface of the oligonucleotide monolayer via carbodiimide coupling. Protein detection was initiated by first capturing the target antigens using the immobilized antibodies and labeling bound antigen with the Au NP probes. To increase the light scattering signal of the immobilized Au NP probes, their diameters were increased by electroless gold or silver deposition. Finally, the light scattering was quantified. Typically, electroless silver deposition is used to grow Au NP probes on oligonucleotide microarrays. Using this amplification method the limit of detection was 3 pM. By adding a second cycle of gold-on-silver deposition on the same microarray, the limit of detection was improved to 300 fM. The higher signal arose from the larger diameter of the Au NPs after the second round of amplification because the light scattering intensity increases dramatically with particle diameter.

Molecular Beacons for Cancer Detection and Analysis

While most approaches that aim to develop new technologies to enable earlier detection of cancer focus on identifying proteins and other biomarkers in blood, researchers at the Emory-Georgia Tech CCNE are developing fluorescent molecular beacons as probes for cancer gene activity in cells. The hope is that by detecting active cancer genes, molecular beacons may be able to spot very small numbers of cancer cells before they develop into aggressive tumors. Toward this end, Drs. Gang Bao and Barbara Boyan have been systematically exploring the many parameters that need to be optimized to detect gene activity in live cells.

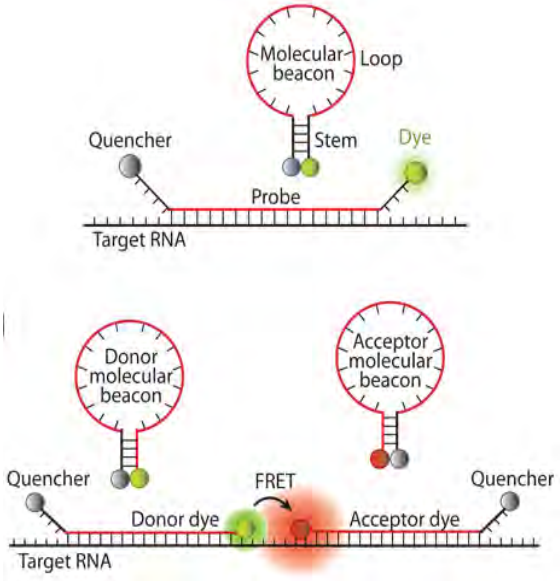

Figure 6 Illustrations of fluorescent probes for live-cell RNA detection. *Top:* Molecular beacons are dual-labeled stem-loop oligonucleotide hairpin probes with a reporter fluorophore at one end and a quencher molecule at the other end. Hybridization with target RNA unfolds the loop, restoring fluorescence *Bottom:* Dual FRET donor and acceptor molecular beacons hybridize to adjacent regions on the same mRNA target, resulting in a FRET signal.(Reprinted from Bao G et al. Fluorescent probes for live-cell RNA detection. Annu Rev Biomed Eng. 11: 25-47).

Drs. Bao and Boyan have largely focused on two types of fluorescent probes (Fig. 6). Molecular beacons form a stem-loop hairpin structure in the absence of a complementary target so that the fluorescence of the fluorophore is quenched. As the probe hybridizes with the target RNA, the fluorophore is spatially separated from the quencher and fluorophore emission is restored, indicating the presence of the target RNA. Unfortunately, degradation of the molecular beacon by nuclease or reaction with nucleic acid binding proteins can lead to false positives. This can be avoided through the use of dual molecular beacons, containing fluorescent

resonant energy transfer (FRET) donor-acceptor fluorophore pairs targeted to adjacent sequences on the target RNA. The FRET signal is observed only when both beacons hybridize, indicating a true positive for the target sequence. The Emory-Georgia Tech team has tested the composition of several backbones and has found that 2'-deoxy ribonucleotides are effective in measuring mRNA during translation whereas 2'-*O*-methyl ribonucleotides measure total cellular mRNA. The researchers have now used these approaches to monitor K-ras and BMP-4 levels in various cell types as well as β1 integrin expression changes when cells are grown on different substrates

In vivo Imaging

Tumor Cell Imaging Using Magnetic Nanoparticles

Magnetic nanoparticles have an extraordinary ability to enhance magnetic resonance image contrast, potentially enabling earlier detection of tumors. They are also capable of selective delivery of chemotherapeutic agents to cancer cells upon magnetic stimulation or through attachment of cell targeting molecules. These properties suggest that a new class of theranostic materials can be developed using these nanoparticles.

A team of investigators at the University of North Carolina CCNE led by Dr. Wenbin Lin has developed several innovative materials for cancer research, with significant implications in realizing early detection by magnetic resonance (MR) imaging and developing effective therapies for brain tumors. In this emerging area of nanomedicine, Dr. Lin and his collaborators are designing nanoscale multimodal contrast agents for magnetic resonance imaging, optical imaging, and X-ray computed tomography and are developing novel nanoparticles for targeted delivery of potent anticancer therapeutics. The team has recently made progress on designing MR contrast agents based on three different nanoparticle platforms: iron oxide nanoparticle clusters, nanoscale metal-organic frameworks (NMOFs), and polysilsequioxane (PSQ) nanoparticles containing organic fluorophores and MR-enhancing gadolinium (Gd) chelates (Fig. 7).

Dr. Lin's group has also made progress in designing platinum-containing nanomaterials for cancer therapy. In one approach, an optical contrast agent (a BODIPY dye) and cisplatin-related anticancer prodrug were successfully incorporated into NMOF particles. These cargoes were released upon degradation of the NMOF frameworks, and the rate of cargo release was controlled by coating the NMOF particles with a silica shell. The potential utility of the new NMOF-based nano-delivery vehicles for optical imaging and anticancer therapy was demonstrated *in vitro* using HT-29 human colon adenocarcinoma cells. Dr. Lin's team has also taken another approach to nanoparticle-based diagnostics that uses novel iodine-containing polymers. These particles were created to carry large payloads of iodine (approximately 63 weight percent) and have potential applications as a new class of contrast agents for computed tomography, adding additional power to this important diagnostic technique.

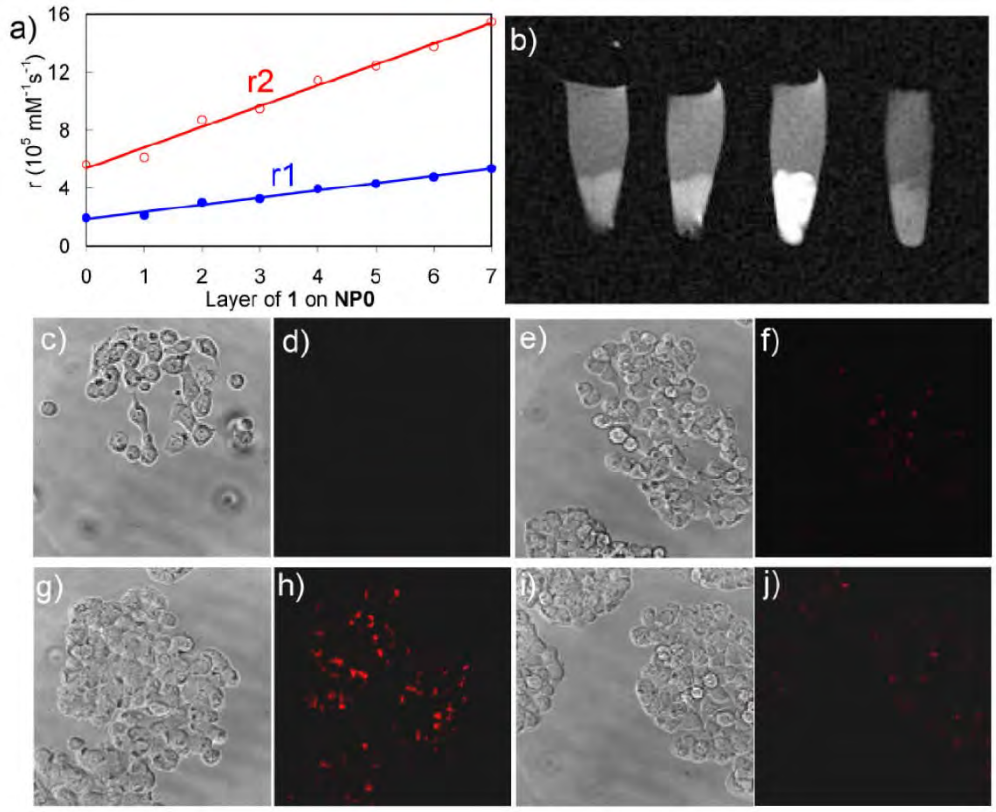

Figure 7: Estimation of r1 and r2 relaxivities for NPnA. (a) Dependence of per particle r1 and r2 values on the number of deposited Gd-DOTA oligomer 1 on NP0. (b) T1-weighted MR images of HT-29 cells that have been incubated with various nanoparticles. From left to right: control cells without any nanoparticle, cells with NP3B particles, cells with NP3B particles that have been non-covalently functionalized with K7RGD, and cells with NP3B particles that have been non-covalently functionalized with K7GRD. (c-j) Phase contrast optical (c, e, g, and i) and LSCFM microscopic images (d, f, h, and j) of HT-29 cells that have been incubated with various nanoparticles: control cells without any nanoparticle (c and d), cells with NP5B particles (e and f), cells with NP5B particles that have been non-covalently functionalized with K7RGD (g and h), and cells with NP5B particles that have been non-covalently functionalized with K7GRD (i and j). (Reprinted from the permission from Kim JS et al. Self-Assembled Hybrid Nanoparticles for Cancer-Specific Multimodal Imaging (2007) J Am Chem Soc. 129(29): 8962–8963. Copyright © 2009 National Library of Medicine).

Near-Infrared Fluorescent Nanoparticles for Targeted Optical Imaging and Drug Delivery

It is well accepted that colloidal particles can preferentially accumulate in tumors after their systemic administration because of the enhanced permeability and retention (EPR) effect. This passive manner of delivery without specific binding to cellular targets (*i.e.*, passive targeting) can be highly effective. However, tumor accumulation of colloidal particles resulting from the EPR effect can only be successful when the blood circulation time of the particles is prolonged. A number of factors, such as size, size distribution, composition, and surface hydrophilicity, can influence the circulation of nanoparticles in the blood. In particular, surface modification with flexible, hydrophilic PEG has proven to be effective in preventing the uptake of various polymer-based nanoparticles by the cells of the mononuclear phagocytic system.

Dr. Chun Li of the University of Texas M.D. Anderson Cancer Center CNPP has developed novel multifunctional nanoparticles suitable for multimodal imaging and theranostic applications. On the basis of pharmacokinetic, biodistribution, and noninvasive imaging data gathered with several types of nanoparticles having average diameter less than 100 nm, including polymeric, semiconductor, and gold nanoparticles, the team has designed nanoparticles coated with PEG that display prolonged blood half-life. Blood half-life is partially a function of the particle size: smaller particles having thicker PEG coating display long blood circulation time. The group has found that other factors, such as compressibility of nanoparticles, can also influence extravasation of nanoparticles from tumor blood vessels into the extravascular space. Also, active targeting with significantly improved tumor deposition can be achieved using nanoparticles coated with homing ligands, as compared to non-targeted nanoparticles. The team has found that the nature of the homing ligand (*i.e.* size, binding affinity, ability to internalize by cancer cells) can have a profound effect on the efficiency of targeted nanoparticle delivery.

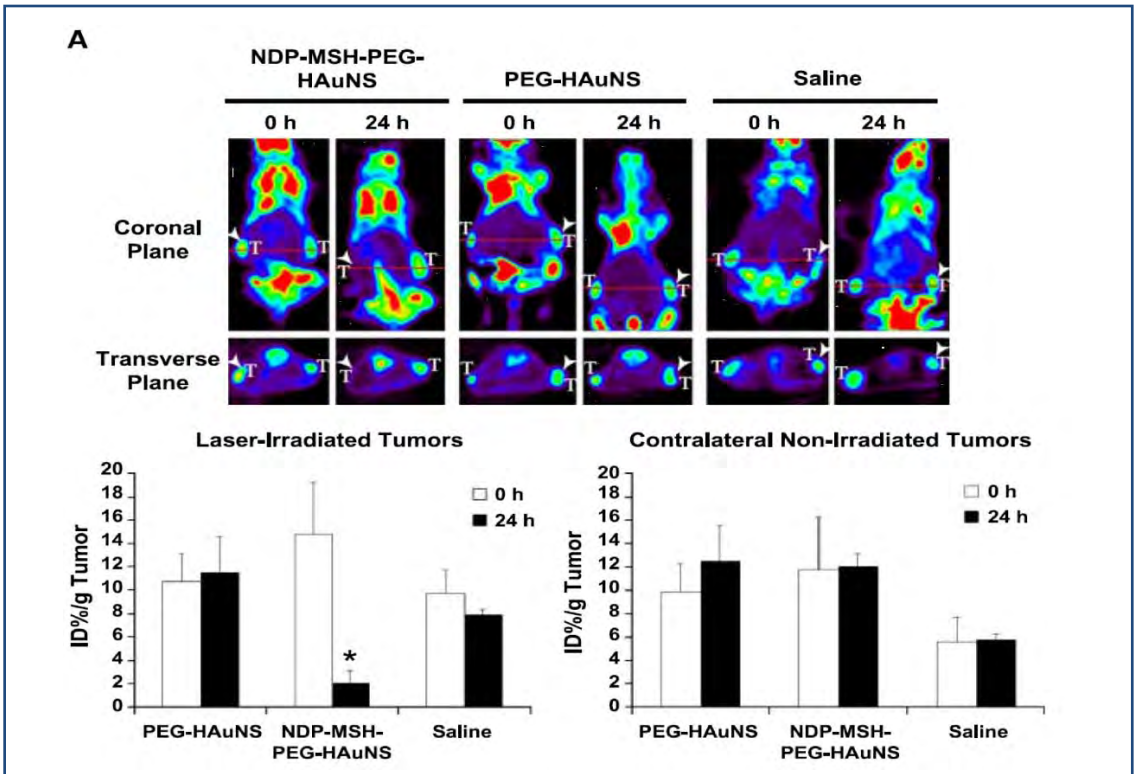

Figure 8: *In vivo* photothermal ablation with targeted NDP-MSH-PEG-HAuNS induced selective destruction of B16/F10 melanoma in nude mice. *A,* [^{18}F]fluorodeoxyglucose PET imaging shows significantly reduced metabolic activity in tumors after photothermal ablation in mice pretreated with NDP-MSH-PEG-HAuNS but not in mice pretreated with PEG-HAuNS or saline. [^{18}F]fluorodeoxyglucose PET was conducted before (0 h) and 24 h after near IR–region laser irradiation (0.5 W/cm^2 at 808 nm for 1 min), which was commenced 4 h after i.v. injection of HAuNS or saline. T, tumor. Arrowheads, tumors irradiated with near IR–region light. [^{18}F]fluorodeoxyglucose uptakes (%ID/g) before and after laser treatment are shown graphically at the bottom. Bars, SD (n = 3). *, $P < 0.01$ for %ID/g posttreatment versus %ID/g pretreatment. (Reprinted from Lu W. et al. Targeted Photothermal Ablation of Murine Melanomas with Melanocyte-Stimulating Hormone Analog–Conjugated Hollow Gold Nanospheres. Clinical Cancer Research (2009) 15; 876. Copyright © 2009 American Association for Cancer Research).

The team's findings have been used to guide the design, development, and evaluation of hollow gold nanospheres (HAuNS), a novel class of core-shell nanostructures that may find wide applications in photothermal ablation therapy, drug delivery, and molecular imaging. The combination of spherical shape, small size (average diameter ~40 nm), absence of a silica core and tunable and strong absorption bands in the near infrared region makes these nanoparticles ideally suited for photothermal ablation applications. Using peptides as targeting ligands, they demonstrated receptor-mediated active targeting and efficient photothermal ablation of melanoma in a murine tumor model. The newly developed nanoparticles are particularly relevant to clinical translation for photothermal ablation of melanoma because these lesions are accessible to near-infrared light penetration. Targeted delivery of nanoparticles to melanoma could increase the efficacy, decrease the energy dose of the laser, and minimize the potential for damage to surrounding normal tissues (Fig. 8).

Moreover, Dr. Li's team has demonstrated a novel strategy called photothermal transfection that selectively induces endo-lysosomal escape of siRNA and efficient RNAi through near-infrared (NIR) light-induced release of siRNA from HAuNS delivery vehicles. Significantly, NIR light-triggered RNAi down-regulated the expression of the NF-κB p65 subunit and enhanced chemosensitivity and apoptotic response to irinotecan, demonstrating that the photothermal transfection technique is a viable approach for effective therapeutic gene silencing. Because NIR light can penetrate deep into tissues and can be delivered at a predetermined time and site, therapeutic RNAi may benefit from this novel transfection strategy. This approach has been shown to be readily applicable to chemotherapeutic agents as well. The data support an effective therapeutic approach whereby NIR light is used for simultaneous modulation of drug release and nanoparticle mediated photothermal killing effect.

Reporters of Therapeutic Efficacy

Targeted Nanosystems for Therapy and Imaging

Nanomaterials have played a promising role in delivering therapeutic molecules effectively to diseased sites, but nanoscale materials can also be harnessed to damage or destroy malignant tissues by converting external electromagnetic energy into heat. Furthermore, most nanomaterial surfaces can be decorated with targeting ligands, enhancing their ability to home to diseased tissues through multivalent interactions with tissue-specific receptors. In spite of these merits, nanotechnology-based cancer therapies have been slow to reach the clinic compared to conventional cancer therapies such as small molecule drugs, whole-body or local hyperthermia, and radiation.

To overcome this barrier, a multidisciplinary collaboration of ANC researchers Drs. Michael Sailor and Erkki Ruoslahti from the UCSD CCNE and Dr. Sangeeta Bhatia from the Harvard-MIT CCNE developed a new cooperative nanosystem that uses the unique photothermal properties of gold nanorods to improve the tumor specificity of targeted nanoparticles for therapy and imaging. The first component of the system are gold nanorod "activators" that populate porous tumor-associated blood vessels and act as photothermal antennas by absorbing and transducing NIR into heat (approximately 43°C). Hyperthermic treatment upregulates the expression of the stress-related protein p32 in tumor cells, which was exploited by coating secondary nanoparticles with LyP-1 – a cyclic peptide specific for p32. 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 ~3 fold increase in doxorubicin deposition compared with individual nanoparticles or an untargeted cooperative system (Fig. 9). Therefore, this approach could significantly reduce the required

dose of anticancer drugs and mitigate toxic side effects.

Magnetic nanoworms make up another potential secondary component of the system. These constructs comprise spherical iron oxide nanoparticles linked together to form worm-like chains, each containing five to 10 magnetic grains of 5 nm in diameter and with an overall global hydrodynamic diameter of 65 nm. Compared with spherical nanoparticle controls this particular geometry was found to bind to tumor cells more efficiently *in vitro* because of multivalent interactions between the nanoworms and targeted cellular receptors, and as a result, they could be used as contrast agent for MRI (Fig. 9). These new cooperative, synergistic therapies using dual or multiple nanomaterials could significantly reduce the required dose of anticancer drugs, mitigating toxic side effects, and more effectively eradicating drug-resistant cancers.

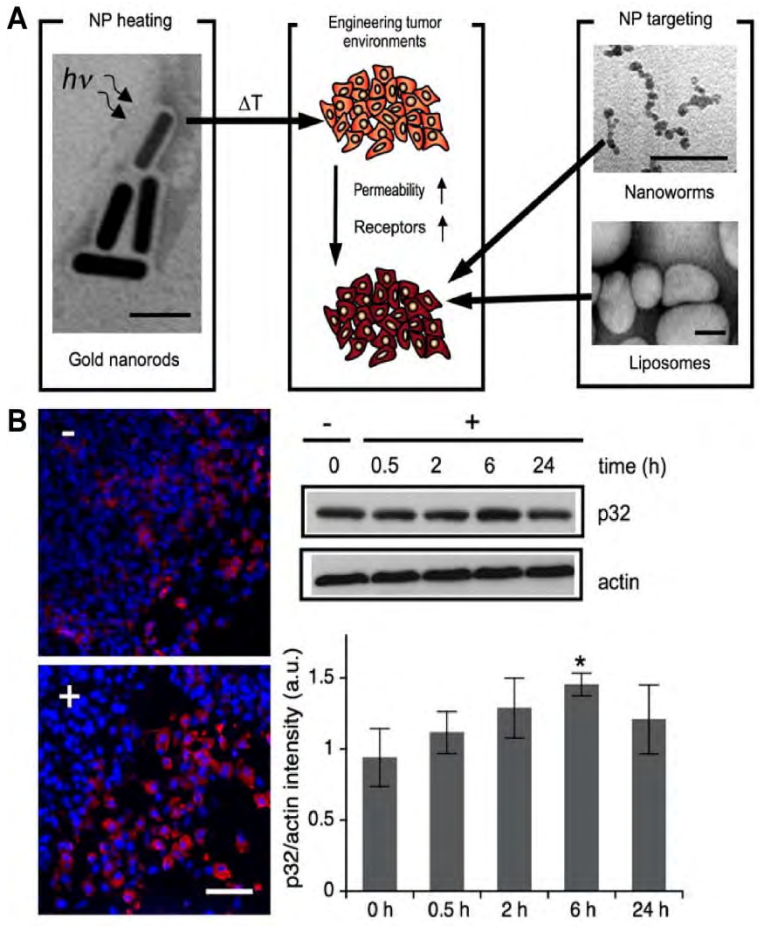

Figure 9: Characterization of the components of cooperative nanosystems. A. Schematic showing the components of the two cooperative nanomaterials systems: the first - gold nanorods (NR), and second - magnetic nanoworms (NW), or doxorubicin-loaded liposomes (LP). Irradiation of the NR with a NIR laser induces localized heating that stimulates changes in the tumor environments. The NW or LP components decorated with LyP-1 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 β-actin control. * indicates $P < 0.05$ for 0 h and 6 h intensity ratio ($n = 3$ ~4). (Reprinted with the permission from Park JH et al. Cooperative nanomaterial system to sensitize, target, and treat tumors. PNAS (2010) 107: 981-6. Copyright© 2010 National Academy of Sciences USA).

Implantable Device for Continuous Cancer Monitoring

Cancer diagnosis is typically performed on tissue samples that have been surgically removed. But biopsies only provide a snapshot of the tumor at a single moment in time. By the time test results are produced they may no longer be accurate. It would be more valuable to monitor the tumor for weeks or months to track how it is responding to treatment. Dr. Michael J. Cima and his colleagues from the MIT-Harvard CCNE have developed the first implantable device with the potential to measure biomarker concentrations as indicators of the local tumor environment. The device, which uses nanoparticle magnetic relaxation switches (MRSw) as reporters, is a cylindrical, 5-millimeter implant made of high-density polyethylene encased in a polycarbonate membrane with 10-nanometer-diameter pores. Packaging the MRSw in this device addresses two key challenges related to using the MRSw *in vivo*: possible immune response to the protein modified nanoparticles and magnetic relaxation (T_2) fluctuations resulting from changes in MRSw concentration. The semi-permeable membrane that covers the MRSw reservoir allows cancer biomarkers or chemotherapeutic agents to diffuse into the device and interact with the MRSw but does not allow diffusion of the MRSw into the tissue environment. The rigid device substrate provides a constant-volume reservoir so the concentration of MRSw remains constant. MRSw are magnetic nanoparticles with a superparamagnetic iron oxide core (about 4 nm in diameter) and a cross-linked dextran shell bearing functional groups that react with target species, such as peptides, oligonucleotides, nucleic acids, receptor ligands, proteins, small molecules, and antibodies. The MRSw aggregate when they react with an analyte, causing a decrease in the transverse relaxation time (T_2) that can be detected by MRI or nuclear magnetic resonance relaxometry. The constant volume reservoir ensures any T_2 changes can be attributed solely to aggregation of the nanoparticles. These MRSw have been used extensively for *in vitro* agglutination assays where the MRSw and analyte solutions are mixed together, and continuous monitoring of glucose with MRSw contained within a dialysis membrane has also been demonstrated *in vitro*.

Dr. Cima's group has also demonstrated detection of a model cancer biomarker, the beta subunit of human chorionic gonadotrophin (hCG-β), in proof-of-principle *in vivo* sensing experiments. MRSws have been shown previously to detect 0.5–5 μg/ml hCG-β, a soluble biomarker that is elevated in testicular and ovarian cancer patients. Serum concentrations of up to 16 μg/ml have been reported in one condition, persistent trophoblastic disease, though hCG-β levels are usually less than 0.005 μg/ml in normal men and women. Two populations of MRSw were prepared, each conjugated with a different monoclonal antibody for hCG-β. Aggregation occurs when both types of MRSw are present with either the hCG-β subunit or the hCG dimer. The high binding affinity of the antibodies favors irreversible aggregation of the MRSw. The local concentration of hCG-β affects the rate of T_2 change such that a low concentration of hCG-β increases the measured signal at a slower rate than a higher concentration. *In vivo* performance was assessed using a commercially available human epithelial cell line (JEG-3) to produce ectopic tumors that secrete hCG in nude mice. Plasma hCG-β concentrations were quantified with an enzyme-linked immunosorbent assay (ELISA) and compared to results using the MRSw device to validate device performance.

Blood Protein Profiling of Glioblastoma Patients: Addressing the Question of Patient Response to Avastin® Therapy

A new barcode chip developed by investigators at the Caltech Nanosystems Biology Cancer Center promises to revolutionize diagnostic medical testing. 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 over broad concentration ranges (~10^5) within 10 min of sample collection. A microfluidic network on the IBBC enables

approximately 15% of plasma (over 99% purity) to be skimmed from whole blood for detection of plasma proteins without pre-processing (Fig. 10A**).** 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 via hybridization to protein specific antibodies that are labeled with complementary singe-stranded DNA (ssDNA) oligomers (Fig. 10B). DNA, unlike antibodies, is stable to the processing used to create the elastomeric microfluidics chips and resists biofouling. Each complementary DNA pair is chosen, via computational and empirical methods, to be orthogonal to the other pairs in order to limit cross reactivity. IBBC protein assays exhibit a sensitivity that matches or betters conventional assays, but the concentration range of IBBC assays is significantly increased, relative to standard assays, through control over the surface chemistry of the glass substrate and the DNA loading on a given spot.

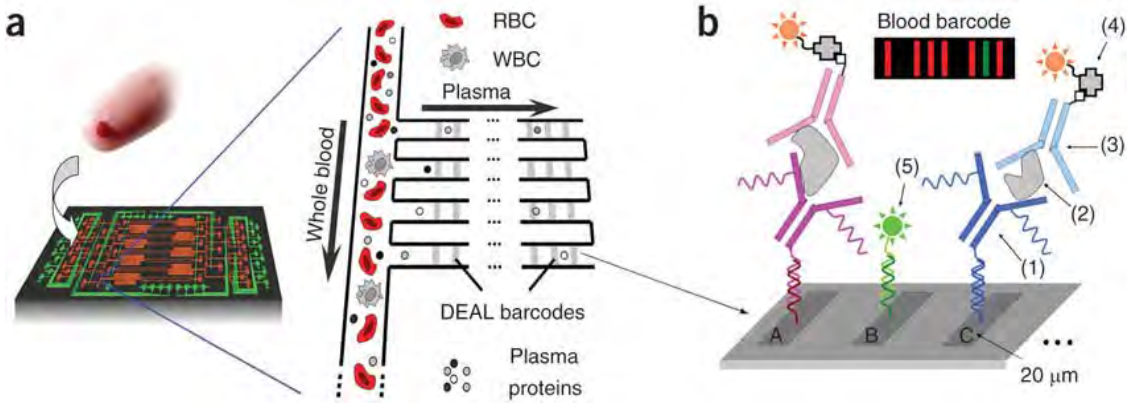

Figure 10: Scheme depicting plasma separation from a finger prick of blood by harnessing the Zweifach-Fung effect. A. Multiple DNA-encoded antibody barcode arrays are patterned within the plasma-skimming channels for *in situ* protein measurements. B. DEAL barcode arrays patterned in plasma channels 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. (Reprinted with the permission from Fan R et al Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. Nat Biotechnol. (2008) 26: 1373-8. Copyright© 2008 Nature Publishing Group).

A potential application of technologies developed by Dr. Heath's team is to identify whether cancer patients are likely to respond to specific therapies. Traditional approaches for assessing response utilize a watch-and wait strategy. A patient is put on therapy regimen A, and the cancer is monitored over a couple of month period using imaging approaches such as Magnetic Resonance Imaging (MRI) or Computed Tomography (CT). If the tumor regresses during this period, the patient is kept on the therapy. If the tumor progresses, the patient is switched to therapy regimen B, and the process is repeated. Working with Dr. Paul Mischel of UCLA, Dr. Heath is currently using the IBBC and DEAL for molecular and functional analysis of clinical glioblastoma tumor samples, to identify patients with the greatest potential for positive response to Avastin® therapy. Avastin®, which was approved in May 2009 for glioblastoma multiforme (GBM) patients, is an angiogenesis inhibitor, and between 20-26% of GBM patients on Avastin® show a measureable tumor response for about 4 months. Avastin® is also FDA approved for other cancers, including colon cancer, breast cancer, non-small-cell lung cancer, and metastatic colon cancer. Unfortunately, there is no diagnostic test that can pre-screen patients to identify potential

responders. As a result, Avastin® is widely prescribed, costing on average $80,000 per GBM patient (more for patients on other cancers because they live longer), and only yielding marginal benefit to 1 in 4 or 5 patients. DEAL and IBBCs have been utilized to identify a panel of biomarkers that can differentiate GBM patients who are responding to Avastin® from those who are not responding has been identified, and is currently being evaluated on a cohort of 400 additional patients. There is a large potential for extending this panel to the other cancers for which Avastin® therapy is approved

The IBBC has also been used to analyze the blood of breast and prostate cancer patients for a number of proteins that serve as biomarkers for disease.

Multifunctional Therapeutics

Nanotherapeutic Strategy for Multidrug Resistant Tumors

Multidrug resistance (MDR) is a major obstacle for successful treatment of cancer. Reversing MDR has been a high priority goal for clinical and investigational oncology, but remains an elusive outcome. Agents targeting the most well-known pathway, the multidrug-resistant transporters (such as MDR1 and MRP) that promote drug resistance to structurally unrelated cytotoxic agents, have proved to be disappointing in clinical trials. Experimental evidence from multiple laboratories implicates a wide range of mechanisms that contribute to the drug resistant phenotype.

Ceramide, a messenger in apoptotic signaling, plays a principal role in the nature of cellular response to anticancer therapies, participating in the reactions to both chemotherapy and radiation. Accumulation of glucosylceramide (GC) is a characteristic of some MDR cancer cells of ovarian, breast, prostate, colon, and lung cancers. Ceramide glycosylation, through glucosylceramide synthase (GCS), allows cellular escape from ceramide-induced apoptosis or programmed cell death. This glycosylation event confers cancer cell resistance to cytotoxic anticancer agents. Investigators have demonstrated the efficacy of targeting ceramide synthesis or degradation pharmacologically to enhance the cytotoxic effects of several clinically relevant drugs. In addition, knocking out the GCS enzyme by siRNA has been shown to reverse MDR.

Mansoor Amiji, Ph.D., and his collaborators at the Northeastern University CNPP have a longstanding interest in targeting *de novo* ceramide production and metabolism. Studies in his laboratory have focused on the generation of different nanoparticles and combination with ceramide to overcome MDR in cancer. In one study, ceramide was combined with the chemotherapeutic drug paclitaxel and delivered to ovarian cancer MDR cells. PEG-modified poly(epsiloncaprolactone) (PEO–PCL) nanoparticles were used to encapsulate and deliver the therapeutic agents for enhanced efficacy. Results demonstrated that the co-therapy does in fact eradicate the complete population of MDR cancer cells when they are treated with their IC50 dose of paclitaxel. More interestingly, when the co-therapy was combined with the properties of nanoparticle drug delivery, the MDR cells were re-sensitized to a dose of paclitaxel near the IC_{50} of non-MDR (drug sensitive) cells, indicating a 100-fold increase in chemo-sensitization via this approach. Molecular analysis of activity verified the hypothesis that the efficacy of this therapeutic approach is indeed a result of a restoration in apoptotic signaling, although the beneficial properties of PEO–PCL nanoparticle delivery appeared to enhance the therapeutic success even further, demonstrating the great potential for clinical use of this therapeutic strategy to overcome MDR. To extend this *in vitro* study to *in vivo*, a separate study was conducted to evaluate whether co-administration with paclitaxel in PEO–PCL nanoparticles could increase intracellular ceramide levels and lower the apoptotic threshold in MDR cells. Upon intravenous administration of paclitaxel combination in PEO–PCL nanoparticle formulations, significant enhancement in antitumor efficacy was observed. Furthermore, the combination of paclitaxel/tamoxifen therapy did not induce any

acute toxicity as measured by body weight changes, blood cell counts, and hepatotoxicity.

To further optimize efficacy of this combination therapy, a novel polymer-blend nanoparticle was designed, including a slow release polymer and a pH-responsive polymer in the same formulation, affording temporal control over release. Paclitaxel was encapsulated in a pH-responsive rapid releasing polymer, poly(beta-amino ester) (PbAE), while ceramide was present in a slow releasing polymer, poly(D,L-lactide-*co*glycolide) (PLGA) within these blend nanoparticles. When particle formulations were administered intravenously to breast cancer bearing mice, higher concentrations of paclitaxel were found in the blood resulting from a longer retention time, and an enhanced tumor accumulation relative to the administration of free drugs was observed. In addition, the PLGA /PbAE blend nanoparticles were effective in enhancing the residence time of both drugs at the tumor site by reducing systemic clearance. *In vivo* studies in both human ovarian and breast cancer MDR xenograft showed that the paclitaxel and ceramide nanoparticle combination therapy reduced the final tumor volume by at least 2-fold compared to treatment with standard paclitaxel therapy alone. The study also revealed that the co-therapy enhances apoptotic signaling in MDR cells. Additionally, evaluation of safety in mice with the combination therapy showed no significant levels of toxicity. These results suggest nanotechnology based targeting ceramide metabolic and cell death signaling pathways is an attractive approach to overcoming MDR.

Dendrimer Nanoparticles for Cancer Diagnosis and Treatment

Taking a critical step toward the development of a multifunctional nanoscale anticancer agent that can detect cancer, treat it, and then report on the success or failure of that treatment, Dr. James Baker, Jr. and his collaborators at the University of Michigan CNPP developed a nanoscale sensor of cell death. This sensor is built using the same biocompatible polymeric dendrimer platform that this research team has already used to image and treat heterogeneous human xenograft tumors in animal models (Fig. 11).

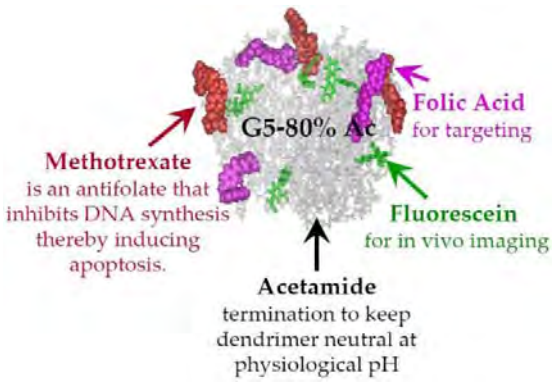

Figure 11: Schematic of multifunctional targeted nanodevice based on the PAMAM dendrimer scaffold. (Reprinted with the permission from Leroueil P. et al. Nanoparticle Interaction with Biological Membranes. Does Nanotechnology present a Janus Face? Acc Chem Res. 2007 May; 40(5): 335–342. Copyright © 2009 National Library of Medicine).

Most approaches to detect apoptosis rely on the human protein annexin V interacting with phosphatidylserine when it is translocated from the inner surface of the plasma membrane to the outer surface as the cells go through cell death. Dr. Baker's group, however, developed a fluorescence resonance energy transfer, or FRET-based assay to assess caspase-3 activation, another indicator of apoptosis. They have utilized poly(amidoamine) (PAMAM) dendrimers containing a caspase peptide substrate linking FRET donor-acceptor pair subunits ; cleavage of the peptide by intracellular caspase separates the FRET pair and restores the donor fluorescence, signaling the presence of caspase. The dendrimer is targeted to cancer cells by an attached folic acid that binds to a receptor that is overexpressed in many tumor types. Dr. Baker's team has shown that the particles are taken up specifically by cells expressing the receptor, and that induction of apoptosis using staurosporine causes an increase in the fluorescence intensity of cells, indicating that this system can serve as a nanodevice that assays caspase activation. Dr. Baker and his team also developed an optical fiber device capable of detecting FRET emissions in tumors and have used it to quantify apoptosis

in live mice with tumors bearing the folic acid receptor.

More recently the group has developed dendrimers containing the chemotherapeutic agent methotrexate, labeled with fluorescein isothiocyanate and bearing riboflavin as a targeting agent. They chose to use riboflavin as a targeting mechanism because it is structurally amenable to covalent modification and it is a possible cancer biomarker. They showed that cells take up the nanoparticles in a riboflavin-receptor-dependent manner, which decreases cell survival and lowers overall toxicity compared to treatment with methotrexate alone. The data establish proof of principle that multifunctional nanoparticles are capable of imaging and treating cancer cells overexpressing the riboflavin receptor. Although this approach has not yet been tested in animal models, it may be an eventual viable treatment option.

Targeting Central Nervous System Tumors with Imaging Nanoprobes

Cancers of the central nervous system (CNS) are among the most deadly and intractable diseases known. Little progress has been made in treating malignant CNS tumors because of three main reasons: the difficulty in differentiating between tumor and healthy CNS tissue, the sensitivity of normal CNS tissue to current therapies, and the blood brain barrier's (BBB) ability to prevent the passage of medicinal substances including drugs and contrast agents. Dr. Miqin Zhang and her team from the University of Washington CNPP have developed a multifunctional nanoparticle platform that serves as both imaging contrast agent and drug carrier and that can pass biological barriers for non-invasive diagnosis, staging, treatment, and treatment response monitoring of brain tumors. The core components of this platform are a superparamagnetic iron oxide nanoparticle and a PEG coating that is subsequently functionalized with tumor targeting chlorotoxin (CTX) and a near infrared fluorescing molecule (NIRF).

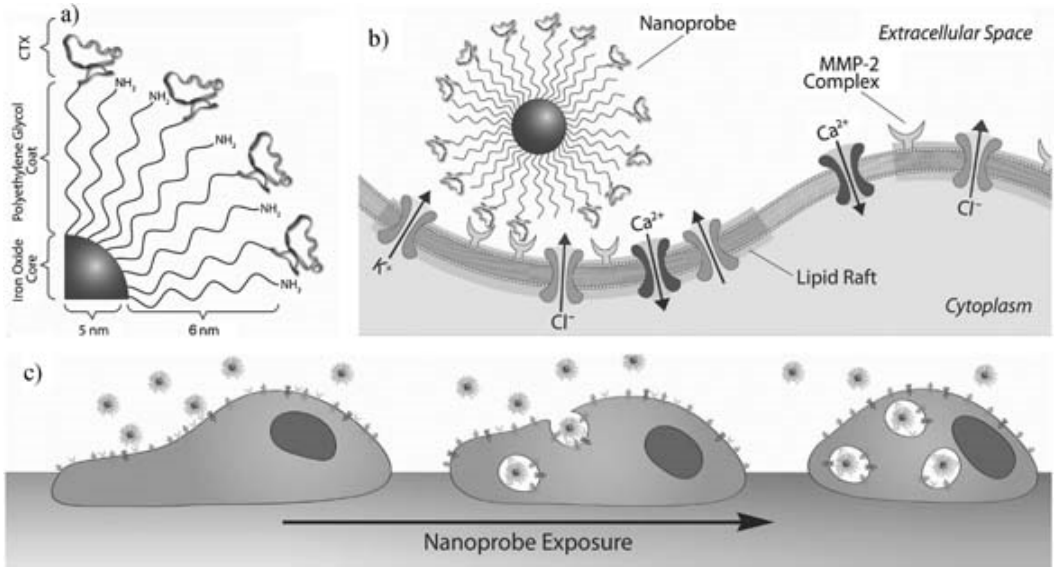

Figure 12: Schematic representations of NPC inhibiting tumor cell invasion. a) Surface chemistry of NPC conjugate. b) NPC binding to lipid rafts of glioma cells containing MMP-2 and select ion channels. c) NPC internalization and effect on cell morphology. (Reprinted with the permission from Veiseh, O. et al . Inhibition of Tumor-Cell Invasion with Chlorotoxin-Bound Superparamagnetic Nanoparticles. Small (2009) 5: 256-264. Copyright © 2009, John Wiley and Sons.)

This nanoparticle system is designed to be detectable by both magnetic resonance imaging (MRI) and fluorescence microscopy and to specifically attack tumor cells. The peptide CTX selectively binds to the membrane-bound matrix metalloproteinase 2 (MMP-2) protein complex that is highly expressed on primary tumors of neuroectodermal origin, but not on normal CNS tissue. Unlike other ligands that only target certain types of brain tumors, CTX targets the majority of brain tumors as well as many other cancers (*e.g.*, breast, prostate, and colon cancers). In this design, the PEG coating not only provides a linkage between the nanoparticles and various functional payloads such as targeting ligands and therapeutic molecules, but also prevents the nanoparticles from aggregating in the blood stream. The PEG coating also increases nanoparticle blood circulation time and biostability, improves cellular penetration of the nanoparticles, and assists nanoparticles in crossing the BBB. Use of NIRF molecules minimizes autofluorescence interference from healthy brain tissue and allows visualization of tissues millimeters in depth resulting from the efficient penetration of photons in the near infrared range (Fig. 12).

Experiments conducted by Dr. Zhang's group showed significant preferential uptake of the nanoparticles by glioma cells over control nanoparticles. Specific targeting of tumor cells has been demonstrated successfully by comparing the uptake of the nanoparticle conjugates by glioma cells and control cells that lack MMP-2 receptors. *In vivo* results demonstrated that the nanoparticle conjugates passed the BBB and bound to genetically engineered intracranial medulloblastoma tumors in mice that bear an intact blood brain barrier. This multifunctional nanoparticle system can be used to detect tumors for diagnosis by MRI and to illuminate and discriminate tumor boundaries during tumor resection in real time using fluorescence imaging, which allows physicians to correlate preoperative diagnostic images with intraoperative pathology at cellular-level resolution.

Multifunctional Nanoparticles for Early Detection of Pancreatic Cancer

Pancreatic cancer has a devastatingly low survival rate (less than 5 percent after 5 years) because it is usually diagnosed at an advanced stage. Initial symptoms, such as pain, jaundice, or weight loss, often do not allow the disease to be caught early enough for surgery and chemotherapy to be effective. A research team from the Emory-Georgia Tech CCNE, led by Drs. Lily Yang, Hui Mao, and Shuming Nie has created tools for the early diagnosis of pancreatic cancer using targeted iron oxide nanoparticles that are clearly visible under magnetic resonance imaging (MRI). Additionally, these particles can be visualized using fluorescence optical imaging resulting from a near infrared dye attached to the targeting ligand.

By incorporating an amino terminal peptide of urokinase plasminogen activator (uPA) into their construct, the researchers were able to show that the particles bind to the uPA receptor (uPAR) and are selectively taken up by cancer cells and tumor endothelial cells but not normal pancreatic cells. Elevated levels of the uPA receptor are associated with tumor aggressiveness, distal metastasis, and overall poor patient prognosis. Significantly, the investigators found that uPAR targeted particles could discriminate between tumor cells and regular pancreatic cells irritated by chronic pancreatitis, a challenging task in clinical diagnosis. Xenograft tumors in nude mice as small as 1 millimeter in diameter can be detected using this approach and visualized by MRI or optical imaging. The technology now needs to be refined so that it is ready to test in patients. Groups of patients that are at increased risk of pancreatic cancer, such as those with inherited cancer risk factors, chronic pancreatitis, or new-onset diabetes, could benefit.

Recently this group has also used the same nanoparticles to visualize mouse mammary tumors derived by injection of human breast cancer xenografts. Figure 13 shows the near-infrared (NIR) imaging of these tumors. Conceivably these particles could be used to visual any tumor types that express significant levels of the uPAR.

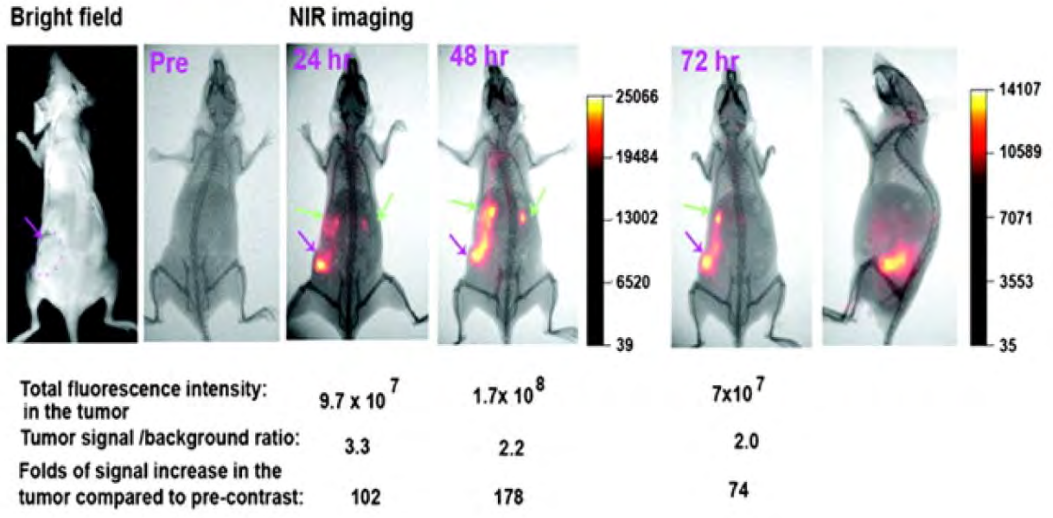

Figure 13. NIR optical imaging of tumor targeting and tissue distribution of nanoparticles over time. A nude mouse bearing a sub-cutaneous 4T1 tumor received 200 pmol per mouse of nanoparticles in 50 μL PBS, which was calculated from the iron oxide nanoparticle concentration. Pink arrows, tumor; green arrows, kidneys. Back and side images are at 72 h. (Reprinted from Yang, L et al. Receptor-targeted nanoparticles for in vivo imaging of breast cancer. Clin. Cancer Res. (2009)15: 4722-4732.)

Prevention and Control of Cancer

PRINT® Technology for Cancer Therapy and Imaging

The exploration and utilization of nanocarriers for the delivery of therapeutics *in vivo* has led to dramatic improvements in the efficacy of various therapies. Over the past few years, intense research and development of novel platforms has resulted in the creation of drug delivery vehicles such as polymeric nanoparticles, micelles, immunoconjugates, DNA-polymer conjugates, dendrimers, and liposomes. Clinically, the success of these carriers has been limited by the lack of control over size, chemical composition, uniformity, cell targeting, and cargo release dynamics. A particle fabrication technology, called PRINT® (**P**article **R**eplication **I**n **N**on-wetting **T**emplates), developed by Dr. Joseph DeSimone of the University of North Carolina CCNE takes advantage of the unique properties of elastomeric molds comprised of a low surface energy perfluoropolyether network to produce monodisperse, shape-specific particles from an extensive array of organic precursors (Fig. 14A).

Understanding the interdependent role of particle size, shape, surface, and matrix composition on the intracellular pathway will lead to a deeper knowledge of the fate of organic nanoparticles *in vivo*. The advent of "calibration quality" particles using PRINT® allows 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 "calibration quality" 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. Once the mechanisms of internalization are established, it will then be possible to use these findings to better engineer the intracellular release of specific cargos (Fig. 14BC). 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.

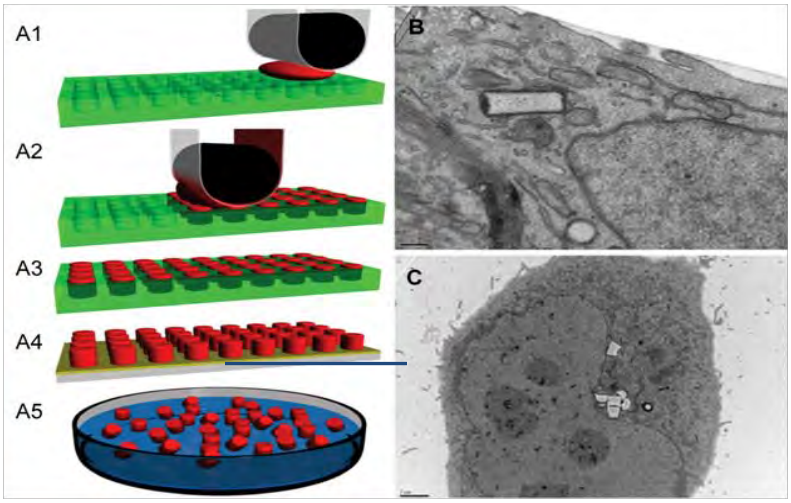

Figure 14: A1-5. Schematic representation of the PRINT process. A1. Empty mold (green), high surface energy polymer sheet (clear), roller (black) is brought into contact with the particle precursor solution (red) and the mold; A2. Roller evenly distributes particle precursor solution into the cavities 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. (Reprinted with permission from Canelas D et al. Top-down particle fabrication: control of size and shape for diagnostic imaging and drug delivery. Wiley Interdiscip Rev: Nanomed Nanobiotechnol (2009) 1: 391–404.Copyright © 2009 John Wiley & Sons, Inc.)

Rapid Isolation and Detection of Cell Free Circulating DNA Biomarkers and Nanoparticles Directly from Whole Blood

Cell-free circulating (cfc) DNA and other cellular nanoparticles represent an important class of biomarkers for the early detection of cancer. Unfortunately, because their isolation is complex, time consuming and expensive they have not been translated into a viable diagnostic. Recently, a new dielectrophoretic (DEP) method has been developed that allows cfc-DNA cellular nanoparticles to be directly isolated and detected from un-diluted whole blood. In earlier work, at AC frequencies in the 3000 – 10000 Hz range and with a 10 V peak-to-peak charge, the separation of cells and micron-sized particles into DEP low field regions and fluorescent nanoparticles into DEP high field regions was carried out in 1xPBS buffer. Under similar conditions high molecule weight (hmw) DNA nanoparticles could also be isolated and concentrated into high field DEP regions. Dr. Thomas Kipps' group from the UCSD CCNE has shown that the microarray DEP device can be used to rapidly isolate and detect hmw-DNA nanoparticles in the 40 to 200 nm range directly from whole blood, and that SYBR Green stained material can be isolated from chronic lymphocytic leukemia (CLL) patient blood samples. At DEP frequency of 5 to 10 kHz spiked OilGreen and SYBR Green fluorescent-stained hmw-DNA separates from the blood and becomes highly concentrated at specific DEP high field regions over the microelectrodes, while blood cells move to the DEP low field regions. The blood cells can then be removed by a simple fluidic wash step while the fluorescent stained hmw-DNA remains highly concentrated. The spiked hmw-DNA could be detected at a level lower than 260 ng/ml, which is within the range for viable clinical diagnostics. Fluorescent 40 nm nanoparticles (used as a model for drug delivery nanoparticles) could also be separated and

easily detected in whole blood. In blood, fluorescent nanoparticles could be detected at concentration of $9.5x10^9$ particles/ ml, well within the range for monitoring drug delivery nanoparticles; for example, the dosage for Abraxane is approximately $7.5x10^9$ particles/ ml blood. Dr. Kipps' team has also demonstrated the detection of significant amount of SYBR Green material from CLL patient samples versus normal blood samples. The effort of Dr. Kipps' group sets the stage for a new generation of diagnostics for early cancer detection.

Research Enablers

Deconstructing Directional Cell Motility in Metastasis through Nanopatterning

Surfaces that have been micropatterned with cell adhesive/non-adhesive regions allow for precise control of the cell's shape, internal organization and function, and motility parameters. In particular, substrates prepared by the reaction-diffusion ASoMic (Anisotropic Solid Microetching) method localize cells onto transparent microislands or tracks surrounded by an opaque, adhesion-resistant background. ASoMic is compatible with several important imaging modalities (*e.g.*, wide-field, digital fluorescence, total internal reflection fluorescence [TIRF] and confocal microscopies), and can be used to study and quantify various intracellular and cellular processes related to cell motility and metastasis. For cells constrained on the islands, the imposed geometry controls spatial organization of the cytoskeleton; analysis of populations of constrained cells eliminates noise. The transparency of the islands allows for real-time analysis of cytoskeletal dynamics. For cells on linear tracks, the high optical contrast between these adhesive regions and the surrounding non-adhesive background allows for straightforward quantification of the key parameters describing cell motility.

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 cover slips and either protecting unetched portions with oligo (ethylene glycol) alkane thiols that inhibit 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 on 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 (Fig. 15). Since this directionality of migration persists even in a mixed cell population (Fig. 15), 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, this type of bi-directional ratchet could be useful to partially purify populations of cells *in vitro*. Drs. Mrksich and Grzybowski have demonstrated that three-dimensional reconstruction of the shapes of cells immobilized onto micropatterned islands quantifies their mechanical properties and can be used to determine mechanical differences between metastatic and non-metastatic cells.

The investigators have also found that in cells on polygonal islands analysis of microtubule growth trajectories establishes a novel mechanism for microtubule targeting to focal adhesions and helps identify new targets for antimotility drugs, and that systems of cells on linear tracks provide information rich "signatures" of cell motility and can quantify cells' metastatic potential as well as cellular response to therapeutic agents. Both types of systems — islands and tracks — constitute new types of microassays in which properties of cancerous cells can be studied in quantitative detail using minimal numbers of cells.

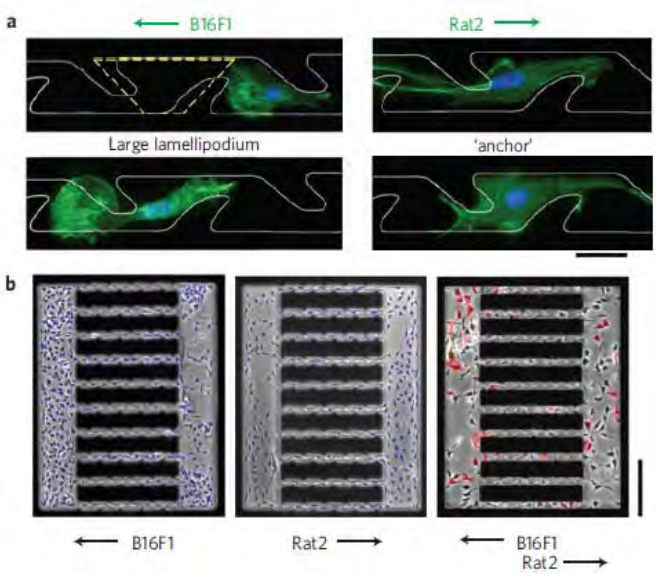

Figure 15: Cells on bi-directional 'lines-with-spikes' ratchets. A. B16F1 and Rat2 cells move in opposite directions on linear ratchets with spikes inclined at 45 degrees (staining: phalloidin, green; Hoechst 33342, blue). B16F1 cells (left column) and Rat2 (right). The trapeziod (delineated by a yellow dotted line) adhesive area is 1,300 um^2. The borders between adhesive and non-adhesive areas are outlined by white dotted lines. The scale bar corresponds to 30 um. B. Distribution of B16F1 (left), Rat2 (middle) and a mixture of B16F1 and Rat2 (right) cells over a pattern of two reservoirs connected by ten 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. (Reprinted with permission from Mahmud G et al. Directing cell motions on micropatterned ratchets. Nat Physics (2009) 5: 606-612. Copyright © 2009 Nature Publishing Group).

Cancer Antibody Functionalized Gold Nanopyramids

While it is a well-known fact that light can rapidly heat gold nanoparticles to a temperature that can kill tumor cells, Dr. Teri Odom and her colleagues at the Northwestern CCNE wondered if it was possible to improve the performance of these nanoscale thermal scalpels. The investigators kept the gold content (parts per million) constant while systematically addressing the roles of size, shell thickness, and surface shape in heat generation in response to near-IR light. Dr. Odom and her collaborators measured temperature changes of aqueous solutions containing various gold nanoparticles after exposures to either constant or pulsed lasers of increasing power. They found increasing laser power resulted in increasing temperature, but the constancy of exposure did not have an impact. The largest temperature changes and therefore photothermal response was garnered by smaller pyramidal nanoparticles (diameter 175 nm) with thin shells (20 nm) and with sharp tips. This work is important because it characterizes basic physical properties of particles and quantifies changes in efficiency of heat generation. Others in the field can use this as a reference when designing their own particles for thermal ablation studies.

Besides their potential use as anticancer therapeutics, pyramidal gold nanoparticles may also improve diagnostic assays that detect cancer biomarkers. Dr. Odom's team developed methods for adding a variety of molecules, including antibodies, to the surface of their gold nanopyramids and is now using the modified particles to image cancer cells. These studies have shown that the maximal light-scattering properties are seen using 250 nm pyramids that are oriented with their tips facing up. Dr. Odom and her colleagues have developed a wide-field imaging technique that rapidly and reliably determine the location, distribution, and orientation of the nanopyramids – and the molecules or cellular structures that they bind to – in intact cells. In addition, they have created nested pyramids in which a smaller shell is stacked inside a larger shell and whose flat faces are parallel and separated by a gap. These

particles exhibited plasmon resonances at both visible and near-infrared wavelengths. They also discovered that increasing the gap thickness to greater than 30 nm resulted in increased Surface Enhanced Raman Spectroscopy (SERS) intensities that exceeded those of thin (5 nm) gap nested nanopyramids. These SERS-tunable nanopyramids have potential for use in chemical or biological sensors.

Diagnostic Nanoarrays

A number of techniques including scanning probe microscopy and confocal microscopy have been developed to image biological entities on the nanoscale. These techniques are cumbersome, relatively time-consuming, costly, and require highly trained personnel. Moreover, the nanotechnology-based imaging techniques developed to date typically reveal the atomic or molecular structure of the specimen under examination but usually do not image other physical properties that have important biological implications. Dr. Stuart Solin and his team from the Washington University CCNE have been developing a series of inexpensive nano-sensors and nano-arrays that are designed to produce images of various physical properties of cancer cells with ultra high spatial and unprecedented temporal resolution. These new sensors and arrays are based on the recent discovery by Dr. Solin's group of electrical transport properties that depend on a device's physical and geometric characteristics. The physical contribution arises from a device's material properties, such as doping level, impurities, and bulk mobility. The geometric contribution comes from the configuration of the device, such as its dimensions, shape, lead contact area, and lead arrangement.

Dr. Solin and co-workers have shown that by careful design, the geometric contribution can be made to dominate the transport properties and have validated this notion by demonstrating a new class of "EXX" phenomena where E = Extraordinary and XX= magnetoresistance (MR), piezoconductance (PC), optoconductance (OC) or electroconductance (EC). They have further shown that EXX devices can function as very effective sensors of the relevant external perturbation, e.g. strain in the case of EPC. The scaling of EXX devices to the micro and nano regimes is a prerequisite to their use in the study of the biological properties of live cancer cells. These EXX devices were prepared using lattice matched GaAs epitaxial layers grown by molecular beam epitaxy (MBE) on semi-insulating GaAs substrates. The device has a simple design of a van der Pauw hybrid structure with a metal (Ti) and semiconductor (GaAs) in a close contact.

Nanoscopic devices having submicron dimensions and the EEC arrays were fabricated using three steps of aligned electron beam lithography and reactive ion plasma etching (RIE). The mesa of the device has been fabricated using negative electron beam resist Hydrogen silsesquioxane (HSQ), followed by controlled Inductively Coupled Plasma (ICP) RIE. The nanoscopic metal features for Ohmic leads and the shunt metal were accomplished by using positive electron beam resist, PMMA. The metallization was done using both thermal and e-beam evaporation. Finally, 250 nm of a Si3N4 dielectric layer was deposited on top of the device using the plasma enhanced chemical vapor deposition technique to isolate the electrical circuit of the devices from the aqueous environment necessary to study a living cancer cell. Individual EEC pixels have exhibited a field sensitivity of 3.05 V/cm whereas cancer cells have a known surface charge density that produces a field of order several kV/cm.

Publication Statistics

ANC investigators have been prolific in publishing their work and findings during the course of the program. After the first five years of the program, almost 1250 publications with an average impact factor of 7.4 were published; 20% of these publications had an impact factor above 10; an average of 5.8 publications were prepared per million dollars of funding.

Selected Publications with High Impact Factor

The research articles published in journals with impact factor above 10 are listed in *Appendix I*.

CHAPTER 4

Development of Translational Technologies

Reflecting an aggressive push for moving the innovative technologies and their proof-of-concept demonstrations in the investigators' laboratories, the ANC Program witnessed a substantial amount of work with animal models, experimentation with human clinical samples, and emerging human clinical trials.

Moving to the Clinic

The ultimate measure of successful research and development lies in its prospective clinical utility. To this end, the ANC has been structured to include researchers from several disciplines including oncology, cancer biology, engineering, chemistry, and physics. It also required that its CCNEs associate with university Cancer Centers and industrial entities involved in technology commercialization. Through the formation of this multi-faceted network, the ANC was able to create a pipeline ranging from technology discovery through technology proof-of-concept to prototype and product development. Currently, several nanotechnology-enabled diagnostic and therapeutic agents developed by ANC investigators have reached the clinical trial stage, and several others are nearing that goal.

Cyclodextrin for Delivering Camptothecin and siRNA

One of several advantages that nanotechnology offers is targeted delivery of therapeutic agents that were previously discarded because of insolubility, high molecular weight or deleterious side effects. Camptothecin is a naturally-occurring compound possessing highly potent anticancer properties against a broad spectrum of tumor cell lines. Camptothecin interrupts cell division and replication by inhibiting the enzyme topoisomerase 1. Unfortunately, camptothecin has significant pharmacological shortcomings, including very poor solubility in water and hydrolysis from its active lactone form to an inactive, yet toxic, carboxylate form. To overcome these hurdles, Calando Pharmaceuticals, founded by Caltech/UCLA CCNE investigator Dr. Mark Davis, has developed Cyclosert™. This novel delivery system is based on cyclic repeating molecules of glucose called β-cyclodextrins, PEG, and L-cysteine. These molecules were used to develop an entirely new, proprietary class of materials called linear cyclodextrin-containing polymers. Animal studies have confirmed that Cyclosert™ polymers are non-toxic and non-immunogenic, even after repeated administration. Cyclosert™ polymers have been synthesized at molecular weights ranging up to 100 kD and can be made biodegradable. The ability to use high molecular weight (>50 kD) Cyclosert™ for systemic drug delivery has the potential to slow renal clearance, increase circulation time, and improve passive accumulation of active drug at target tissues. Additionally, Cyclosert™ polymers can be tuned to be neutral, positively charged, or negatively charged. This feature is unique to Cyclosert™ technology and provides great flexibility for formulation and delivery.

Camptothecin is covalently attached to Cyclosert™ through a glycine linker, resulting in a 4000-fold increase in camptothecin

solubility while stabilizing it in its active lactone form. Nanoparticles of camptothecin conjugated to Cyclosert™, denoted as IT-101, are typically between 30 and 60 nm in diameter, which avoids rapid kidney clearance. Their hydrophilic character and close-to-neutral surface charge allows them to evade uptake by macrophages, which do not recognize them as foreign entities. Similarly, they can circulate for extended times in the blood stream. A long circulation half-life leads to a preferential accumulation of Cyclosert™ nanoparticles in tumors with abnormally leaky vasculature. Currently, Calando Pharmaceuticals is conducting an open-label, dose-escalation clinical phase I study of IT-101 in patients with solid tumor malignancies.

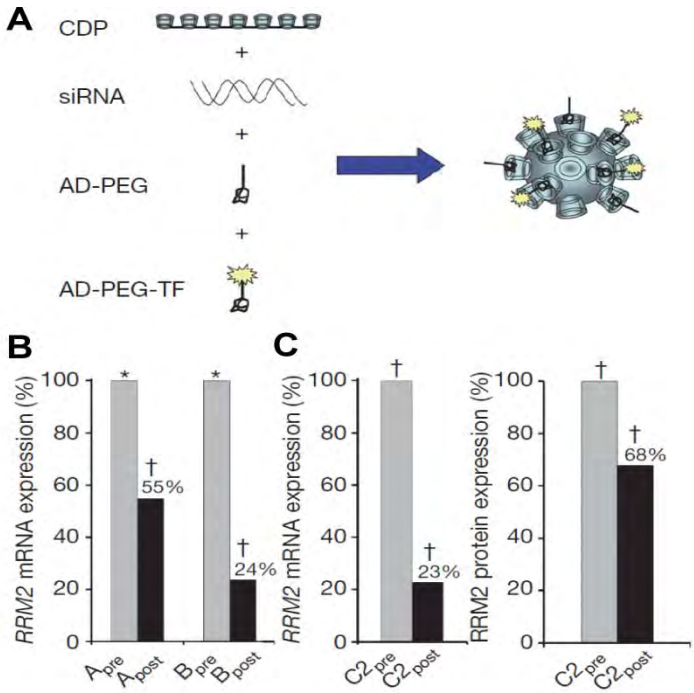

Figure 16: A. Schematic representation of targeted nanoparticles. B-C: RRM2 mRNA and protein expression in tumour tissue. B. qRT–PCR analysis of *RRM2* mRNA levels in samples from patients A and B before and after dosing. *RRM2* mRNA levels are normalized to TATA box binding protein (*TBP*) mRNA levels. 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 et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature (2010) 464: 1067-70. . Copyright© 2010 Nature Publishing Group).

The discovery of RNAi and, more specifically, the demonstration that siRNA could function in mammalian cells without eliciting an immune response opened the pathway for creating human therapeutics with siRNA. Potent siRNA molecules against almost any gene target can be found. However, a major hurdle to the creation of human therapeutics with siRNA is stabilizing the siRNA for delivery to its target. With the RONDEL™ (RNAi/oligonucleotide delivery) polymer delivery system, Calando Pharmaceuticals has developed a nanoparticle formulation of siRNA, denoted as CALAA-01. RONDEL™ siRNA delivery consists of mixing together siRNA, a short cyclodextrin-containing polymer, a PEG steric stabilization agent, and human transferrin as a targeting ligand for binding to transferrin receptors that are typically upregulated on cancer cells (Fig. 16). This four-component construct self-assembles into 60-80 nm nanoparticles. Upon delivery to the target cell, the targeting ligand binds to membrane receptors on the cell surface and the RNA-containing nanoparticle is taken into the cell by endocytosis. There, chemistry built into the polymer functions to release the siRNA from the delivery vehicle. These nanoparticles were rationally conceived to satisfy a range of pharmacologic and formulation considerations.

In collaboration with Dr. Antoni Ribas from the Caltech/UCLA CCNE, 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 (a first for systemically delivered nanoparticles of any kind). A reduction was found in both the specific messenger RNA and associated protein levels when compared to pre-dosing tissue (Fig. 17). Most notably, Dr. Davis' team detected the presence of an mRNA fragment that demonstrates that siRNA-mediated mRNA cleavage occurs specifically at the predicted site in a patient who received the highest dose of the nanoparticles. Together, the data demonstrates that siRNA administered systemically can produce a specific gene inhibition (reduction in mRNA and protein production) by an RNAi mechanism of action.

New PET Imaging Agent

The advent of imaging approaches such as Positron Emission Tomography (PET) has enabled noninvasive measurements of molecular and cellular processes throughout the body in preclinical and clinical settings. PET imaging has numerous diagnostic applications, allows early evaluation of treatment responses, and is useful in drug development by providing measurements of tissue pharmacokinetics and target occupancy. However, major challenges need to be addressed to widen the utility of PET in clinical practice. Areas of intense investigation concern the limited availability of PET probes for molecular and therapeutic stratification of disease processes, as well as for *in vivo* monitoring of immune responses.

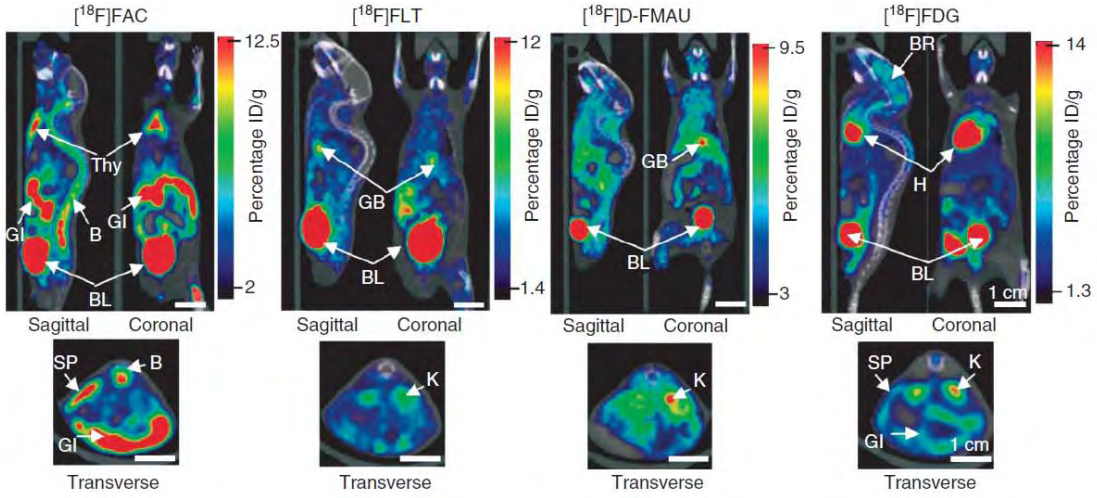

Figure 17: [18F]FAC has better selectivity for lymphoid organs compared with other PET probes for nucleoside metabolism and glycolysis. C57/BL6 mice were scanned by microPET-CT using [18F]FAC, [18F]FLT, [18F]DFMAU and [18F]FDG. Mice were imaged 60 min after intravenous injection of probes. B: bone; BL: 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 Radu CG et al. Molecular imaging of lymphoid organs and immune activation by positron emission tomography with a new [18F]-labeled 2-deoxycytidine analog. Nat Med (2008) 14: 783-788. Copyright © 2008 Nature Publishing Group).

Drs. Caius Radu, Owen Witte, and Michael Phelps at the NSBCC CCNE have recently developed a new positron emission tomography (PET) imaging agent, [18F]FAC (1-(2'-deoxy-2'-[18F] fluoroarabinofuranosyl) cytosine), using a microfluidic circuit for rapid radiochemical synthesis. This new PET probe allows visualization of immune organs, is sensitive to alterations in lymphoid mass and immune status, and can be used to monitor

immunosuppressive therapy. This probe accumulates in cells expressing high levels of deoxycytidine kinase (DCK), an enzyme in the nucleoside salvage pathway that is preferentially expressed in immune cells. FAC PET can highlight areas of immune activation *in vivo* during anticancer immune responses and in autoimmune disease (Fig. 17). In comparison to FDG, which accumulates to high levels in innate immune cells such as macrophages, FAC labels proliferating adaptive immune cells. Genetic deletion of DCK in mice results in loss of FAC signal, and these animals are deficient in producing cells of the adaptive immune response.

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, such as gemcitabine, cytarabine, or fludarabine, in a variety of cancers. In the current clinical trial, the biodistribution of D-FAC and L-FAC probes is being determined in eight healthy volunteers. Recruitment of patients with autoimmune disorders as well as patients with lymphomas, pancreatic, and ovarian cancer is underway.

Novel Nanotechnology-Based MRI Contrast Agent

Angiogenesis, the development of new blood vessels from preexisting vessels, is a key step in tumor growth, invasion, and metastasis formation. Inhibition of tumor angiogenesis is considered an attractive approach to suppress cancer progression and spread. Adhesion receptors of the integrin family promote tumor angiogenesis by mediating cell migration, proliferation, and survival of angiogenic endothelial cells. Integrins up-regulated and highly expressed on neovascular endothelial cells, such as $\alpha_v\beta_3$-integrins, have been proposed as relevant targets for anti-angiogenic therapies. Although small molecular integrin antagonists or blocking antibodies suppress angiogenesis and tumor progression in many well-defined animal models, anti-angiogenic compounds have had limited success in the clinic, in part resulting from an unpredictable heterogeneous response of spontaneous tumors to treatment. To date, patients have been treated with these new agents without regard to the induced expression level of their vascular targets by the tumors.

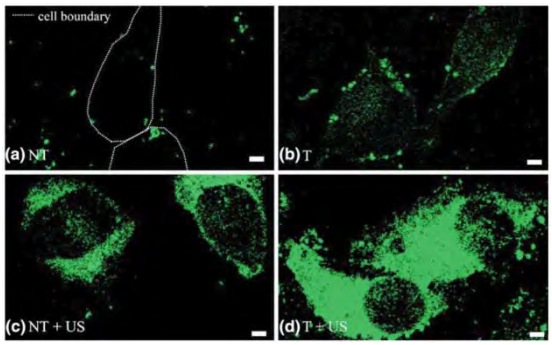

Figure 18: *Ultrasound energy* plus targeting augment lipophilic delivery to C32 melanoma cells. Fluorescent lipid transferred from nanoparticles is green. (a) Confocal micrographs under normal conditions for nontargeted (NT) cells show minimal nonspecific internalization (dashed line indicates cell boundary). (b) $\alpha_v\beta_3$ integrin targeted (T) cells demonstrate specific targeting of nanoparticles to cell surface (green dots) and delivery of lipids in cell cytoplasm (diffuse green). (c) and (d) After ultrasound (US) insonification for 5 min, marked enhancement of cytoplasmic lipid delivery (diffuse green) was observed for both nontargeted (c) and targeted (d) cells, with the most dramatic effect for the targeted nanoparticles *with insonification* (d). The cell components observed are the nucleus (dark circular region) cell cytoplasm (bright interior), and cell membrane (bright borders). Scale bar: 2 mm. (Reprinted from Kaneda MM et al. Perfluorocarbon Nanoemulsions for Quantitative Molecular Imaging and Targeted Therapeutics. Ann Biomed Eng (2009) 37: 1922-33. Copyright © 2009 National Library of Medicine).

Drs. Samuel Wickline and Gregory Lanza from the Washington University CCNE hypothesized that a palette of ligand-targeted nanoparticles would provide increased sensitivity for noninvasive early detection and characterization of nascent tumors (primary or metastatic), as well as targeted destruction of the cancer and its routes for metastasis. The versatility, ease of administration, and relative noninvasiveness of this agent should improve the diagnosis and

treatment of primary or recurrent tumors, even in outpatient settings.

To prove this hypothesis, Drs. Wickline and Lanza have developed integrin-targeted perfluorocarbon nanoparticles that combine molecular imaging with local drug delivery. This combination allows for the verification and quantification of therapeutic delivery, and additionally provides prognostic information about the expected response to treatment. Specifically, the ligand-targeted emulsion technology consists of a perfluorocarbon nanoparticle core surrounded by a lipid monolayer (Fig. 18). This lipid layer both stabilizes the particle and provides a virtually unlimited number of anchoring sites for targeting ligands and payload molecules. Water-insoluble payloads, such as chemotherapeutic agents, are incorporated into the lipid monolayer. The result is an oil-in-water emulsion of particles with an average size of approximately 250 nm. $\alpha_v\beta_3$-integrin is used as the targeting ligand and gadolinium-tetraazacyclododecanetetraacetic acid (Gd-DOTA), an imaging agent, is used as the payload. Drs. Wickline and Lanza demonstrated that the constructed perfluorocarbon nanoparticles are able to detect tumors by imaging angiogenesis via the $\alpha_v\beta_3$-integrin biomarker and can recognize tumors as small as 1-2 mm in size. These nanoparticles can also prove useful in monitoring response to anti-angiogenic therapies. The investigators are currently testing this technology in clinical settings in Australia and the United States with Kereos, Inc., co-developer of the technology.

Superparamagnetic Nanoparticles for Detection Lymph Node Metastases

Magnetic nanoparticles (MNPs) have become important tools for clinical cancer imaging. A number of monocrystalline iron oxide preparations have been developed over the last decade and shown to significantly improve the accuracy of nodal cancer staging. Despite these advances, the full potential of next generation targeted MNPs for clinical use has not yet been realized. This will require development of more efficient *in vivo* targeting strategies through clever synthetic approaches to optimize pharmacokinetics for imaging, minimize nonspecific macrophage/monocyte uptake, and maximize target uptake by harnessing cellular internalization and trapping, and multivalency.

Recently, Dr. Ralph Weissleder and his team from the MIT-Harvard CCNE have developed intravenously administrable lymphotropic contrast agents, such as long circulating dextran-based iron oxides (LCDIOs). This technology has prompted several clinical trials to determine the efficacy of contrast-enhanced (CE) lymph node imaging. LCDIOs have a monocrystalline, inverse spinel, superparamagnetic iron oxide core coated with densely packed dextrans to prolong their time in circulation. The LCDIO interact strongly with the magnetic field in an MRI instrument, with each nanoparticle acting as a single large magnet. The nanoparticles slowly accumulate in macrophages of normal lymph nodes, decreasing nodal signal intensity on T_2-weighted images and causing nodes to appear homogeneous and hypointense. Nodes completely replaced by tumor lack uptake of LCDIO, and generally remain homogeneous and hyperintense by T_2-weighted imaging. Signal intensity changes are relatively easy to interpret in normal nodes or nodes completely replaced by tumor (Fig. 19). Dr. Weissleder is now leading a clinical trial to determine if LCDIO can be used to identify small and otherwise undetectable lymph node metastasis in patients with prostate cancer.

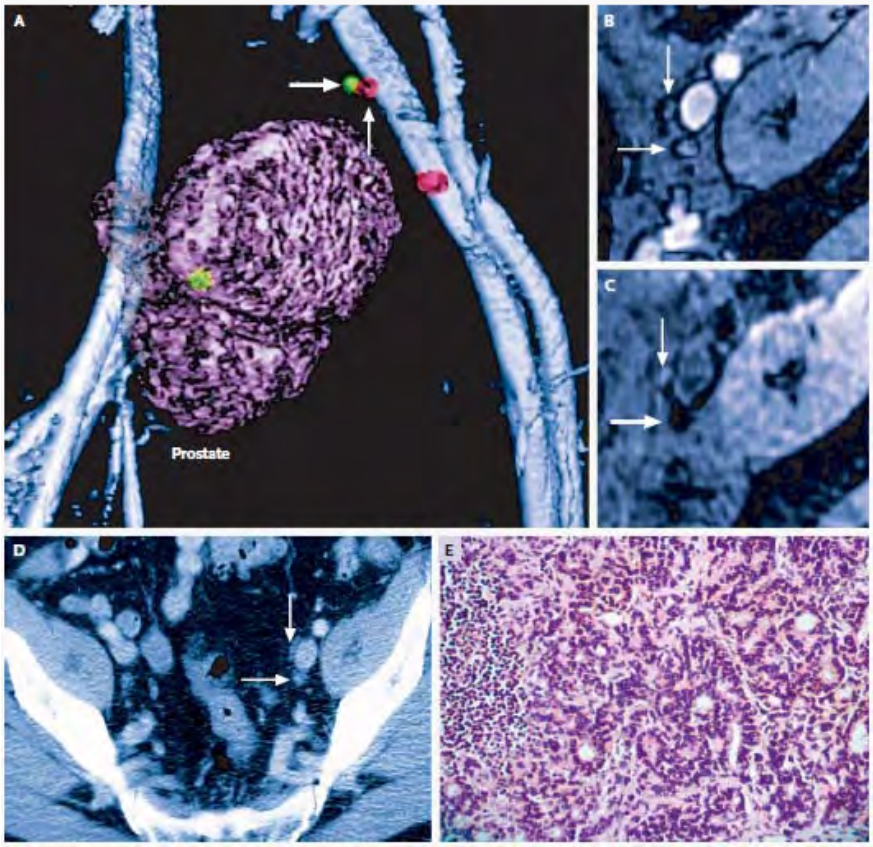

Figure 19: Three-Dimensional Reconstruction of Pelvic Lymph Nodes (Panel A), Conventional MRI (Panel B), MRI with Lymphotropic Superparamagnetic Nanoparticles (Panel C), Abdominal Computed Tomography (CT) (Panel D), and Histopathological Findings (Panel E). Panel A shows a three-dimensional reconstruction of the prostate, iliac vessels, and metastatic (red) and nonmetastatic (green) lymph nodes, to assist in the planning of surgery and radiotherapy. There is a malignant node (thick arrow) immediately adjacent to the normal node (thin arrow) posteromedial to the iliac vessels. In Panel B, conventional MRI shows that the signal intensity is identical in the two nodes (arrows). In Panel C, MRI with lymphotropic superparamagnetic nanoparticles shows that the signal in the normal node is decreased (thick arrow) but that it is high in the metastatic node (thin arrow). In Panel D, abdominal CT fails to differentiate between the two lymph nodes (arrows). In Panel E, histopathological examination of the malignant lymph node reveals sheaths of carcinoma cells (hematoxylin and eosin, ×200). (Reprinted from Harisinghani MG et al. Noninvasive Detection of Clinically Occult Lymph-Node Metastases in Prostate Cancer. N Engl J Med (2003) 348:2491-2499. Copyright © 2003 New England Journal of Medicine).

Polymeric Nanoparticles for Targeted Anticancer Drug Delivery

Nanotechnology approaches in which a constant dose of chemotherapy is delivered directly to cancer cells over an extended period may result in alternative or complementary therapeutic options for patients with early-stage cancer. The challenge lies in the design of nanoparticles that are specifically and differentially taken up by the targeted cells and release their payload over an extended period to achieve a clinical response. Using prostate cancer as a model cancer and the following design criteria, Drs. Robert Langer and Omid Farokhzad from the MIT-Harvard CCNE aimed to develop drug-encapsulated nanoparticles for prostate cancer targeting. The first design criterion was the use of biodegradable and biocompatible

components that were previously approved by the FDA for clinical use. They chose poly(d,l-lactic-*co*-glycolic acid) (PLGA) as the controlled release polymer system because its safety in clinical use has been well established.

The second criterion was surface functionalization with nucleic acid ligands known as aptamers for targeted delivery and uptake in a cell-specific manner. Aptamers are DNA or RNA oligonucleotides that, through intramolecular interactions, fold into unique tertiary conformations capable of binding to target antigens with high affinity and specificity, analogous to antibodies. The researchers chose aptamers as targeting molecules because this class of ligands, unlike antibodies, is non-immunogenic and exhibits remarkable stability in a wide range of pH≈(4 –9), temperature, and organic solvents without loss of activity.

The third design criterion was resistant to uptake by tissue macrophages and by non-targeted cells, thus increasing nanoparticle residence time at the site of administration. They chose to develop poly(ethylene glycol) (PEG)-functionalized nanoparticles because they had previously shown that pegylated polymeric nanoparticles are considerably more effective against systemic clearance than similar particles without PEG. PEG has also been used to improve the pharmacokinetic properties of liposomes, macromolecules, and small molecule drugs.

The fourth criterion was use of a chemotherapeutic agent currently in clinical use for the management of prostate cancer. Docetaxel, when used systemically, can prolong the survival of patients with hormone-resistant prostate cancer. The investigators postulated that controlled release of docetaxel targeted to prostate cancer cells could result in enhanced cytotoxicity and antitumor efficacy, making it a potential therapeutic modality for the management of localized prostate cancer. The combination of the above design criteria may facilitate the translation of therapeutically effective nanoparticle-aptamer bioconjugates into clinical practice.

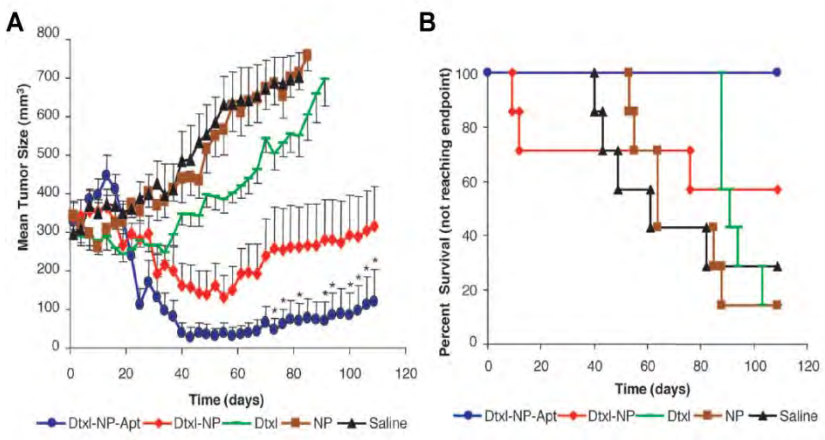

Figure 20: Comparative efficacy study in LNCaP s.c. xenograft nude mouse model of prostate cancer (PCa). A. PCa was induced in mice by implanting LNCaP prostate epithelial cells s.c. in the flanks of nude mice and allowing the tumors to develop to appreciable size over 21 days (≈300 mm^3). The comparative 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 and demonstrated that targeted NPs are significantly more efficacious in tumor reduction as compared with other groups. Data represent mean ± SEM of seven mice per group. B. The Kaplan–Meier survival curve demonstrates that 100% of the Dtxl-NP-Apt group was alive on day 109, whereas the other groups had animals reaching our study end points and were euthanized on various days throughout the study period (end points defined as tumor load of 800 mm^3 or BWL >20%). (Reprinted with permission from Farokhzad OC et al. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy *in vivo*. PNAS. (2006) 103: 6315-20. Copyright © 2006 National Academy of Sciences USA).

Drs. Langer and Farokhzad encapsulated rhodamine-labeled dextran (as a model drug) within nanoparticles formulated with poly(d,l-lactic acid) (PLA)-*b*-PEG block copolymer and surface functionalized these nanoparticles with nuclease-stabilized A10 2′-fluoropyrimidine RNA aptamers that recognize the extracellular domain of the prostate-specific membrane antigen (PSMA). PSMA is a well characterized antigen expressed on the surface of prostate cancer cells that participates in membrane recycling and becomes internalized through ligand-induced endocytosis. Their data demonstrated that these fluorescently labeled, targeted nanoparticle-aptamer bioconjugates differentially bound and got taken up by LNCaP prostate epithelial cells, which express the PSMA protein efficiently and with high specificity. No binding or uptake was detected in PC3 prostate epithelial cells, which do not express the PSMA protein. The team has also developed docetaxel-encapsulated, PEGylated PLGA nanoparticle-aptamer bioconjugates that bind to the PSMA protein on the surface of prostate cancer cells, resulting in significantly enhanced cellular toxicity as compared with non-targeted nanoparticles. This construct exhibited improved efficacy and reduced toxicity *in vivo*. Moreover, after a single intratumoral injection of this construct containing docetaxel, 100% of mice used in the experiment survived the 109-day study. In contrast, docetaxel alone had a survivability of only 14% (Fig. 20).

This technology is being developed by BIND Biosciences. The company's lead compound that targets solid tumors is planned to enter clinical development at the end of 2010. To read more about BIND Biosciences, please go to page 67.

Carbon Nanotube X-ray Source

Clinical trials are scheduled to begin in late 2010 on a new type of CT scanner, developed by Dr. Otto Zhou at the UNC CCNE that uses carbon nanotubes (CNTs) as the x-ray source. This new scanner, developed through a joint venture with Xintek, founded by the UNC CCNE members, and Siemens, a leader in medical imaging, contains 52 nanotube x-ray sources and detectors arranged in a ring, a configuration that eliminates the need to move the x-ray source and increases the precision and speed of CT scanning, which in turn, could make CT scanning a preferred method for detecting small tumors.

Figure 21: Volume-rendered image of the 600-view FDK reconstruction. (Reprinted with permission from Bian J et al. Investigation of Sparse Data Mouse Imaging Using Micro-CT with a Carbon-Nanotube-Based X-ray Source. Tsinghua Sci Technol. 2010 February 1; 15(1): 74–78. Copyright © 2010 National Library of Medicine).

The basic design of the x-ray tube has not changed significantly since x-rays were first developed into an imaging tool: a thermionic cathode is used to produce electrons that strike on a metal target to generate x-ray. This design has several intrinsic drawbacks that have limited the effectiveness and advancement of x-ray technologies. These limitations include the high cathode operating temperature (~1000°C), which prevents miniaturization and novel source configurations that can increase imaging speed and accuracy; high imaging doses that can cause radiation damage; and low temporal and spatial resolution, which affects the size and accuracy of the features that can be detected. CNT-based field emission x-ray sources have the potential to not only overcome these limitations but also enable new novel imaging modalities (Fig. 21). For example, traditional CT

scanners use a single x-ray source that takes approximately 1,000 images from multiple angles by mechanically rotating either the x-ray source or the object being scanned at high speed. Dr. Zhou and colleagues created a scanner with multiple x-ray sources, called a multipixel scanner. The machine required no mechanical motion but switched rapidly among many x-ray sources, each taking an image of the object from a different angle in fast succession. The team's newest innovation combines this multiple-x-ray-source innovation with a principle called multiplexing, in which all the x-ray sources are turned on simultaneously to capture images from multiple views at the same time. This new technology should reduce imaging time and radiation dose and improve image resolution by reducing motion-induced blur.

Surface Enhanced Raman Spectrometry Gold-Based Nanoparticles for Colorectal Cancer Detection

Molecular imaging of living subjects provides the ability to study cellular and molecular processes that have the potential to impact many facets of biomedical research and clinical patient management. Imaging is currently possible by using positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), computed tomography (CT), optical bioluminescence and fluorescence, high frequency ultrasound (HFUS), and several other emerging modalities. However, no single modality currently combines high sensitivity, high spatial and temporal resolution, and high multiplexing capacity, low cost and high-throughput capability.

Dr. Sanjiv Sam Gambhir and his colleagues at the Stanford CCNE have attempted to develop new imaging strategies having all of these capabilities. Their approach has been to use Raman spectroscopy, a well-established bioanalytical tool with many instrumental advantages including excellent sensitivity to small structural and chemical changes, minimal sample preparation, and high spatial resolution. Raman spectroscopy can also differentiate the spectral fingerprint of many molecules, resulting in very high multiplexing capabilities. Narrow spectral features are easily separated from background broadband autofluorescence since Raman is a scattering, not absorption/emission, phenomenon. In addition, Raman-active molecules are more photostable than most fluorophores, which are rapidly photobleached.

Although Raman scattering is a very inefficient process (only one in ten million photons is inelastically scattered), the use of surface enhancers such as gold or silver nanoparticles effectively increases the Raman scattering process by several orders of magnitude, thus enabling pM sensitivity. This effect is known as surface enhanced Raman scattering (SERS) and is a result of a plasmonic phenomenon where molecules absorbed onto nano-roughened noble metal surfaces experience a dramatic increase in the incident electromagnetic field producing high Raman intensity. For years, scientists have reported the use of SERS to image biological processes within living cell cultures and excised tissues.

Dr. Gambhir's team has harnessed this SERS phenomenon through its use of gold nanoparticles for *in vivo* diagnostic applications. His team has developed ten different Raman nanoparticles consisting of a 50-nm gold core with a unique Raman active layer absorbed onto the gold surface and coated with glass, for a total diameter of 120 nm. Each of these ten nanoparticles has a unique Raman spectral fingerprint, which allows for multiplexed imaging. So far, Dr. Gambhir's team has demonstrated that an optimized Raman microscope has the potential to non-invasively image deep tissues as well as multiplex 10 spectrally-unique batches of SERS nanoparticles in living mice (Fig. 22).

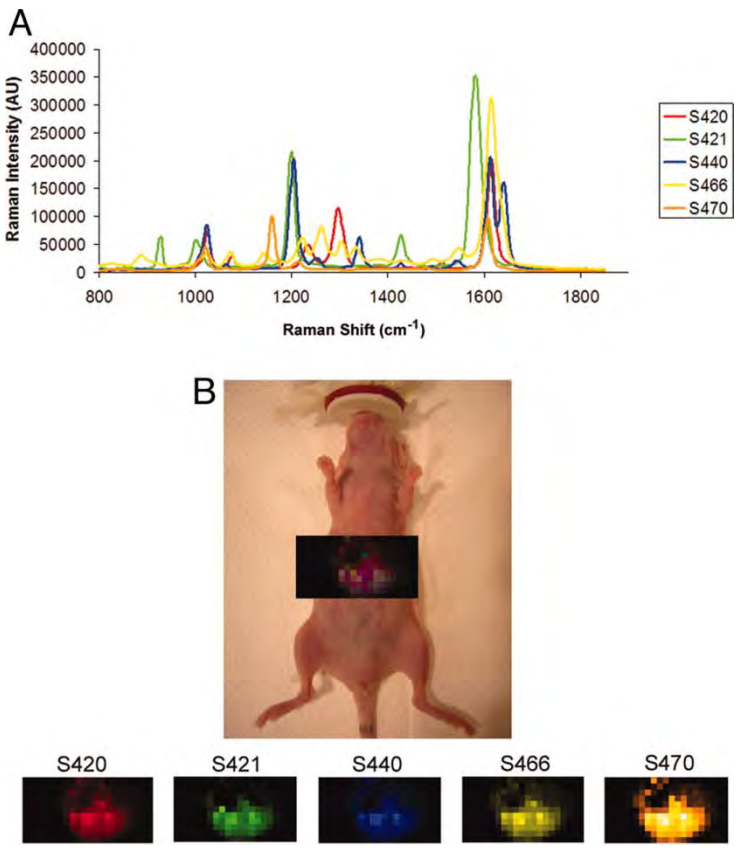

Figure 22: Demonstration of deep-tissue multiplexed imaging 24 h after i.v. injection of five unique SERS nanoparticle batches simultaneously. (A) Graph depicting five unique Raman spectra, each associated with its own SERS batch: S420 (red), S421 (green), S440 (blue), S466 (yellow), and S470 (orange). It is noteworthy that their peaks have very little spectral overlap, allowing easier spectral unmixing and resulting in better deep-tissue detection. (B) Raman image of liver overlaid on digital photo of mouse, showing accumulation of all five SERS batches accumulating in the liver after 24 h post i.v. injection. Panels below depict separate channels associated with each of the injected SERS nanoparticle batches. Individual colors have been assigned to each channel, and the resulting mixture shows a purple color that represents a mixture of the five SERS nanoparticle batches accumulating simultaneously. It should be noted that all channels show accumulation in the liver; however the channels are not all homogenous in their distribution throughout the liver. (Reprinted with permission from Zavaleta CL et al. Multiplexed imaging of surface enhanced Raman scattering nanotags in living mice using noninvasive Raman spectroscopy. PNAS (2009) 106: 13511-13516. Copyright © 2009 National Academy of Sciences USA).

In collaboration with Oxonica, Inc., Dr. Gambhir has developed a Raman endoscopic probe that could be sent through an accessory channel of a clinical endoscope/colonoscope for early detection of gastrointestinal cancers. It is intended to use this Raman endoscope in conjunction with Raman active gold nanoparticles targeted to a specific cancer to detect early stage and flat lesion cancers with greater sensitivity that what is currently used. Currently, Dr. Gambhir is in the process of getting FDA approval for using this technology in a clinical trial for detection of colorectal cancer.

Chemically Engineered Adenovirus Nanoparticles to Improve Immune Gene Therapy in Chronic Lymphocytic Leukemia

Effective and non-toxic therapies against cancer could be achieved by activating the human immune system to generate a host anti-tumor immune response. Towards this end, Dr. Thomas J. Kipps and his research group at the UCSD CCNE have focused on using adenovirus vectors to develop a new series of molecules that improve the therapeutic potential of various tumor necrosis factor (TNF) molecules, a large family of proteins with diverse roles in immune regulation in chronic lymphocytic leukemia (CLL). Adenovirus vectors are potentially suitable for transferring genes into CLL B cells. Adenovirus is a 36-kb double-stranded DNA

virus that can infect many different types of cells efficiently and with low pathogenicity. In particular, adenovirus vectors can introduce foreign genes into non-replicating cells, theoretically making them good candidate vectors for gene transfer into postmitotic CLL B cells. Another advantage is that adenovirus vectors are relatively stable and can be produced and concentrated to high titers, making it feasible to infect cells at a relatively high multiplicity of infection (MOI).

The first TNF family derived molecule developed by Dr. Kipps' team is a recombinant protein that binds and activates human CD40 B lymphocytes. This molecule (ISF35) constructs replication defective adenovirus vectors using serotype 5 adenovirus in which the E1 region of the virus genome was replaced with the gene encoding either human or mouse CD154 flanked in the 5′ direction with a heterologous cytomegalovirus promoter, and in the 3′ direction with a bovine polyadenylation signal (Ad-CD154). Since soluble mouse CD154 binds human CD40 and mouse CD154 was found to be expressed more effectively on human B leukemias and lymphomas than human CD154, the mouse CD154 was used in these studies. This also allowed them to distinguish the transgene product from endogenously expressed human CD154 in transduced human cells.

Construct ISF35 is a novel CD40-binding protein designed to maximize stable, high-level surface-expression of this potent immuno-stimulatory molecule on cells transduced to express this protein. As a single agent in pre-clinical studies, ISF35 has demonstrated compelling activity against B cell leukemias and lymphomas resulting in acute and long lasting responses against these diseases. The *in vivo* activation of malignant B lymphocytes and the up-regulation of pro-apoptotic factors by ISF35 enhance the sensitivities of these cancers to standard therapies including patients who have developed resistance to standard treatment protocols.

In an ongoing Phase I clinical study, which is conducted by Memgen (the company that commercially develops ISF35), patients with CLL received intravenous infusions of autologous leukemia cells transduced *ex vivo* to express ISF35 using a replication-defective adenovirus vector (Ad-ISF35). This treatment was well tolerated, did not have dose-limiting toxicity, and had apparent clinical activity. Moreover, injection of autologous, ISF35-expressing CLL cells induced upregulation of death receptors and pro-apoptotic proteins on bystander, non-infected CLL cells, resulting in acute cytoreductions in leukemia cell counts and reductions in the size of lymph nodes and spleen of the treated patients.

The benefit of the technology developed by Dr. Kipps' team is not limited to CLL treatment. Because it is a potent immunostimulatory molecule, this approach could provide the foundation for therapies directed toward solid tumor cancers such as malignant melanoma, breast, colon, ovarian, and lung cancers.

Nanotechnology Characterization Laboratory

Recognizing the need for accelerated clinical translation of promising nanotechnology-based therapies and diagnostics, the NCI established the NCL in 2004 as part of the ANC. Soon after its launch, the NCL entered into a formal intra-governmental collaboration with the FDA and NIST.

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, allowing NCL to support the most promising and innovative new nanomedicines. 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. Nanomaterials are selected for characterization through an application process in which applications are evaluated based on published criteria, with a focus on demonstrated proof-of-concept anti-cancer efficacy and potential for clinical translation. 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 has recently begun collaborating with FDA's National Center for Toxicological Research (NCTR) in Jefferson, AR. 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). Additionally, in collaboration with NIST and ASTM, NCL coordinated an inter-laboratory study involving more than 60 labs that helped to expose sources of data variability in experiments on nanomaterials.

To date, several ANC Investigators have submitted nanomaterials to the NCL for pre-clinical characterizations, including:

The founders of BIND Biosciences, Drs. Robert Langer and Omid Farokhzad from the MIT-Harvard CCNE, have been collaborating with the NCL to examine the extent to which BIND's particles evade the immune system, bind to target sites, accumulate in target tissues, and provide the desired drug release profile.

Kereos Inc., funded by Drs. Greg Lanza and Samuel Wickline at the Washington University CCNE, has recently developed a nanoemulsion with melittin (a cationic peptide derived from the venom of the honeybee) for cancer therapy. The NCL is currently evaluating these nanomaterials.

Arsenic trioxide (ATO) is one of the front-line therapies for treatment of acute promyelocytic leukemia. Dr. Thomas O'Halloran and colleagues at the Northwestern University CCNE have developed a nanobin formulation of arsenic trioxide. The encapsulation of the arsenic inside the nanobin is intended to modulate its toxicity and improve its efficacy. NCL began characterizing Dr. O'Halloran's arsenic nanobins in February of 2009. Since then, the NCL has conducted physicochemical characterization, *in vitro* immunological characterization, *in vitro* toxicological characterization, and four *in vivo* studies. The NCL's physicochemical characterization of the arsenic nanobins included hydrodynamic size measurements, particle stability studies, and zeta potential measurements using dynamic light scattering (DLS), as well as measurement of the concentrations of arsenic and other metals using inductively coupled plasma mass spectrometry (ICP-MS). The NCL's *in vitro* toxicological evaluation compared the toxicity of the nanobins to approved forms of arsenic trioxide in a variety of *in vitro* cell lines. *In vitro* immunological characterization included testing of the sterility, pyrogenicity, and blood contact properties of the arsenic nanobins, as well as examining their effects on immune cell function.

Dr. Sanjiv Sam Gambhir from Stanford CCNE has developed nanoscale spherical gold silica composites for Raman image-guided surgery. Dr. Gambhir submitted nanomaterials to the NCL in May of 2010 and NCL has conducted a three-day acute toxicological mass balance *in vivo* study and is analyzing the distribution of the particles to tissue using inductively-coupled plasma mass spectrometry (ICP-MS).

The NCL has also collaborated with Dr. Mansoor Amiji from the Northeastern CNPP. Dr. Amiji has produced chemotherapeutic-loaded nanoemulsions that may be able to deliver certain cancer drugs across the blood brain barrier for the treatment of glioblastoma. The NCL has assisted Dr. Amiji with physicochemical, *in vitro* immunological, and *in vitro* toxicological characterization of several nanoemulsion production lots. In 2008, the NCL developed a high pressure liquid chromatography (HPLC) method for detecting paclitaxel in biological matrix. This method has

proved useful for *in vivo* studies on a variety of paclitaxel-containing nanoparticles, including Dr. Amiji's paclitaxel-containing nanoemulsions.

Avidimer Therapeutics, Inc. was a spin-off company of Dr. James Baker Jr. from the University of Michigan CNPP. In May of 2007, NCL began assisting Dr. Baker with the preclinical characterization of the folate-targeted, methotrexate-conjugated dendrimer for targeted drug delivery. The NCL characterized dendrimers with spectroscopic and chromatographic methods to determine the number of targeting and drug molecules conjugated to the dendrimer and evaluated the targeting efficiency of these dendrimers in *in vitro* cell lines. In 2008, NCL performed an *in vivo* repeat-dose efficacy study, administering these dendrimers intravenously to mice bearing nasopharyngeal carcinoma xenografts and monitoring the animals' body weight, tumor size, and tumor ulceration.

Dr. Kattesh Katti from the University of Missouri CNPP has developed gum arabic-stabilized gold nanoparticles for liver imaging. He submitted nanomaterials to the NCL in May of 2007 and the NCL characterized the size, sterility, and blood-contact properties of two nanoparticle samples.

Dr. Miqin Zhang from the University of Washington CNPP has developed chlorotoxin-conjugated iron oxide theranostic nanoparticles. Dr. Zhang submitted nanomaterials to the NCL in July of 2010, and the NCL is characterizing their physicochemical and *in vitro* properties.

CHAPTER 5

Technology Transfer and Commercialization

In previous chapter, we described academic efforts to develop technologies with a translational value. The translational process and path to commercialization is achieved predominantly through small start-up companies, which are span-off from university laboratories. ANC investigators have produced extensive portfolios of patent filings and disclosures – over 250 total – that are listed in *Appendix II.* These patents are the foundation for technology licensing to spin-off companies.

ANC Industrial Partnerships and Company Profiles

Overall, ANC investigators have played founding or key technological roles in at least fifty (50) industrial companies, an outstanding achievement considering that the ANC initiative began only five years ago. Furthermore, large and established companies also recognized the promise of nanotechnology and have established direct involvement in the ANC by assigning their own researchers to projects within both CCNEs and CNPPs. The following section profiles, several partnerships and small companies that have been associated with the ANC program. *Appendix III* provides an abbreviated summary of industrial entities that are associated with the Program.

Affinity Biosensors' core technology, the suspended microchannel resonator (SMR), was invented in 2003 by ANC investigator Dr. Scott Manalis and his research group at the MIT. In 2005, Dr. Manalis partnered with Dr. Ken Babcock and MEMS manufacturer Innovative Micro Technology (IMT) to obtain a 3-year, $2.04 million grant from the Institute for Collaborative Biotechnologies, for which Dr. Babcock serves as the principal investigator. A key goal of this program was to develop a commercial fabrication process for SMR sensors, which was completed in spring 2006. The resulting sensors exceeded expectations in performance and manufacturing yield. Based on this success, Drs. Manalis and Babcock founded Affinity Biosensors in April 2006 to develop and market whole products that utilize SMR mass sensing as the core element. Innovative Micro Technology will be Affinity Biosensors' strategic supplier of the SMR sensor "chips." Affinity Biosensors first explored business opportunities in biomolecular detection and diagnostics.

During summer 2006 it became clear that the validation needed to initiate such a business would possibly require years of basic research in the Manalis laboratory and additional collaborative effort, and that the business propositions were themselves long term. At the same time, the Manalis group demonstrated breakthrough measurements of particle characteristics with SMR, and Dr. Babcock recognized that instruments for particle metrology could form the basis for a compelling business opportunity that could be pursued in relatively short order. Considerable effort was then expanded to acquire additional validation data, develop a business plan, and garner information from particle engineering experts and potential customers. In the past, Affinity Biosensors had successfully obtained SBIR Phase

I and Phase II grants as well as initial venture funding.

AuraSense, LLC, is a biotechnology company founded by Drs. Shad Thaxton and Chad Mirkin at Northwestern CCNE to develop and exploit the commercial potential of biofunctionalized nanoparticles. These nanoparticles are biocompatible and possess versatility to be used as therapeutics and intracellular assays for combating a broad range of diseases, including heart disease, cancer, and bacterial infection. For example, AuraSense is developing a technology that uses gold nanoparticles to sweep cholesterol out of a patient's bloodstream. The nanoparticles are designed to work like high-density lipoproteins (HDL), which can help protect the body from heart disease or stroke. Also, these nanoparticles include constructs that overcome one of the most difficult obstacles to gene regulation: safe and effective delivery into cells. AuraSense particles exhibit high stability, high binding specificity, and unparalleled transfection efficiency into numerous cell and tissue types. Needing no carriers or transfection agents, they provoke minimal immune response and no known toxicity.

Avidimer Therapeutics was formed in December 2003 to develop novel nanoscale targeted therapeutics based on the dendrimer technology invented by ANC investigator Dr. James Baker, Jr. Dr. Baker invented avidimer technology at the University of Michigan, where he is the Ruth Dow Doan Professor of Medicine and Head of Allergy and Immunology at the University of Michigan Medical School. Dr. Baker is also Director of the Michigan Nanotechnology Institute for Medicine and Biological Sciences (M-NIMBS), which is focused on the use of nanotechnology in biomedical applications, and serves as Avidimer's chairman and chief scientific officer.

Dendrimers, nanometer-size polymers that serve as an inert scaffolding, are the key to Avidimer Therapeutics technology and are well-suited to serve as a foundation for treating cancer cells without harming healthy cells. At approximately 5 nm in diameter, dendrimers are about the same size and shape of a hemoglobin molecule. Therapeutic and/or diagnostic agents are covalently attached to this scaffolding through chemical linkers. Additionally, targeting vectors - molecules that associate strongly with cell surface markers expressed selectively on disease cells (*e.g.*, cancer) and not on healthy cells or tissues - are attached to the dendrimers. These targeting vectors serve as a precision guidance system that directs the system to sites of disease while ignoring or bypassing healthy tissue.

The constructs formed by simultaneously attaching targeting vectors, drugs, and imaging agents to dendrimers are referred to as avidimers (*i.e.*, "smart drugs"). A fully configured avidimer is less than 15 nm in diameter; nevertheless, its shape and flexibility permit the Avidimer to maneuver freely through the body without encountering the resistance to extravasation, target recognition, and cellular uptake observed with carrier systems of larger dimensions. Avidimers are excreted primarily in the urine in less than 72 hours without causing apparent harm to test subjects. In the context of cancer, avidimers offer dramatically improved tumor-specific delivery, which should result in improvements in both efficacy and safety relative to the corresponding untargeted drugs. Additionally, by incorporating an anticancer drug into an avidimer, the drug's biodistribution can be altered in a manner controlled by the targeting vector, thus potentially broadening its spectrum of activity to include tumor types to which the untargeted drug fails to show activity.

One other strength of Avidimer Therapeutics' technology is that it is capable of creating avidimers with multiple copies of a targeting vector and drug to the dendrimer. The ability to attach multiple targeting vector molecules results in significantly tighter binding of the avidimer to complementary cell surface markers (i.e., greater avidity) and higher efficiency of internalization of the avidimer into target cells. By having multiple copies of the drug bound to the avidimer, the delivery payload is increased significantly. This capability to attach multiple molecules of both the chosen targeting vector and drug is particularly valuable for therapeutics intended to treat cancer, where high intra-tumoral pressures create substantial opposition to the delivery of traditional particulate systems. In addition, by selecting appropriate linker chemistries, avidimers can be

designed to either retain the attached drug on the dendrimer throughout its lifetime or allow the drug to disassociate (*i.e.*, cleave) from the dendrimer following delivery to the intended target. Maintaining the drug attached to the dendrimer in transit to the disease target is important in sparing healthy tissue, *i.e.*, minimizing toxicity, but in some cases, the drug must be released from the dendrimer at its intended destination to express biological activity; in other cases, the drug retains its activity when attached to the dendrimer. The versatility of the dendrimer and its various linker chemistries provide a platform from which a broad portfolio of products can be derived that incorporate a diverse set of drugs and targeting vectors, all leveraging the same underlying technology platform and discovery and development methodologies.

BIND Biosciences, Inc., is a biopharmaceutical company developing therapeutic targeted nanoparticles to produce best in class drugs. The company was founded by Drs. Robert Langer Omid Farokhzad (MIT-Harvard CCNE). To read more about the technology and the company please go to pages 50 and 67, respectively.

B3 Biosciences is an outgrowth of research that has been carried out in the laboratories of ANC members Dr. Bruce Sullenger (UNC CCNE) and Dr. Andrew Ellington (Stanford CCNE). The company's focus is the development of platform technologies for the delivery of nucleic acid drugs to tumors. In particular, these researchers have shown that nucleic acid binding species (aptamers) directed against cell surface receptors can direct the internalization of siRNAs into particular tumor cell types. The same aptamers and associated pro-apoptotic siRNAs have proven useful in inhibiting tumor growth in animal models. Additional aptamers directed against cell surface receptors have been found to act as agonists, and such receptor activating aptamers (RAPTERs) have the potential for use in immunotherapy treatments of cancer.

The business and management team at B3 includes Dani Bolognesi, formerly of Trimeris, a company that launched a $300 million anti-HIV drug (Fuzion). B3 is currently carrying out research in facilities located in both Chapel Hill, North Carolina, and Austin, Texas, and is quickly forming clinical alliances with researchers in both academia and industry. The collaborative work performed by Dr. Sullenger, a surgeon, and Dr. Ellington, a chemist, stands as an example of how interdisciplinary interactions can yield interesting new technologies for the treatment of cancer. Recently, B3 Biosciences partnered with pharmaceutical giant Roche to further develop the company's technology.

Calando Pharmaceuticals seeks to develop and commercialize new therapeutics based on the scientific discovery of RNA interference, or RNAi, using the company's proprietary RNAi/Oligo Nanoparticle Delivery ("RONDEL™") technology as a means of achieving systemic intracellular delivery of nanoparticles containing RNAi and other oligonucleotide-based therapeutics to treat cancer and other human diseases and conditions. The RONDEL™ technology was developed by ANC investigator and company cofounder Dr. Mark Davis of the Caltech. Calando answers the delivery issue with RNAi therapeutics with its targeted, cyclodextrin-containing polymers that form the foundation for its RONDEL™ delivery technology. To read more about the technology please go to page 45.

Calhoun Vision, founded in 2000, uses technology developed by ANC investigator Dr. Robert Grubbs of the Caltech CCNE to develop light-adjustable intraocular lenses that will enable patients to achieve optimal vision without the need for glasses after cataract surgery. The technology is based on a material whose shape can be adjusted by light. The refractive power of a lens constructed from the material can be adjusted after implantation to correct any errors in refraction. Other lens configurations are also being developed. The products are being tested and used by cataract surgeons in Mexico and Spain.

Carestream Molecular Imaging, a division of Carestream Health, Inc., develops and markets high-performance digital imaging systems, imaging agents, film, and accessories under the Kodak brand for the life science research and drug discovery/development market segments. With revenues of more than $2.5 billion, Carestream Health is a leading provider of medical and dental imaging systems and health

care IT solutions; molecular imaging systems for the life science research and drug discovery/development market segments; and x-ray film and digital x-ray products for the nondestructive testing market. The company emerged in 2007 when Onex Corporation (TSX: OCX) of Toronto, Canada, purchased Eastman Kodak Company's Health Group.

In September 2005, the University of Texas M.D. Anderson Cancer Center and Kodak established a formal collaborative relationship enabling the exchange of ideas, materials, and techniques. The goal was to evaluate the *in vivo* pharmacological properties of silica and polymer nanoparticles and assess the potential applications of these particles as carriers for NIRF imaging probes. The nanoparticles were based on Kodak's existing platform technology originally developed for its photographic film business and leverage Kodak's 50 years of dye chemistry and nanoparticle expertise. With the award of the NCI CNPP R01 grant, Kodak augmented its existing team of drug delivery and imaging science researchers, nano-fabrication engineers and chemists, dye chemists, analytical chemists, near-infrared fluorophores (NIRF) instrumentation engineers, and biologists to accelerate the development and translation of nanoparticulate optical imaging probes into clinical reality.

Cellular Bioengineering was founded in 2003 to develop disruptive technologies for biomedical and biodefense applications. The University of California, San Diego, has licensed technology invented by ANC investigator Dr. Michael Sailor to the company. Dr. Sailor's team has developed a method to construct encoded nanoparticles that act as robust, nontoxic tags. The key feature of these tags is a nanostructure that is programmed during electrochemical synthesis to display a complex reflectivity spectrum, referred to as a "spectral barcode." The reflectivity spectrum can be decoded using simple, low-power optical spectrometers. The intended application is high-throughput screening and encoded bead-based assays for *in vitro* diagnostics and development of therapeutics.

Enlight Biosciences is a Boston-based company established in partnership with major pharmaceutical companies to develop breakthrough innovations that will fundamentally alter drug discovery and development. Dr. Sanjiv Sam Gambhir of the Stanford CCNE is a co-founder and serves on the Scientific Advisory Board. Funded and guided by top pharmaceutical companies, including Abbott Laboratories, Johnson & Johnson, Eli Lilly & Company, Merck & Company, Novartis, and Pfizer, Enlight proactively addresses critical unmet industry needs with innovations drawn from academic laboratories, startups, and ideas generated internally by the Enlight team.

In recent years, most traditional life science investors have diverted their funding towards late-stage therapeutic programs despite the importance of developing impactful enabling technologies. As a consequence, important discoveries of great strategic value to pharmaceutical companies are not being commercialized. In addition, enabling technology and platform startup companies typically function independently of their potential pharmaceutical customers in the early stages, and do not always design their products to address the core unmet needs of the pharmaceutical industry. With diminishing access to innovative venture-backed technology platforms, pharmaceutical groups have been left to conduct translational technology development themselves. As they attempt to address similar core needs, there is a costly duplication of efforts and a distraction of focus from drug discovery. Enlight provides a unique mechanism through which pharmaceutical companies partner to foster rapid and tailored development of high impact enabling technologies and platforms that directly address their most pressing common needs.

Endra, Inc., is a startup created by Enlight Biosciences to commercialize photoacoustic tomography (PAT). Dr. Sanjiv Sam Gambhir of the Stanford CCNE serves on the Board of Directors at this company. PAT is an emerging imaging modality that combines the most compelling features of optics and ultrasound, providing both high optical contrast and high ultrasound resolution at depth. PAT can be used to generate powerful images based on inherent soft-tissue contrast without external contrast agents, and is also extremely well-suited for use

with existing and specialized photoacoustic contrast agents. The equipment is inexpensive, safe, and well understood, making the platform ideal for clinical use. PAT's unique properties make it superior to other modalities in generating information-rich structural and functional images in multiple critical disease areas, including applications within cancer, cardiovascular disease, dermatology, women's and men's health, and inflammation.

Photoacoustic signals are induced by pulsed laser illumination. When laser energy is absorbed by biological tissues, the resulting very minimal heating of the tissues generates ultrasonic waves. The waves can be detected by an ultrasonic transducer and then used to create an image of the optical absorption distribution inside the tissues. Different soft tissue types in the body differentially absorb laser light of different wavelengths, providing high inherent contrast. Endra's photoacoustic products are unique in their ability to provide rapid functional images of structures such as tumors, blood vessels, lymph nodes, and other tissues at a fraction of the cost of most current medical imaging devices, thereby addressing critical unmet needs in pre-clinical, clinical, and point of care imaging settings.

GE Global Research has an ongoing collaboration with Dr. Shan Wang of the Stanford CCNE to develop magnetic nanoparticles with high-saturation magnetization and high permeability for use in magneto nano-sensors. The current focus of this collaboration is on the preparation of clusters of superparamagnetic iron oxide (SPIO) nanoparticles as an approach to increasing the magnetic permeability of the particles while maintaining superparamagnetism. To this end, approaches involving the synthesis of SPIO nanoparticles from molecular precursors are being evaluated and characterized in collaboration with Dr. Wang with regard to size, composition, and magnetic properties.

Homestead Clinical Corporation is a biomarker discovery company with a focus on identifying and validating organ-specific, secreted biomarkers for disease analysis via serum profiling. Homestead has acquired the rights to license four technologies developed by Caltech CCNE investigators Drs. James Heath and Leroy Hood. Three of these technologies relate to DNA-Encoded Antibody Libraries (DEAL) technology and one relates to quantitative nanowire biosensors. Those technologies, coupled with quantitative transcript analysis, high-throughput and high-sensitivity mass spectrometry proteomics, and massively parallel SPR, combine to give Homestead benchtop-to-bedside capability.

Homestead, which was formed in Seattle from a biotech incubator that is being funded by various venture capital groups, is currently working on validating several identified biomarkers for glioblastoma, drug toxicology, and prostate cancer. Datasets related to a number of other cancers have been collected. The company has entered into preliminary discussions with three biotech/pharma companies on conducting joint projects.

Insert Therapeutics is a clinical-stage biopharmaceutical company positioned to become a market and technological leader in systemic delivery of therapeutic agents of all types. Insert was founded in August 2000 and based upon drug delivery technology developed in the laboratory of the Caltech CCNE investigator Dr. Mark Davis. The company utilizes modified cyclodextrin molecules as building blocks to create an entirely new class of drug delivery materials known as linear cyclodextrin-containing polymers. To learn more about this technology and clinical trials, please go to page 45.

Kereos is a St. Louis-based startup backed by more than a dozen venture capital firms, as well as the venture arms of Genentech and Philips Medical Systems. In collaboration with Drs. Gregory Lanza and Samuel Wickline, both investigators at the Washington University CCNE, the company is developing molecular imaging agents and targeted therapies based on ligand-targeted perfluorocarbon emulsion technology. Linking vascular biomarker targeting ligands with imaging or drug payloads through the "nanodroplet" core, Kereos' emulsion technology is especially well-adapted for visualizing and/or treating angiogenesis. To read more about its developing technology and its clinical testing, please go to page 48.

Integrated Diagnostics leverages powerful emerging technologies to develop diagnostic products that enable physicians and patients to manage complex and important diseases such as cancer, lung diseases, and CNS diseases through blood tests that can monitor tens to hundreds of disease markers simultaneously. The company plans to develop a pipeline of game-changing diagnostic products that enable the diagnosis and prognosis of a variety of diseases. The company's work is based on the concept of a systems view of disease where pathophysiology arises from disease-perturbed networks of proteins, genes and other molecules.

Liquidia Technologies is developing the PRINT® technology for the prevention and treatment of human disease, including cancer. The company was founded by Dr. Joseph DeSimone (UNC CCNE). To read more about the technology and the company please go to pages 38 and 68, respectively.

MagArray, Inc., is a startup company founded in 2005 to commercialize the magneto-nano sensor technology developed by Dr. Shan Wang (Stanford CCNE). To read more about the technology and the company please go to pages 21 and 69, respectively.

Molecular Biomarkers was founded by Caltech CCNE investigators Drs. James Heath, Michael Phelps, and Hsian-Rong Tseng. The primary goal of the company is to identify early-stage technologies that are coming out of the Caltech CCNE and related projects and to bring them to the point where they can exist as stand-alone companies, basically bridging the gap between scientific demonstrations, such as are carried out within the context of the Caltech CCNE projects, and robust platforms that are attractive vehicles for commercialization.

Momentum Biosciences is a private business advisory group committed to accelerating the transfer of new technologies from the laboratory to the commercial environment. Momentum Biosciences houses 6,500 square feet of laboratory space and support for startup ventures interested in moving their technology from academia to industry. CCNE investigators Drs. Michael Phelps, James Heath, and Owen Witte all serve on the Board of Directors. Having seen Los Angeles-based inventions moving to the more established biotech hubs of San Francisco and San Diego and recognizing that a typical biotech company will spend approximately $1.5 million in the first year, the majority of that valuable capital going towards infrastructure, faculty at UCLA and Caltech founded Momentum Biosciences to create a local home for entrepreneurial academics and their new ideas. To date, Momentum has housed over 10 companies in a variety of life and physical science disciplines, helping build the biotech economy of Los Angeles. These companies include Sofie Biosciences and Integrated Diagnostics, both of which have roots in research conducted by Caltech CCNE investigators.

Nanoparticle BioChem, Inc., focuses its efforts on the design and development of various types of metallic nanoparticles and bioconjugates by its co-founder, Dr. Kattesh Katti of the University of Missouri-Columbia CNPP. To read more about the technology and the company please go to page 69.

NanoInk is an emerging growth technology company specializing in nanometer-scale manufacturing and application development for the life science and semiconductor industries. With dip-pen nanolithography (DPN®), which was invented by Dr Chad Mirkin from the Northwestern CCNE, the company possesses a nanofabrication technology that allows for unmatched flexibility and accuracy, and high-resolution Nanoencryption™ technology. As a result, NanoInk is able to offer customers innovative solutions to fight counterfeiting and illegal diversion of products. Other key applications include nanoscale additive repair and nanoscale rapid prototyping. Located in the new Illinois Science and Technology Park north of Chicago, NanoInk currently has over 100 issued or pending patents and patent applications filed worldwide and has licensing agreements with Northwestern University, Stanford University, University of Illinois at Urbana-Champaign, and the Georgia Institute of Technology.

DPN® is used to build nanometer scale structures and patterns by literally drawing materials directly onto a surface. DPN® users can build at resolutions ranging from many micrometers down to 15 nanometers, using

virtually any material. This combination of ultrahigh resolution and material flexibility makes for numerous commercial applications, including high-density assays for virus capture and other medical screening. NanoInk also has developed an anti-counterfeiting technology for the pharmaceutical supply chain, called Nanoencryption™ technology. Nanoencryption technology is a layered pharmaceutical brand protection solution based on proprietary nanolithographic encryption technology. An industry first, Nanoencryption technology provides the only true covert, forensic level track-and-trace brand protection at the unit level. Nanoencryption technology ensures brand security through all points in the supply chain, providing authentication of prescription drugs while working to eliminate risks to consumers.

Nanosphere is a nanotechnology-based molecular diagnostics company offering proprietary breakthrough technologies that provide a unique and powerful solution to greatly simplify molecular diagnostic testing. Its mission is to improve the diagnosis and treatment of disease by enabling earlier access to, and detection of, new and existing biomarkers. Nanosphere was founded in 1999 by Northwestern CCNE investigator Dr. Chad Mirkin. His research discoveries made possible the consistent manufacturing and functionalization of gold nanoparticles with oligonucleotides (DNA or RNA), or antibodies that can be used in a wide variety of diagnostic applications to detect nucleic acid or protein targets, respectively. Dr. Mirkin then invented the biobarcode assay in 2003, making it possible to detect proteins at concentrations many orders of magnitude lower than conventional immunoassays. This technology enables applications in the detection of markers for diseases including many forms of cancer, coronary heart disease, Alzheimer's disease, and HIV.

Since its founding, Nanosphere has made continuous enhancements to the original technology advances by coupling gold nanoparticle chemistry and capabilities with multiplexed array analysis, microfluidics, human factors instrument engineering, and software development to produce the Verigene® System, a full-solution, molecular diagnostics workstation. The company is now a fully integrated diagnostics company with established cGMP manufacturing operations, leading-edge research and development teams, and veteran customer service and support teams. Nanosphere's technology for ultrasensitive protein detection can facilitate the discovery of new biomarkers for both clinical applications and drug development.

Additionally, Nanosphere is setting a new standard in public health and safety through the development of improved bio-security systems. Rapid identification of biological pathogens is critical to national security and public health. In October 2002, from a field of 12,000 applicants, Nanosphere secured one of ten contracts awarded by the U.S. Government Technical Support Working Group, a joint venture of the U.S. State and Defense Departments, to apply its platform to detection of biological agents in the water supply. Nanosphere is currently optimizing its platform for specific use as a field-deployable system to enable bio-defense agencies to detect signature nucleic acid sequences of known biological pathogens (*e.g.*, anthrax, plague, and others). Nanosphere's nanoparticle probe technology is well-suited for rapid and accurate testing for nucleic acid and protein targets in industrial sensors. This type of detection has widespread applications, including drinking water testing and the first-responder market, such as fire and police departments. The company is in dialogue with several hazardous materials teams and is preparing for deployment of systems that will be used for the rapid analysis of undisclosed, but known, substances in the field.

NanoVici, a subsidiary of OncoVista (San Antonio, Texas), focuses on targeted nanoparticle drugs for cancer treatment using technology developed by Dr. Shuming Nie from the Emory-Georgia Tech CCNE. The company's key technology platform is a ternary nanoparticle platform consisting of a biocompatible polymer backbone, a cancer drug, and a molecular targeting ligand. Targeted applications and clinical markets include human ovarian cancer, breast cancer, and head and neck cancer. Clinical work is being conducted at the Winship Cancer Institute at Emory University.

PreDx creates novel functional imaging agents that enable real-time visualization of specific physiological events or disease. Dr. Thomas Meade, an ANC investigator from the Northwestern CCNE, founded the company in 2006. The company intends to transform conventional diagnostic imaging techniques, such as magnetic resonance imaging (MRI), from their current role as anatomical imaging tools into powerful metabolic imaging agents. PreDx is developing unique agents, or metabolic probes, that may provide information about the physiological or pathological state of a cell or tissue. These agents turn contrast enhancement effects on or off in the presence of a specific enzyme or other functional reporter system. In addition to diagnostic imaging, the company is working to develop therapeutic and research applications of its technology platform.

Sofie Biosciences was founded by a group consisting of UCLA and Caltech faculty, including CCNE investigators Drs. Michael Phelps and James Heath. Company founders also include imaging industry professionals who are committed to accelerating the transfer of new healthcare technologies from the laboratory to the clinical and commercial environment.

T2 Biosystems focuses on developing distributive diagnostics that use magnetic resonance imaging (MRI) technology to provide immediate, accurate diagnostic testing for nearly any health condition, in nearly any setting. T2's scientific founders include Drs. Robert Langer, Michael Cima, Tyler Jacks, and Ralph Weissleder, all from the MIT-Harvard CCNE. The company's proprietary NanoDxTM detection system originated from the work of its founders and combines a powerful nanoparticle assay technology with miniaturized MRI-based detectors. In the near term, the company plans to offer benchtop systems for use in hospital, laboratory, and medical office setting. Longer term, the company will offer much smaller, more portable instruments for use wherever immediate medical decision-making is key. Such settings would include not only hospitals, clinics, and doctors' offices, but also ambulances, schools, and military situations. T2 will ultimately offer tiny devices for wearable, handheld, or even implanted applications.

Vivonetics was founded in 2003 to commercialize dual FRET molecular beacons and the modified molecular beacon nanotechnologies developed by Dr. Gang Bao from the Emory-Georgia Tech CCNE. Vivonetics's diagnostic products could potentially offer a competitive advantage over existing methods, such as antibodies, real-time quantitative PCR, and *in situ* hybridizations. Molecular beacon diagnostics will have improved sensitivity, be less labor-intensive, require less time for results, and be quantifiable. In addition to the diagnostic market, molecular beacons can be applied to basic research, particularly to identify and characterize cancer stem cells. To read more about the molecular beacon developed by Dr. Bao, please see page 26.

XinRay Systems is a joint venture between Xintek (see below) and Siemens Medical Solutions. The company is developing and manufacturing multipixel x-ray sources for a broad range of applications, including diagnostic medical imaging, homeland security, and industrial inspection. Xintek was founded by UNC CCNE investigator Dr. Otto Zhou. Beginning in 2005, Xintek and Siemens established formal collaborations, and in September 2007 the two companies announced a new joint venture company, XinRay Systems, to further develop the technology created by the collaborations. In 2005, the University of North Carolina and Xintek announced a major advance by placing multiple carbon nanotube sources in an array. In this multipixel configuration, all the x-ray energy sources (*i.e.*, carbon nanotubes) can fire at once from different angles, and, equally important, the sources can fire repeatedly, in one-millionth of a second.

Xintek. The company was founded in October 2000 by UNC CCNE investigator Dr. Otto Zhou. Xintek's mission is to identify and commercialize applications using carbon nanotubes (CNTs). The company manufactures CNT materials, CNT-enabled cold diode and triode cathodes, and x-ray tubes. Xintek's primary focus is on developing CNT-based field emission technologies for x-ray and display applications.

Please go to page 52 to learn more about technology developed by Otto Zhou, which gave foundation to Xintek and XinRay Systems.

Small Business Innovation Research (SBIR) Program

The ANC has played a vital role in attracting a number of high-quality research proposals to be funded through the NCI Small Business Innovation Research (SBIR) Program (http://sbir.cancer.gov) on topics that support the mission of the ANC. The SBIR program provides early-stage technology financing to promote innovation for developing and commercializing novel technologies. Each year, the SBIR Program together with the ANC office, proposes and posts announcements for contract topics. Three topics focused on cancer nanotechnology have been offered since FY 2006. These topics are:

- **Nanotechnology Sensing Platforms for Improved Cancer Detection.** The goal is to develop nanotechnology-based devices with improved sensitivity and specificity for early detection and post-treatment monitoring of cancer signatures using genomic and proteomic means operating in both *in vitro* and *in vivo* environments.
- **Multifunctional Therapeutics and Theranostics Based on Nanotechnology.** The goal is to develop an *in vivo* nanoparticle-based delivery platform with improved efficacy as compared to currently used treatments, and to incorporate an imaging agent to provide real-time feedback and monitoring of therapy.
- **Nanotechnology Imaging Agents or Devices for Improved Detection of Cancer.** The goal is to develop nano-enabled platforms that can provide increased resolution both spatially and, more importantly, temporally in detecting cancer that would ultimately offer clinicians a way to maximize the chance of positive clinical prognosis. The platforms can be used for early detection/imaging of initial onset of disease, or be used as post-treatment monitoring to detect/image recurrence of disease.

The SBIR awards are divided into phases as follows:

- **Phase I - Feasibility Study.** A small business may submit a Phase I proposal in response to the topics published in an open NCI solicitation. A Phase I SBIR award is typically funded at $150,000 for a six-month period to demonstrate the feasibility of a concept. The awarded companies also begin to pursue commitments for follow-on funding during this phase.
- **Phase II - Development.** Upon successful completion of a Phase I project, the program manager(s) may invite a company to submit a Phase II proposal for consideration. A Phase II proposal is more extensive than the Phase I proposal and should demonstrate the company's potential for rendering a product or process.

In the last five years, several companies associated with the ANC program have applied for the contract topics. The successful applicants include the following companies:

BIND Biosciences (http://www.bindbio.com) was founded in 2007 by Drs. Robert Langer and Omid Farokhzad from MIT-Harvard CCNE. BIND Biosciences is engaged in developing targeted therapeutics that deliver high drug concentrations to target cells and tissues with precisely controlled pharmacokinetic and pharmacodynamic properties resulting in increased efficacy and reduced toxicity. Its Nanoengineering™ platform allows it to precisely engineer libraries of drug encapsulated targeted nanoparticles that differ systematically in their biophysicochemical properties; to screen for targeted nanoparticles with optimal drug PK; biodistribution; cell- or tissue-specific targeting; and drug exposure kinetics; and also to manufacture candidate drug encapsulated targeted nanoparticles using scalable unit operations. Its targeted nanoparticles consist of targeting ligand, surface functionalization,

polymer matrix, and therapeutic payloads. To read more about this innovative technology, please go to page 50.

BIND Biosciences' proprietary pipeline is focused on improving the efficacy and expanding the applicability of existing drugs. Its technology can be used to deliver a broad range of payloads including emerging therapeutic modalities such as RNAi, allowing us to address multiple therapeutic areas and indications. The company's initial development efforts are in the therapeutic areas of cancer and cardiovascular disease.

BIND submitted a proposal in response to the FY2006 "Multifunctional Therapeutics and Theranostics Based on Nanotechnology" SBIR announcement. The goals of BIND's SBIR Phase I contract were to (1) characterize a targeted nanoparticle therapeutic for prostate cancer selected as a candidate for clinical trials, and (2) develop a clinical manufacturing process for the nanoparticle to lay the foundation for Investigational New Drug (IND) enabling toxicology and efficacy studies, and eventual clinical evaluation of these targeted nanoparticles for prostate cancer therapy. BIND successfully completed its Phase I contract in March 2008. Since completing its Phase I contract with the SBIR program BIND Biosciences has raised over $41 million dollars in private funding and hired 30 employees. The substantial venture capital funds raised by BIND made the company ineligible for Phase II funding by the SBIR program.

BIND also works very closely with the NCL in Frederick, MD to characterize its lead concept as they prepare to file an IND with the FDA. BIND's lead concept is projected to enter Phase I clinical trials in 2010. In addition to cancer therapeutics, BIND is leveraging its technology to develop treatments for cardiovascular and inflammatory diseases. Thus, BIND Biosciences has progressed from a small startup in 2007 to a promising biotechnology company on the verge of filing an IND with the FDA in just three years with the assistance of NCI and NCL. BIND Biosciences' investors are ARCH Venture Partners, DHK Investment, Endeavour Vision, Flagship Ventures, NanoDimension AG and Polaris Venture Partners.

Liquidia Technolgies Inc. (http://www.liquidia.com) was founded in 2004 by Dr. Joseph DeSimone from the UNC CCNE. The company designs, develops, and manufactures proprietary PRINT® product platforms for a wide range of life sciences and materials science applications. PRINT® technology is based on the techniques of imprint lithography used to etch the intricate patterns on computer microchips. PRINT® technology enables the design and manufacture of precisely engineered nanoparticles with respect to particle size, shape, modulus, chemical composition, and surface functionality. To read more about PRINT® technology, please go to page 38.

Liquidia has demonstrated that cellular uptake of nanoparticles is a function of nanoparticle size, shape, and surface charge. For example, nanoparticles possessing the size and shape of rod-like bacteria were internalized at higher rates than particles of other sizes and shapes. Understanding these dynamics will allow Liquidia to rationally design nanoparticles that target specific cells and tissues, modulate circulation times, release drugs only where and when needed, and carry a large therapeutic payload.

One of Liquidia's major research efforts is in vaccine development. Liquidia is developing particle-based vaccines based on the PRINT® technology to combat cancer, infectious, and emerging diseases. By using simple dissolvable PRINT® particles to deliver antigen, vaccine effectiveness is increased while requiring less antigen and no adjuvant. Liquidia is developing both conjugate polysaccharide- and nucleic acids-based vaccines. Liquidia plans to file an IND for a vaccine product with the FDA no later than 2011. Ultimately, precise control of particle design and manufacture using PRINT® technology may lead to treatment regimens with enhanced efficacy and reduced side effects of therapeutics and vaccines.

Liquidia is also developing inhalation products. For diseases such as tuberculosis and cystic fibrosis, delivery of therapeutic agents to specific regions of the lung is crucial. Inhaled delivery of therapeutics holds great potential for treating these diseases. More efficient and targeted delivery made possible by Liquidia's PRINT® technology may lead to enhanced

efficacy and optimized therapeutic index of therapeutic agents for these diseases. Additionally, Liquidia is investigating the applications of PRINT® technology for aerospace and defense, energy storage, energy efficient building materials and solid state lighting applications laying the foundation of a more diversified company.

The goals of Liquidia's Phase I SBIR contract were to (1) design and fabricate PRINT nanoparticles, (2) synthesize the targeting ligand and attach it to the nanoparticles, (3) conduct *in vitro* experiments to assess the efficacy and toxicity of the drug-loaded nanoparticles, and (4) conduct a preliminary biodistribution study in animal models. The Phase I contract was successfully completed in June 2008.

Since completing its Phase I contract with the SBIR office in 2007 Liquidia Technologies has raised over $46 million dollars in private funding,(making the company ineligible for Phase II funding from the SBIR program. The company has expanded to 48 employees and is well on its way to establishing itself as a major player in the nanotherapeutics industry. Liquidia has alliances with CTI Molecular Imaging, University of North Carolina, and North Carolina State University. The company's investors are New Enterprise Associates (NEA), Pappas Ventures, Firelake Capital Management, Canaan Partners, and Siemens Venture Capital.

MagArray (http://www.magarray.com) was founded in 2005 by Dr. Shan Wang of the Stanford CCNE to commercialize the magneto-nano sensor technology developed in his laboratory. This technology – MagArray™ chip – contain arrays of magnetic sensors that can be used to detect surface binding reactions of biological molecules that have been labeled with 10 to 100 nm sized magnetic particles. To read more about MagArray™ technology, please go to page 24.

The company's core technology uses magnetic nanotags (MNTs) as the detection moiety that is quantified using inexpensive giant magnetoresistive (GMR) sensors. The advantage of MNTs is that very small quantities can be detected using GMR sensors, yielding a more sensitive means of detecting individual molecules in biological samples. Although MagArray chips are in some ways similar to fluorescence-based DNA array chips, the use of magnetic labeling tags leads to many distinct advantages, such as better background rejection, no label bleaching, inexpensive chip readers, potentially higher sensitivity, the ability to measure multiple binding reactions in homogeneous assays simultaneously and in real time, and seamless integration with magnetic separation techniques. So far, MagArray chips have been used successfully to perform quantitative analytic bioassays of both protein and nucleic acid targets. MagArray has also demonstrated the ability to simultaneously detect as many as 20 proteins with a linear dynamic range of six logs. It is possible to reduce the total assay time to less than 30 minutes using techniques such as analyte incubation in a microfluidic channel, making the MagArray system suitable for use in physician office, laboratory, or point of care applications.

Based on the success of their Phase I SBIR contract MagArray was invited by NCI to submit a proposal for a Phase II SBIR contract that was awarded in 2009. In addition to the SBIR funds received from NCI, MagArray has secured funding from several federal agencies and private foundations.

Nanoparticle Biochem, Inc., (http://www.nanoparticlebiochem.com/) was co-founded by an interdisciplinary team of chemists, physicists, and radiologists including ANC member Dr. Kattesh Katti of the University of Missouri, Columbia CNPP. NBI's core technologies lie in the synthesis of a wide spectrum of gold, silver, palladium, iron oxide, and platinum nanoparticles functionalized with an array of chemical and biochemical matrices. Gum arabic-coated gold and silver nanoparticles allow systematic variations in metal content, thus providing unprecedented means of developing mono- and multi-nanolayers with assorted metal content for a variety of materials and biomedical research applications. The company's nanomedicine research concentrates on developing products for the diagnosis and treatment of cancer. For example, NBI's therapeutic efficacy studies carried out in prostate tumor bearing mice have demonstrated unprecedented 82 percent reduction in tumor volume after a single dose administration of the

radioactive gold nanoparticle, GA-^{198}AuNP, making inoperable prostate tumors operable (Fig. 23). NBI also conducts nanoparticle-based research of antimicrobial agents that could have important applications for the production of antimicrobial textiles for the defense, health, hospitality, and hygiene industries.

NBI was awarded two Phase 1 SBIR awards from the NCI in 2008. The first award was made under the contract topic "Multifunctional Therapeutics Based on Nanotechnology" and the second under the contract topic "Nanotechnology Imaging and Sensing Platforms for Improved Diagnosis of Cancer." Also, NBI has used seed money from a Missouri Technology Incentive Program (MoTIP) grant to develop additional SBIR applications, results of which are pending from NIH and the Environmental Protection Agency. NBI has also been awarded a Phase 2 SBIR by the NCI for continuation of the Multifunctional Therapeutics concept (these needs to be confirmed before we can use it).

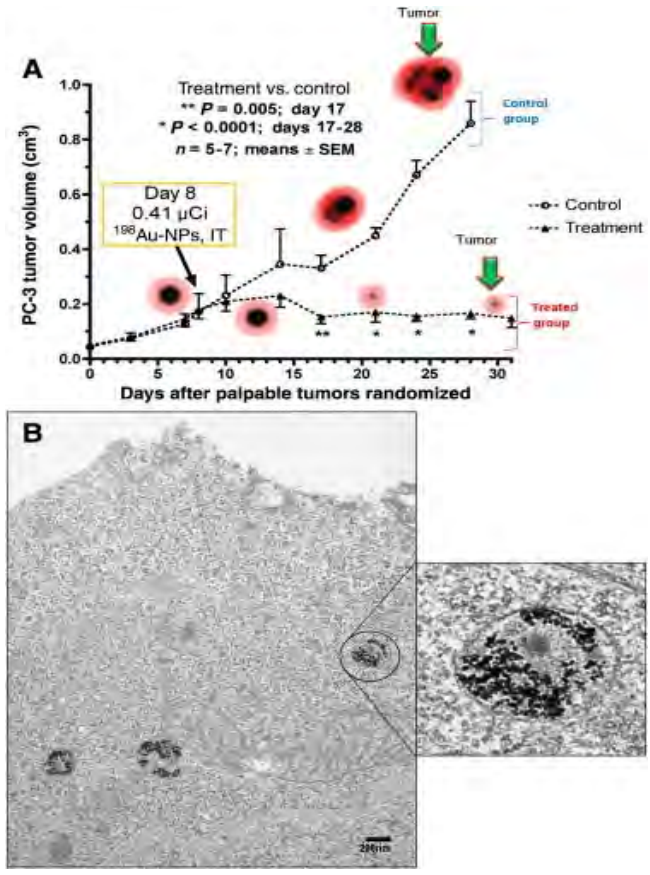

Figure 23: Tumor therapy using GA-^{198}AuNPs. (A) Therapeutic efficacy of GA-^{198}AuNPs in prostate tumor–bearing SCID mice. Subcutaneous tumors were generated in SCID mice by PC-3 engraftment. Mice bearing palpable tumors were randomized for treatment (n = 7) and control (n = 7) groups followed by intratumoral (IT) injections of GA-^{198}AuNPs (408 µCi per animal) or DPBS, respectively. Graph represents mean tumor volume following 30 days of treatment. **(B)** TEM image showing uptake of GA-AuNPs in prostate cancer cells. (Reprinted with permission from Chanda N et al. Radioactive gold nanoparticles in cancer therapy: therapeutic efficacy studies of GA-^{198}AuNP nanoconstruct in prostate tumor–bearing mice Nanomedicine: Nanotechnology, Biology and Medicine (2010) 6: 201-209. Copyright © 2010 Elsevier).

CHAPTER 6

Bioinformatics and Data Sharing

Cancer nanotechnology is inherently interdisciplinary, and researchers in this field can only work efficiently if they can integrate their own data with those obtained from other disciplines. At present, progress in the field is being impeded by the lack of a knowledge-management infrastructure for comparing and combining results within and across disciplines. Providing researchers with access to nanoparticle characterization data (physico-chemical, *in vitro* and *in vivo* biological behavior), along with the conditions under which the characterization was done, will expedite the use of nanoparticles in biomedicine. The OCNR staff believes bioinformatics can meet this need for information exchange, as it encompasses both terminology standardization and data handling that promotes interdisciplinary communication, allows data and protocol storage, and facilitates search, retrieval and modeling of data output.

Connectivity with caBIG®

In 2004, the NCI launched the cancer Biomedical Informatics Grid (caBIG®) initiative as part of its mission to advance research on cancer and improve clinical outcomes for patients. NCI recognized that the ability to connect people, organizations, and data through information technology would be critical to fulfilling NCI's mission. caBIG®, overseen by the NCI Center for Bioinformatics and Information Technology (CBIIT), began with a three-year Pilot Phase, in order to test the ability of a complex informatics initiative to achieve measurable goals and produce deliverables toward enhancing cancer research, and to assess the opportunities and challenges of connecting a disparate biomedical community on a national and eventually international scale.

The caBIG® Pilot Phase concluded in March 2007, followed by a transition to an Enterprise Phase. In the Enterprise Phase, an expanding number of organizations – including additional Cancer Centers, the pharmaceutical and biotech community, and the commercial IT sector – are being invited to achieve connectivity through the adoption of caBIG® tools and infrastructure. caBIG® is sharing its experience, expertise, and tools with the larger biomedical community with the hope that this infrastructure can be applied to diseases beyond cancer.

caBIG® has become the underlying information infrastructure for the ANC. To date, a major focus of the effort at the CBIIT for the ANC has been the development of a cancer Nanotechnology Laboratory Portal (caNanoLab).

caNanoLab

To address the challenges of data sharing among nanoscience researchers, the caBIG® team worked with ANC members to create caNanoLab. The goal of this effort is to lay the

basic groundwork for a system in which primary nanotechnology research data are standardized and shared within the scientific and clinical community. This initiative will have an enormous impact on nanomedicine since it further facilitates translational research. The caNanoLab Web portal is designed to facilitate data sharing in the research community. Currently, the caNanoLab portal supports the submission and retrieval of physical and *in vitro* characterizations for nanoparticles. The project is expanding to include support for *in vivo* characterizations of nanoparticles and their functionalizing entities, which are analogous to those required for small molecules and other medical devices. These characterizations include rigorous testing to determine toxicity and PK/ADME properties and additional *in vivo* characterization of nanoparticles to address additional challenges stemming from the relationship of particle structural properties to biological activity.

At present, caNanoLab allows researchers to submit and retrieve information on nanoparticles including the composition of the particle, the function of the particle (*e.g.* therapeutic, targeting, diagnostic imaging), the experimental characterization of the particle from physical (*e.g.* size, molecular weight) and *in vitro* (*e.g.* cytotoxicity, immunotoxicity) assays, the protocols for these characterizations, and related publications. To date, caNanoLab provides access to publicly available data from characterizations performed at the NCL; information on almost 700 nanoparticles; a list of almost 1100 peer-reviewed publications on cancer nanotechnology; and 26 protocols used to perform nanomaterial characterization assays.

The caNanoLab project is coordinated by the caBIG® Integrative Cancer Research Nanotechnology Working Group (caBIG® ICR Nano WG), which includes members from the NCI, EPA (the Evironmental Protection Agency), FDA, NIBIB (National Institute of Biomedical Imaging and Bioengineering), NIST and other organizations. The goal of this working group is to develop nanotechnology standards and the application of these standards in the caNanoLab nanotechnology resource. The main two goals of the caBIG® ICR Nano WG are to develop standards for data sharing and to standardize terminology in nanomedicine (ontology).

Nanotechnology Data Sharing Standards

In 2008, the caNanoLab project worked with caBIG® ICR Nano WG to develop a *Nanotechnology Data Sharing Standards* position paper that identifies common data elements leveraged across nanotechnology resources. *Nanotechnology Data Sharing Standards* includes support for:

- Nanomaterials and their composition: nanomaterials (*e.g.* dendrimer) and their associated properties (*e.g.* branch, generation) and composing elements (*e.g.* core). Includes functionalizing entities (*e.g.* small molecule, antibodies) associated with the nanomaterial that allow the material to function as a targeting, therapeutic, and/or diagnostic agent.
- Physico-chemical characterizations: assay conditions and measurements associated with the physical (*e.g.* size, molecular weight) and chemical (*e.g.* surface chemistry) properties of nanomaterials.
- *In vitro* characterizations: assay conditions and measurements that assess a nanomaterial's interaction with cellular components including cytotoxicity and blood contact properties.
- *In vivo* characterizations: characterizations performed on nanomaterials to determine the safety, efficacy, pharmacokinetics, and toxicology properties of nanomaterials in animal models so that nanomaterials can be transitioned for use in clinical applications.

CHAPTER 7

Meetings

The OCNR Staff and ANC investigators have actively participated in government, industry, and scientific meetings and events dedicated to cancer nanotechnology or fields supporting it. The OCNR Program Staff has also organized numerous meetings, workshops, and conference sessions to introduce the concepts of nanotechnology and nanomaterials in cancer biology and oncology. These efforts included sessions on cancer nanotechnology at the American Association for Cancer Research (AACR) meetings, Biotechnology Industrial Organization (BIO) International Forum, Materials Research Society (MRS) workshops and conferences, Institute of Electrical and Electronic Engineers (IEEE) meetings, American Physical Society (APS) meetings, Nano Science and Technology Institute (NSTI) meetings, and others.

Annual Meeting of the American Association of Cancer Research

The Program Staff and ANC investigators have been responsible for organizing nanotechnology-related mini-symposia at the AACR Annual Meeting. These symposia were designed to address the growing need for knowledge of how nanotechnology will contribute to the evolution of medicine and in particular, oncology.

In 2005, the ANC organized an educational session titled, "*Nanotechnology: Accelerating Progress in Cancer Research.*" This session provided an informative overview of multiple platform nanotechnologies and their current and envisioned applications in cancer research and the clinic. In particular, the session featured novel ways to look at cancer through the perspective afforded by nanotechnology. The program featured a detailed look at nanotechnologies for the early detection of cancer using noninvasive procedures, as well as a discussion on the use of multifunctional nanoparticulates for the targeted delivery of therapeutic and preventive actions. The session was led by Dr. Anna Barker, and presentations were given by Drs. Piotr Grodzinski, Mauro Ferrari (University of Texas Health Science Center) and James Heath (Caltech CCNE).

In 2006, the ANC set up another education session with the title, "*Harnessing the Power of Nanotechnology for Diagnostics and Treatment of Cancer.*" This session introduced the concepts of nanotechnology and nanomaterials in the context of the 'nano-environment' of the cancer cell. Drs. Anna Barker, Joseph DeSimone (UNC CCNE), James Heath (Caltech CCNE) and Chad Mirkin (Northwestern CCNE), discussed the opportunities and challenges in creating nanodevices for early detection, diagnosis, and treatment of cancer.

A session titled "*Novel Approaches to Drug Delivery in Cancer*" was held at the AACR Annual Meeting at Los Angeles in 2007. The session was organized by Dr. Mark Davis (Caltech CCNE). At this session, Dr. Davis discussed issues such as control of size and surface charge of nanoparticles and some of the features of nanoparticles that make them particularly interesting for cancer therapeutics such as multivalent targeting to cell surface receptors. Dr. Dong Shin (Emory-Georgia Tech CCNE) talked about limitations to drug delivery systems for cancer and how nanotechnology can provide new approaches to drug delivery and

imaging. He also focused on binary and tertiary structures of therapeutic nanoparticles and discussed the development of ongoing novel nanotherapeutics. Dr. Anna Wu (Stanford CCNE) talked about engineering antibodies and antibody fragments for use as targeting agents.

Also at the 2007 AACR Meeting, Dr. Jan Schnitzer (Sidney Kimmel Cancer Center CNPP) organized a session titled, "Nanotechnology in Discovery, Imaging & Therapy of Cancer." This session presented some of the most promising new advancements in the application of nanotechnology to cancer research including significant progress in using human tumor samples and in moving towards clinical testing. The speakers included: Drs. James Baker (U Michigan CNPP), Moungi Bawendi (MIT-Harvard CCNE), Scott Manalis (MIT CNPP), Jan Schnitzer (Sidney Kimmel Cancer Center CNPP) and Samuel Wickline (Washington University CCNE).

In 2008, Dr. Mauro Ferrari (University of Texas Health Science Center) organized a methods workshop to present examples of leading research in the nanotechnology based cancer diagnosis (*in vivo* imaging and laboratory diagnosis) and therapy. Two ANC investigators were invited to give presentation: Drs. Joseph DeSimone (UNC CCNE) and Shad Thaxton (Northwestern CCNE)

In 2009, the ANC sponsored a session at AACR titled "*Cancer Diagnostics Using Nanotechnology Platforms*." This session introduced the broader cancer research community to the promise of applying cutting edge nanotechnology solutions to cancer diagnostics being developed by ANC investigators. Speakers at this session included: Drs. Michael Cima and Ralph Weissleder (both from MIT-Harvard CCNE), Joseph DeSimone (UNC CCNE), Piotr Grodzinski, James Heath (Caltech CCNE) and Chad Mirkin (Northwestern CCNE).

In 2010, the ANC also sponsored an AACR session titled, "*Cancer Nanotechnology – Path to the Clinic*," which described the fundamentals of the science and technology enabling nanotechnology applications in oncology. The session also outlined the translational path of these technologies to the clinical world. Speakers at this session included: Drs. Anna Barker, Mark Davis (Caltech CCNE), Sanjiv Sam Gambhir (Stanford CCNE), Piotr Grodzinski, and Chad Mirkin (Northwestern CCNE).

Annual ANC Investigators Meetings

The ANC has held four Annual Investigators Meetings During the first four years of the ANC's first phase, the NCI held an Annual Investigators Meeting to which all investigators, staff, postdoctoral fellows, and graduate students were invited. These meetings, which were not open to the broader scientific community, were meant to encourage the development of cross-institutional collaborations and to leverage the discoveries being made at the CCNEs and CNPPs by disseminating experimental results among the ANC community.

The first Investigators Meeting, held in October 2006 and hosted by the UCSD CCNE, featured sessions on commercialization, *in vivo* diagnostics and imaging, bioinformatics, targeted nanotherapeutics, nanomaterials in biological matrices, clinical pathways for nanotechnology-based products, and *in vitro* diagnostics. The meeting also offered a session on multidisciplinary research training and team development. One of the primary outcomes of this meeting was the formation of several new collaborations across ANC institutions that resulted from discussions held at the meeting.

The second Investigators Meeting, held in October 2007 and hosted by the UNC CCNE, was structured to build on the foundation of the 2006 meeting in order to further accelerate progress in using nanotechnology to develop novel diagnostic, imaging, and therapeutic tools for use in detecting and treating cancer in its earliest stages. The 2007 meeting, attended by over 280 investigators, postdoctoral fellows, and students, featured eight scientific sessions, three plenary talks, a keynote presentation, and over 110 poster presentations. Representatives from

all eight CCNEs and the 12 CNPPs attended the Investigators Meeting. In addition to the formal presentations on ANC research projects, the meeting was preceded by tutorial sessions on several multidisciplinary topics relevant to cancer nanotechnology and a session on the clinical pathway. More information on these tutorial sessions can be found in chapter 9.

The main meeting featured sessions on cancer targeting strategies; *in vivo* nano delivery platforms; nanotherapeutics; clinical applications of quantum dots; nanotechnology devices for early diagnosis of cancer; nanotechnology contrast agents for medical imaging; controlling cancer metastasis with cancer nanotechnology tools; and toxicity, characterization and data sharing for nanomaterials. The meeting closed with a plenary talk on clinical translation.

The third Investigators Meeting, held in October 2008 and hosted by the Northwestern CCNE, was attended by 285 investigators, postdoctoral fellows, and students, featured six scientific sessions, two plenary talks, an after-dinner keynote presentation, and approximately 100 poster presentations. Representatives from all eight CCNEs and the 12 CNPPs attended the Investigators Meeting. In addition to the formal presentations on ANC research projects, the meeting was preceded by four tutorial sessions on several multidisciplinary topics relevant to cancer nanotechnology and a session on the clinical pathway. The main meeting offered sessions on imaging; monitoring therapeutic responses; clinical translation; multifunctional nanotechnology platforms; biomarkers and drug development; nanotherapeutics; nanomaterial development; and diagnostic nanotechnology devices.

The fourth and final Investigators Meeting was held in October 2009 and was hosted by the Caltech CCNE. Once again, this meeting highlighted scientific advances of the ANC partners, fostered collaborations, and provided a venue for working group discussions. Keeping an eye on the future, the ANC also hosted tutorial sessions to provide education and guidance to the next generation of young nanoscientists. Finally, clinical translation and technology commercialization were addressed in a special translational session where successful ANC spinoff companies presented their accomplishments. Scientific sessions were held on nanotherapeutics; diagnostic techniques based on nanotechnology; nanotechnology in the clinic; contrast agents and imaging techniques; nano science policy; molecular analysis of circulating tumor cells; and nanomaterial characterization and nanoinformatics.

NCI Strategic Workshops

As the ANC program approached the midway point of its five-year funding, NCI held a series of three strategic workshops on cancer nanotechnology covering the areas of 1) *in vitro* diagnostics and prevention, 2) *in vivo* diagnosis and imaging, and 3) therapy and post-treatment. The purpose of these workshops was to assess the current status of cancer nanotechnology, determine the needs and gaps of the field, and develop the vision for the future for the next phase of ANC. To each of these meetings, OCNR Program Staff invited several experts from academia, industry, the non-profit sector, and the federal government. The detailed findings and recommendations of these workshops can be found in the publication by Nagahara et al. "*Strategic Workshop on Cancer Nanotechnology*" (2010) Cancer Res. 70 (1): 4265-8.

There were several recommendations that appeared as common themes throughout the three workshops. These include:

The Technologist and the Clinician: The audience overwhelmingly pointed out the continued need for technologists, biomedical researchers, and clinicians to work together in order to make the most out of the opportunities that nanotechnology can generate. Many applauded NCI's efforts in creating a multidisciplinary team science environment, and expressed hope that such efforts would continue to be expanded going forward. It was believed that the ANC program provided a

boost to the field of cancer nanotechnology. In addition, the attendees recommended that the NCI should consider new mechanisms for creating strategic partnerships with other agencies and other fields to maximize the impact that nanoscience will have on cancer research and clinical oncology.

Multifunctional/Multimodal Nanotechnology Agents: Prevailing throughout the three workshops was the notion that the paradigm shifting power of cancer nanotechnology will occur when an agent/platform combines two or more modalities - diagnostics, imaging, and/or therapy. Clearly, a strong advantage for a nanoparticle system is the potential for a plug-and-play-like approach that integrates multifunctionality and multimodality. However, maintaining a more pragmatic vision, the participants suggested that a single modality nanoparticle be developed first and subsequently translated to the clinic. The increase in the complexity of the multi-modal solution should then occur gradually.

In addition, each of the individual workshops identified overarching themes to guide the future of the ANC programs. These included:

***In Vitro* Diagnostics and Prevention Workshop:** One of the keys to the growing number of cancer survivors is the emergence of early diagnostics. The participants at this workshop believed it paramount that the ANC should promote advances in the development and adoption of new nanotechnology methodologies that enable cancer to be discovered earlier in its development and ultimately to prevent it from occurring in the first place. A positive feedback loop mechanism (diagnosis, treatment, and monitoring of treatment results) will be important for pushing this field forward. Early detection methods will be enabled by improved early-stage biomarkers and followed by more effective therapies designed to target early stage disease. As a result, developing new early detection methodologies becomes even more important in the quest to reduce the incidence and mortality from cancer. The long term vision for developing new *in vitro* diagnostics is to be able to take a blood sample, for example, and determine the presence of low-abundance biomarkers, characteristics of cancer that would ideally identify the type of tumor present, specify the appropriate therapy, and predict the outcome of that therapy.

Therapy and Post-Treatment Workshop: Targeted cancer therapies represent a glimpse into the future of oncology with ERBB and VEGF based therapies being the first successful examples of using targeted approaches. Similarly, it has been demonstrated that nano-carrier-based delivery can improve the efficacy of anticancer drugs and reduce the associated toxicities. The participants at this workshop shared a common vision that nanoparticles will be able to improve the therapeutic index for a wide variety of anticancer drugs, and that this improvement alone will be of great potential benefit. Moreover, multifunctional aspects and the monitoring therapeutic response using "smart" nanoparticles will also represent a paradigm-changing event in oncology.

This workshop group also recommended that the NCI continue its efforts to work with the FDA and clinicians to address the unique features of nanoparticles and the opportunities to change the approval paradigm as far as modularity and personalized therapies are concerned. The group also recommended that the NCI and its NCL continue their efforts to develop bioanalytical methods suitable for characterization of nanoparticles and to fund efforts for mathematical modeling that might help drug developers rationalize their choice of a specific nanoparticle for a particular application. To accomplish these goals, the audience identified several critical needs, including: the development of relevant animal models of human cancer; the development of a streamlined approach to evaluate toxicology, pharmacokinetics, and the efficacy of potential nanotherapeutics, essentially expanding the scope of the NCL's mission; and the creation of an infrastructure for translational nanotechnology research that would feed promising therapeutics into the nation's clinical trials apparatus.

***In Vivo* Diagnosis and Imaging Workshop:** *In vivo* imaging is perhaps the most significant use of nanotechnology that is relatively close to the clinic. Improving cancer diagnosis by detecting tumors at ever small stages, via *in-vivo* imaging, opens new opportunities for improving

treatment, as well as for understanding the metastatic processes. Currently, imaging provides limited information about tumor type, with subsequent surgery and then pathology being used to identify the tumor and determine the appropriate course of therapy. A vision that this workshop's participants shared is to develop *in-vivo* imaging techniques that will provide specific information about tumor type and tumor environment and largely eliminate the need for surgical biopsy prior to determining the proper course of therapy. Moreover, the group believed that nanotechnology-enabled imaging methodology would be capable of monitoring the response to therapy in real time. This, in turn, would reduce the time lapse to determine if therapy is effective, would greatly improve the quality of life for patients by getting patients off ineffective drugs that could cause adverse side effects, and would decrease the likelihood that drug resistance might develop before an effective therapy is established for particular patients.

Best Practices in Nanotechnology

The ultimate goal of this meeting was to generate a white paper that would aim to inform the broader drug development community about the specific ways in which nanotechnology can best be used to reduce the time and expense of developing new agents to treat cancer more effectively and with fewer side effects.

This meeting featured presentations on a variety of aspects of the development and commercialization of clinically relevant nanotechnology-enabled therapeutics. Specifically, talks were focused on the current status in preclinical models; contract research organizations; the NCL; the FDA's position on nanotherapeutics; legal, environmental, health, and safety concerns; nanotechnology's role in personalized medicine, and financing and venture capital. There were also presentations given by the industry researchers who described the efforts that their companies have made toward commercializing nanotherapeutics.

The presentation provided impetus for two panel discussions on *"Pathways to the Clinic"*. During these panels several challenging areas were identified that need to be overcome in order to accelerate the clinical translation of nanotechnology-based therapeutics. These discussions served as the foundation for working group discussions held at the end of the meeting, when attendees broke into two groups to develop an overall approach to using nanotechnology to address one of two problems posed to the groups by the NCI:

- How to design nanoparticles for the delivery of highly toxic chemotherapeutics, and
- Design and develop nanotechnology platforms capable of crossing the blood-brain barrier.

The first group concluded that it is important from early on in the development effort to remember that any formulation benefits from having simple, scalable methods for manufacturing. The optimal nanoparticulate systems would be made from biodegradable materials, including lipids and organic polymers. Any nanoparticulate anticancer agent should be designed from the start to have the longest circulation time possible in order to give an increasing fraction of the initial dose time to accumulate in tumors. However, this requirement means that a nanoparticle should also be designed to release its drug payload only after it has been taken up by a tumor; otherwise, long-circulation time would likely result in increased toxicity since healthy tissue would then also be exposed to drug with each pass through the body. An alternative approach would be to use a prodrug that would only be converted into an active, toxic molecule under the unique physiological properties of the tumor or to include within the nanoparticle a drug antidote that would be inactivated within a tumor.

The second group concluded that success at developing a nanoparticle capable of carrying a payload across the blood-brain barrier would

have broad implications for the treatment and imaging of diseases beyond cancer. This group also noted that there is little effort in academia or the pharmaceutical industry to study the blood-brain barrier or develop methods for breaching it, and as a result, a successful effort would meet a huge unmet need and present a compelling case for investment.

The best approach for developing such a nanoparticle would be to hijack an existing transporter to take molecules into the brain. Surveying a list of ligands known to function as molecular transporters would be the first step, and then looking to see if any of those ligands were also overexpressed on tumors; chlorotoxin would be an example. That ligand, or a mimic, would serve as both a transport and targeting agent for the appropriate nanoparticle. The group cautioned, however, that it will be important to consider how and when this nanoparticle would be used in relationship to other therapies, particularly with regard to cancer therapy. Prior or simultaneous treatment with another drug might impact the targeted transporter. The group also said it would be necessary to examine the effect of ligand density on nanoparticle behavior, particularly regarding the avidity of transport receptor binding.

Initial screening assays should rely on normal mouse models to study transport across the blood-brain barrier. After optimizing transport in the normal mouse, the nanoparticle should then be tested in genetically engineered mouse models of cancer since orthotopic models are not good at representing the intact blood-brain barrier. This group also recommended that the nanoparticle construct be designed to utilize a two-stage mechanism, such as prodrug activation, to minimize the potential for non-target tissue toxicities.

CHAPTER 8

Collaboration and Programmatic Integration within the Alliance

One of the core features of the ANC is its heavy emphasis on promoting team science and collaborations across the program. The OCNR Program Staff played an active role in creating and driving multidisciplinary teams drawing on synergistic elements of the expertise within different Centers and projects.

Examples of Scientific Collaborations within the Program

Over the past five years, ANC investigators developed several trans-institutional collaborative projects, including the following:

Nanotechnology-Derived Positron-Emitting Probes for Molecular Imaging

Collaboration of Drs. Michael Phelps (Caltech CCNE), Joseph DeSimone (UNC CCNE), and Anna Wu (Stanford CCNE)

Positron emission tomography (PET) is a sensitive non-invasive imaging technology for measuring biochemical processes at the whole body level in living subjects. As a result, PET imaging in cancer provides powerful means to:

- identify early disease;
- differentiate benign from malignant lesions;
- examine all organs for metastasis;
- stratify patients based on potential sensitivity to targeted therapies; and
- provide an early readout of response to therapy.

The major roadblock to increasing the applications of PET in preclinical and clinical research - and aiding drug discovery and molecular imaging diagnostics in the process - is a convenient and low-cost source of a diverse array of PET probes. Integrated microfluidic technology, with intrinsic advantages of speed, chemical economy, flexibility, user-friendliness, safety, modularity and low cost, is a prime technology platform for producing radiolabeled PET probes. The goal of Dr. Michael Phelps' lab is to develop new technology platforms that accelerate the discovery and development processes of new PET probes and facilitate a broader use and value of PET imaging. This joint project - which brought together the expertise of eight research groups covering the fields of radiochemistry, microfluidics, polymer materials, device prototyping, molecular imaging and antibody engineering from the Caltech, UNC and Stanford CCNEs - aimed to design, fabricate, and test three generations of microfluidic devices for automated production of [^{18}F]-labeled PET imaging probes. Compared to the conventional approach, accelerated synthesis of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), the most commonly used PET tracer for imaging altered glucose metabolic states in cancer, was accomplished with improved radiochemical yield and purity using

Generation-I devices. Design modifications in Generation-II devices enhanced reliability and increased the generality of this radiochemical technology platform. The rapid synthesis of a different PET tracer, [^{18}F]-3′-deoxy-3′-fluoro-L-thymidine ([^{18}F]FLT), a PET imaging probe for DNA replication and cell proliferation, has been demonstrated in the Generation-II devices.

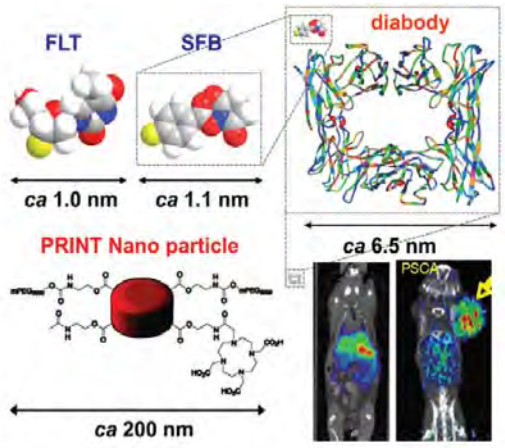

Figure 24: Illustration of various categories of small molecules, biomolecules and synthetic nanoparticles which can be labeled with positron-emitting radionuclides (*e.g.* F-^{18}) using our microfluidics. Micro-PET: PRINT nanoparticles (Cu-^{64}, left) and anti-PSCA (prostate stem cell antigen) minibody (I-^{124}, right). (Reprinted from The NCI Alliance for Nanotechnology in Cancer Bulletin (2008) 1 (1): 1-3).

In collaboration with Dr. Joseph DeSimone, at the UNC CCNE, and scientists at Liquidia Technologies, Inc., chemical- and solvent-resistant poly(perfluoropolyether) (PFPE) elastomers (developed at UNC and commercialized by Liquidia) were utilized to replace the original polydimethylsiloxane (PDMS) materials for the fabrication of the Generation III chips. Using the Generation III chips with improved chemical inertness and device robustness, the team was able to further expand the chip-based radiochemistry for syntheses of a wide range of PET probes. The team also worked with Dr. DeSimone's group to study *in vivo* biodistribution of the nanoparticles produced by their particle molding technology known as PRINT. The PRINT process enables the production and harvesting of monodisperse, shape-specific nanoparticles made from a variety of polymers. The PRINT nanoparticles decorated with 1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid (DOTA) can be labeled with the radioisotope ^{64}Cu and sequentially imaged in small animals via microPET (Fig. 24) to obtain their *in vivo* biodistribution properties. These PRINT particles are presently being designed to reach new understandings and therapies in cancer prevention, diagnosis, and treatment.

Dr. Anna Wu, from the Stanford CCNE, has accumulated extensive experience exploring the potential of engineering antibody fragments as PET probes with improved specificity and well-controlled pharmacokinetics. Dr. Wu's research group tested the feasibility of performing automated syntheses of [^{18}F]-labeled antibody fragments in a microfluidic setting. The group has been developing a new generation of microfluidic devices, in which two sequential reactions — radiosynthesis of N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and labeling of small quantities of antibody fragments with the *in situ* prepared [^{18}F]SFB — can be conducted in an automated fashion.

Such collaborative cross-disciplinary efforts significantly advanced the goals of the project and provided opportunities to develop nanotechnologies and microfluidics platforms for molecular imaging and cancer-related research. The group envisions that a microfluidic-based platform along with the existing widespread commercial supply of [^{18}F]-fluoride will provide an enabling technology for routine probe production and for academic and commercial scientists to accelerate their discovery and development of new tracers for PET in research, drug discovery and development, and molecular imaging diagnostics in patient care.

Tackling Metastasis through Team Science: Cancer Biologists Lead the Charge Synergizing their Discoveries behind Common Nanotechnology Platforms

Collaboration of Drs. Leland W. K. Chung (Emory-Georgia Tech CCNE), Jianjun Cheng (Washington University CCNE), Douglas Hanahan (UCSF CNPP), and Kattesh V. Katti (U Missouri CNPP)

Metastatic cancer is lethal and disseminating cancer cells are extremely difficult to kill. This problem can only be resolved by pursing an increased understanding of cancer biology, new technology for early cancer detection and novel therapy for the management of advanced cancer metastases even after cancer has spread to vital organs. ANC scientists Drs. Douglas Hanahan, Jianjun Cheng, Kattesh Katti, and Leland Chung worked together in a way that would not have been possible without the ANC initiative. The group met at various ANC PI meetings and followed up with emails and teleconferences. Unlike other traditional team science projects, this team scientists did not know each other when they began their efforts, had no prior joint publications, and were not likely to meet each other because of disparate scientific disciplines. Yet remarkably, because of their common passion to defeat cancer metastasis, and the collaborative network generated by the NCI ANC program, they came together and shared ideas, chemical reagents, cell lines, and laboratory models.

Dr. Hanahan's laboratory has worked on understanding the molecular events underlying multi-step carcinogenesis, cell proliferation, and angiogenesis. His group created a transgenic pancreatic cancer progression model that recapitulated the histopathology and behavior of human disease. Dr. Cheng is a polymer and material scientist with a strong commitment to translational research. He holds numerous patents on the design and synthesis of novel drug-nanoparticle conjugates for cancer drug delivery. Dr. Katti's laboratory has synthesized gold and silver nanoparticles for improved cancer diagnosis and therapy. His laboratory discovered bombesin, an effective targeting ligand for the delivery of these metallic nanoparticles to human cancer epithelial cells. Dr. Chung is a cancer biologist with a special interest in prostate cancer bone metastasis. His laboratory established a number of cancer metastasis models closely resembling human androgen-independent and lethal bone-metastatic prostate cancer.

Recently, Dr. Chung's laboratory, in collaboration with Dr. Lucjan Strekowski's laboratory at Georgia State University, discovered a class of near-infrared (NIR) heptamethine cyanine dyes that have the unique ability to be taken up by human and mouse cancer cells but not normal cells. Since pancreatic cancer and hormone-refractory bone-homing prostate cancers are considered the most aggressive and lethal forms of human cancers, Drs. Hanahan and Chung collaborated to demonstrate that one of the heptamethine cyanine dyes – IR-783 (Sigma-Aldrich) – can be readily taken up by several pancreatic and prostate cancer cell lines and pancreatic and prostate tumor xenografts in mice (Fig. 25).

Although this NIR dye can be photosensitized by light to yield tumoricidal derivatives, it requires high concentrations difficult to achieve in live animals. This problem attracted the attention of Dr. Cheng, who then worked with his graduate student, Rong Tong, and Dr. Chung's postdoctoral fellow, Dr. Xiaojin Yang, to develop a family of novel IR-783-taxol and taxotere (or IR-MUT-1) conjugates. They then evaluated the cytotoxicity and biodistribution of these organic dye-drug conjugates and demonstrated that these conjugates could accumulate in cancerous but not in normal tissues for a prolonged period of time (over 4 days). The attractive and promising properties of the dye-drug conjugates in pancreatic and prostate tumor models will soon be expanded into large scale studies of efficacy and safety with the hope that these designed drugs can be applied in human cancers as a new class of highly effective targeted therapeutics with minimal toxicity to normal tissues and cells.

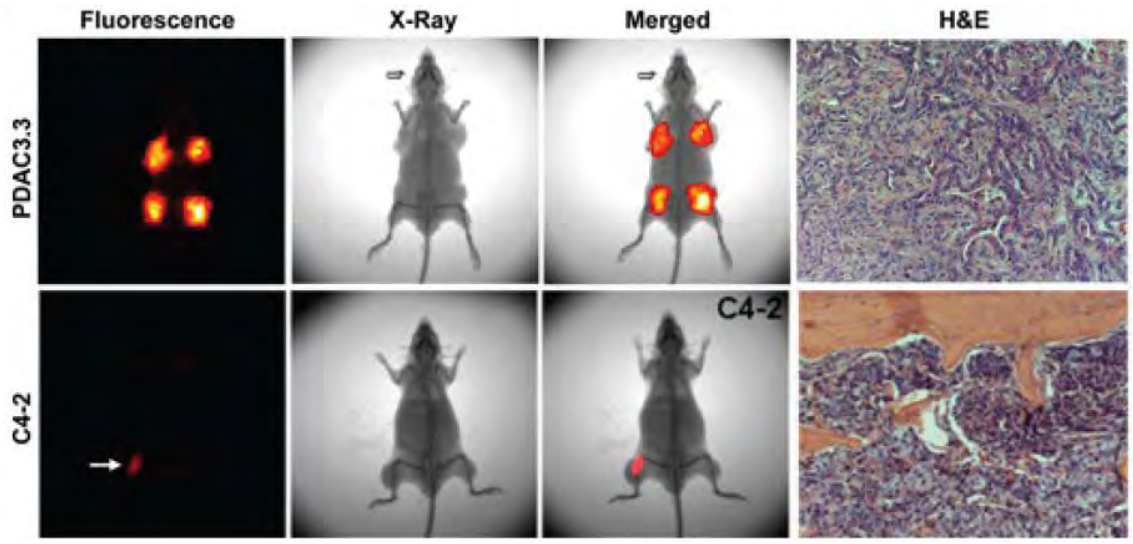

Figure 25: Imaging pancreatic tumors grown subcutaneously and prostate tumor grown in bone of immune compromised mice. A pancreatic tumor cell line, PDAC3.3, and a bone metastatic prostate tumor cell line, C4-2, were implanted respectively at subcutaneous and intratibial site of nude mice. Tumor growth can be clearly imaged without interference background (left panels). The presence of the tumors was confirmed by histopathology (right panels). (Reprinted from The NCI Alliance for Nanotechnology in Cancer Bulletin (2008) 1 (2): 1-4).

Dr. Katti's laboratory has now completed a series of basic studies documenting the effectiveness of bombesin as a cancer cell-surface-specific ligand that can guide gold nanoparticles to cancer cells without accumulating in normal cells. A collaboration with Dr. Chung's laboratory demonstrated that bombesin-guided gold nanoparticles can be delivered systemically to cancer cells. These new guided nanoparticles offer promise for cancer detection and also can be activated by external energy to induce focal hyperthermia to specifically kill cancer cells, which has the potential to improve cancer metastasis therapies.

Nano Mother Ships Designed to Detect and Treat Cancer

Collaboration of Sangeeta Bhatia (MIT-Harvard CCNE), Michael Sailor (UCSD CCNE), and Erkki Ruoslahti (UCSD CCNE)

A key nanotechnology objective is to build molecular devices that surpass the function of single molecules. Ultimately these enhanced nanodevices would provide modern medicine with integrated therapeutic and diagnostic function within a single *in vivo* delivery device. Until recently, multi-functional hybrid nanosystems have been studied *in vitro*, but there have been specific obstacles in moving to animal studies. The poor stability of nanodevices led to toxicity issues and poor targeting, while the natural clearing process of the animal's circulation system limited the nanodevice circulation time and subsequent effectiveness.

The multidisciplinary, multi-CCNE team of Drs. Sailor, Bhatia, and Ruoslahti came together through the support and nurturing of the ANC to address these obstacles. The team focuses on engineering multifunctional nanoparticles that exploit biological processes to guide the targeting, self-assembly, and remote function of materials to treat tumors in mouse models of cancer. Specifically, this team is developing long-circulating "nano mother ships" of micellar hybrid nanoparticles (MHN) (Fig. 26, Right). These MHN are composed of magnetic particles and quantum dots for dual mode imaging (magnetic resonance and fluorescence) and the anti-cancer drug doxorubicin within a single PEG-phospholipid micelle. This team's efforts

have led to a publication in *Angewandte Chemie* that provides the first demonstration of a single nanodevice capable of utilizing multi-modal imaging and targeted drug delivery to tumor tissue both *in vitro* and *in vivo*.

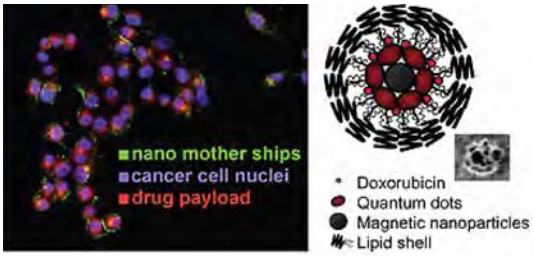

Figure 26: (Left) **The image shows the mother ships in green, sticking to MDA-MB-435 human carcinoma cells, whose nuclei appear blue.** The ships have just discharged their payload into the cancer cells: the anti-cancer drug doxorubicin (red in the image). (Right) **Structural diagram of nano mother ship design** (inset Transmission Electron Micrograph of MHN). (Reprinted from The NCI Alliance for Nanotechnology in Cancer Bulletin (2008) 1 (3): 1-4).

MHNs conjugated to a targeting peptide dock and merge contents into a specific cell, utilizing the cells endosomal system to eventually allow doxorubicin to reach the nucleus. Each MHN carries multiple iron oxide nanoparticles to enhance MRI brightness for locating the tumor in body and quantum dots for NIR fluorescence detection to enhance tumor visualization during surgery. Dr. Sailor's group concentrated on developing and evaluating the multi-functional nanoparticles containing the magnetic iron oxide nanoparticles and quantum dots for stability and imaging efficacy in this study. Synthesis of the "nano mother ship" involved combining magnetic nanoparticles and quantum dots (both coated with hydrophobic chains), followed by encapsulation into micelles of PEG-modified phospholipid (60-70 nm in size, see Fig. 26, inset TEM micrograph). The investigators found that as the ratio of magnetic nanoparticles to quantum dots increases, the fluorescence spectra was found to decrease, though detection was observed at sub-nanomolar QD concentrations.

Dr. Ruoslahti's research focuses on identifying unique tumor vasculature "zip codes" that can be used to identify homing peptides as targeting elements to deliver nanoparticles into tumors and other sites of disease. The targeting ligand that he identified, known as F3, binds specifically to endothelial cells in tumor blood vessels and transports payloads into tumor vasculature *in vivo*. Dr. Bhatia's group then utilized its expertise in cell and animal imaging to evaluate the targeting, detection, and delivery of the "nano mother ships" for *in vitro* and *in vivo* studies. They demonstrated an increase in both NIR fluorescence and MRI contrast within cells incubated with these hybrid nanoparticles. The F3 ligand on the surface of the hybrid particles was observed to chaperone doxorubicin into cancer cells though the endosomal pathway, which facilitated the release of the drug into the nucleus. The inherent red fluorescence of the drug provided a means to visualize this delivery process (Fig. 26, Left). Distinct fluorescence and MRI contrast was observed in nude mice bearing tumors, and treated with the "nano mother ships."

With any *in vivo* study, toxicity must be a consideration. However, the potentially toxic nature of cadmium quantum dots was not observed in this encapsulated form. Perhaps more importantly, the cytotoxicity of doxorubicin delivered in the hybrid nanoparticles was significantly greater than equivalent levels of free or untargeted doxorubicin-containing hybrid particles. Also, the PEG-coated MHN remained in circulation significantly longer than was observed with previous formulations.

Nanoparticle Measurements: Monitoring Nanoparticle-Biomolecule Conjugates Utilizing Mass Sensing with Resonating Microchannels

Collaboration of Drs. Joseph DeSimone (UNC CCNE) and Scott Manalis (MIT CNPP)

Current research exploring the therapeutic and diagnostic applications for nanomaterials is helping to identify critical parameters for the compatibility of various materials with biological systems. The most current findings identify physical attributes such as size, shape, hydrophilicity, and surface chemistry as key factors contributing to the fate of a nanomaterial *in vitro* and *in vivo*. Therefore, one of the keys to advancing the field of nanotechnology with regards to biomedical applications involves developing standardized, accurate, and reproducible methodologies for the characterization of the physical properties of a given nanomaterial. Ensemble (or bulk) characterization techniques are simple and fast, but do not directly reveal information about sample homogeneity. Single nanoparticle analysis techniques based on microscopic imaging reveal homogeneity, but are slow and labor intensive.

The collaboration between the Dr. DeSimone's and Dr. Manalis's laboratories was established to address the issue of applying suspended microchannel resonators to enhance the characterization of PRINT® nanoparticles. The PRINT platform, developed by Dr. DeSimone and his collaborators, uniquely leverages micro- and nano-fabrication techniques from the electronics industry to precisely control the size and shape of particles ranging in size from tens of nanometers to hundreds of microns. The PRINT technology is being utilized for numerous applications, one of which is engineered drug therapies. Since the PRINT® process allows for the modification of size, shape, modulus/flexibility, chemical composition, and surface functionality, it is vital to have instrumentation to monitor the effect of these modifications on the outcome of the final "formulated" nanoparticle.

The miniaturization of mechanical resonators to the nanoscale has enabled mass to be resolved with a precision equivalent to a single gold atom when measured in vacuum. However, mass measurements in fluids have been over twelve orders of magnitude less sensitive (typically on the order of a nanogram), in part resulting from viscous damping from fluid that surrounds the resonator. In order to reduce this damping, the Manalis lab has developed the suspended microchannel resonator (SMR), which consists of a vacuum-packaged hollow cantilever. In addition to being able to resolve the mass of cells or particles with femtogram precision, the SMR can also measure mass density and charge. Not only will this technology provide exquisitely accurate determination of the mass of manufactured nanoparticles, it will also provide precise measurements to determine the efficiency of numerous particle modifications. For example, this technology will be able to determine the efficiency of functionalization (targeting peptide or monoclonal antibody) of the surface of the nanoparticle. Also, this technology can be utilized to monitor particle degradation elicited by change in pH or other biologic factors.

Photodynamic Therapy to Treat Ovarian Cancer

Collaboration by Drs. Tayyaba Hasan (MGH-Harvard CNPP) and Andrew Ellington (Stanford CCNE)

The primary research focus of Dr. Tayyaba Hasan's laboratory at the MGH-Harvard CNPP lies in the development of the photodynamic therapy (PDT) platform to treat malignancies, pathogenic infections, and other diseases. PDT is a photochemistry-based approach to disease therapy in which a light-activatable molecule - the photosensitizer - is excited with light to generate reactive oxygen species that destroy a variety of biomolecules. PDT is approved for the treatment of various malignant and non-malignant diseases, and it is routinely used for obstructive esophageal cancer and cases of advanced and early lung cancers. In addition, the FDA has approved a PDT-based therapy developed in Dr. Hasan's laboratory as a first line treatment for age-related macular degeneration. However, efforts to use PDT more

widely in the treatment of cancer have been limited because of associated toxicities that arise when the photosensitizer is inadvertently activated in non-targeted tissues. Combining nanotechnology with PDT could produce a powerful tool for selective destruction of diseased cells and tissues. One of the diseases in which targeted delivery of PDT is very important is in ovarian cancer. PDT can be effective on drug-resistant cancer cells and has shown promise in clinical studies of ovarian cancer. It is, however, dose limited as a result of the fact that the disseminated nature of the disease requires diffuse light to illuminate the entire peritoneal cavity.

In order to potentiate mechanism-based combination treatments with PDT using nanotechnology, the Hasan laboratory has developed a nano-construct termed nanocell in which the PS is non-covalently trapped inside a polymer nanoparticle and these nanoparticles, along with additional therapeutic and imaging agents, such as the anticancer therapeutic Avastin®, are then encapsulated inside liposomes. Experiments in Dr. Hasan's laboratory have demonstrated that these nanocells will deliver Avastin® and a photosensitizer known as benzoporphyrin derivative monoacid (BPD) to ovarian cancer cells and transport the two active agents into the tumor cells (Fig. 27). The investigators have since shown that these untargeted nanocells can serve as contrast agents capable of detecting ovarian cancer *in vivo* in an orthotopic mouse model using a fluorescence microendoscope.

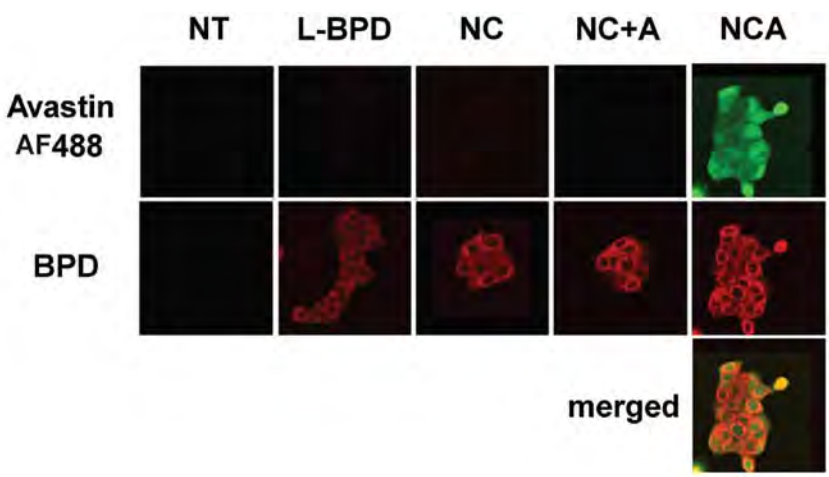

Figure 27: OVCAR-5 cells were incubated with various constructs for 90 minutes and imaged using confocal microscopy. Avastin®-Alexa 488, as shown in green, is only delivered intracellularly when encapsulated in a nanocell (NCA). Free Avastin®-Alexa 488 (NC+A) is cell-impermeant and is not delivered intracellularly. (Reprinted from The NCI Alliance for Nanotechnology in Cancer Bulletin (2010) 1 (1): 1-5).

However, for the treatment of complex sites such as the peritoneal cavity it is likely that enhanced selectivity will be required in order to prevent collateral damage to adjacent organs. To develop an ovarian cancer targeting agent, Dr. Hasan has been collaborating with Dr. Andrew Ellington at the Stanford CCNE to identify and test aptamers that target the epidermal growth factor receptor that is overexpressed by ovarian tumors. This collaboration was initiated as a result of the ANC Annual Meeting held in Chicago in September 2008 and was sparked by a poster describing EGFR targeting aptamers presented by Dr. Na Li, a postdoctoral fellow in Dr. Ellington's laboratory. Aptamers have rapidly emerged as a novel class of ligands that are capable of binding to target molecules with high affinity and specificity. Dr. Ellington is a leader in the field of aptamer design and one of the originators of aptamer selection technologies. Dr. Ellington's group has developed a 2'F-Py modified extended anti-EGFR RNA aptamer with demonstrated specificity for EGFR expressing cell lines. This is a stable RNA aptamer on which a complimentary oligonucleotide is hybridized to provide a fluorescence probe for tracking and an

amino terminal group for conjugation to the nano-constructs.

In just nine months, the collaborators have already demonstrated that this aptamer successfully targets the photosensitizer-containing nanocell construct developed by Dr. Hasan's laboratory specifically to cells expressing EGFR. Confocal microscopy experiments establish the distinct localization of these nanoparticles and the aptamers. In the future, nano-constructs will be combined to give the high payload of therapeutic and imaging agents and are expected to localize at EGFR rich sites. The Hasan and the Ellington groups plan to continue the collaboration and to use these aptamers for conjugation to the nanoparticles to target ovarian cancer. Using both *in vitro* and *in vivo* models of disease, their aim is to study this targeted nanocell construct through the custom designed microendoscope to improve early detection of microscopic malignancies *in vivo*, online monitoring of treatment effectiveness, and initiation of secondary treatments, where necessary, to enhance overall survival.

Working Groups

The ANC is a network of organizations that share the common goal of advancing the science of nanotechnology in cancer. As such, there are many advantages to not only sharing the scientific efforts of the individual ANC participants across the whole of the ANC, but also collaborating through working groups on several interests. Since the program's inception, ANC members working closely with ANC Program Staff have created several working groups. The working groups included:

***In vitro* Diagnostics Working Group** – focused on the development of new, highly selective, and specific *in vitro* assays for early diagnosis of the disease, including the design of new sensor platforms, availability of biomarkers and new biological assays is expected to be carried out here.

Imaging Working Group – focused on the development of *in vivo* imaging techniques that can provide more specific information about tumor type and tumor environment and thus virtually eliminate the need for surgical biopsy prior to determining the therapy.

Therapy and Post-Therapy Monitoring Working Group – focused on the development of novel localized therapeutic solutions, nanoparticle-based formulation of new drugs, and methodologies to monitor outcomes of therapy.

Informatics/ Data Sharing Working Group – focused on the development of new bioinformatics tools, databases, and data-sharing methodologies, including the development and adoption of the cancer Nanotechnology Laboratory Portal (caNanoLab) by all members of the ANC. For more information on caNanoLab, see page 71.

Nanotechnology Tools and Fabrication Working Group – focused on designing, synthesis, fabrication, and characterization of new nanoscale structures, devices, and systems and to use them as a tool-kit to in oncology research.

Toxicology Working Group – focused on toxicity challenges in nanomedicine. This group leveraged and extended existing concepts from *in vivo* characterizations performed on small molecules and biomedical devices to develop new models in support of toxicity challenges in nanotechnology.

The Communications and Integration Working Group (CIWG) – focused on supporting interactions and providing a forum for frequent updates on the operation of individual centers and projects within the program to further promote interactions amongst the ANC community. The group has developed and contributed to several ANC-wide initiatives including *The Alliance for Nanotechnology in Cancer Bulletin*, *Nanotech News*, *ANC Calendar*, and *Image Gallery*. Recently, the CIWG has produced the *Best Practices Operations Manual*, a resource tool for those involved in the operation of NCI-sponsored CCNEs. Detailed information on these initiatives can be found in "Outreach and Education" chapter of this book.

Program Collaborations with Other NIH Programs and Federal Agencies

The ANC program collaborates with and supports the development of other nanotechnology initiatives within NCI and NIH. There are several Funding Opportunity Announcements, including:

Image-Guided Drug Delivery in Cancer (PA-CA-09-253) prepared jointly with Cancer Imaging Program at DCTD will support the development of quantitative *in vivo* imaging methods in image-guided drug delivery (IGDD) to interrogate tumor-drug interaction, study biodistribution, pharmacokinetics, pharmacodynamics, and therapeutic response

Advanced In Vivo Imaging to Understand Cancer Systems prepared jointly with Cancer Imaging Program at DCTD will provide an opportunity for new collaborations among cancer complexity researchers, cancer imagers and experts in cutting-edge applications such as nanotechnology

Cancer Diagnostic and Therapeutics Agents Enabled by Nanotechnology (PAR-CA-10-286) prepared jointly with SBIR program will support pre-clinical optimization and testing of promising nanotechnology-derived cancer therapeutics, *in vivo* imaging agents and *in vitro* diagnostics

ET-Cure Supplements to Parent Grant awarded jointly with the NCI Center to Reduce Cancer Health Disparities (CRCHD) provide training opportunities for scientists from underserved populations in nanotechnology, clinical proteomics, bioinformatics and cancer health disparities

NCI-NSF Training Grants were awarded in 2005 to establish training programs for U.S. science and engineering doctoral students through the Integrative Graduate Education and Research Traineeship Program (IGERT)

In addition to these grant opportunities, we also established a small contract program to support studies in nanomaterials biodistribution and toxicity in larger animals and re-formulation effort that attempts to resurrect drugs that failed in free systemic delivery resulting from 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 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 Trans-NIH Nanotechnology Task Force in 2006 to coordinate efforts in this area across the agency.

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 were two joint meetings: in Beijing in 2008 and Washington, DC in 2010.

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

The ANC maintains also close consultation with the FDA on regulatory review of nanotechnology enabled devices and nanomaterials for biomedical application.

CHAPTER 9

Outreach and Education

Strong educational resources are instrumental to the effective introduction of newly developed technologies, especially within a complex area of research that crosses between biomedicine, engineering, and oncology. This need to increase our knowledge and understanding of how nanotechnology can be used to improve cancer diagnosis and treatment applies to the many disciplines within the scientific community involved in nanotechnology research, as well as to the public at large. The OCNR staff remains committed to the development of educational resources and participation in various activities that demonstrate how nanotechnology can be leveraged to improve cancer research and clinical care, including an extensive Web site, trainings at all levels from K-12 students to oncologists, diversity trainings, seminars series, symposia, and various scientific publications.

Training

Nanotechnology research is by nature an interdisciplinary endeavor. Investigators with basic science, engineering, molecular biology, and clinical backgrounds must work closely together in order to design new drugs and diagnostic tools that combine nanostructured materials, biological molecules, and novel instrumentation. The ANC has implemented numerous training and career development mechanisms to build an interdisciplinary, biologically inspired nanotechnology workforce, and to support the research teams working on NCI-funded nanotechnology projects. In addition to the Multidisciplinary Fellowships in Cancer Nanotechnology Research and NRSA award for postdoctoral and senior fellow trainees with interdisciplinary training (listed on the pages 16 and 17), the ANC initiated and coordinated several educational activities in cancer nanotechnology for different audiences ranging from physicians to K-12 students.

ANC Investigators Training and Education Activities

Each of the eight CCNEs has also developed their own internal multidisciplinary training and educational programs, including:

The Emory-Georgia Tech CCNE successfully convened the Frontiers of Cancer Nanotechnology Seminar Series to educate a multidisciplinary research community on new and novel nanotechnologies. Each presentation was broadcast live over the Internet and archived on the CCNE Web site for broadest possible access. The Center also ran a postdoctoral fellowship program and developed new nanotechnology courses for a multidisciplinary audience. Emory-Georgia Tech CCNE fellows were required to be assigned to at least two principal investigators. The program included scientists with a wide range of backgrounds and skills, ranging from electrical engineering to molecular biology. Participants within these fellowships worked closely with CCNE investigators on nanoparticle platform development as well as clinical translation for both cancer therapy and imaging.

The UCSD CCNE successfully developed and implemented four nanotechnology forums and two workshops and offered an extension course

during the summer of 2007. The UCSD also established a new Department of NanoEngineering within its Jacobs School of Engineering effective July 1, 2007. Two CCNE investigators - Drs. Sadik Esener and Michael Heller - served on the leadership team for this new department. Another example of active engagement in training was an exchange of students and postdoctoral fellows between the UCSD and MIT-Harvard CCNEs. The students in this program worked on a joint collaboration project involving Drs. Sangeeta Bhatia (MIT-Harvard CCNE), Michael Sailor and Erkki Ruoslahti (both from UCSD CCNE).

The Caltech CCNE developed a strong interdisciplinary student exchange process as an integral part of carrying out its research, with students from pathology and immunology departments routinely working in nanofabrication laboratories. A pilot program was developed for Caltech CCNE graduate students and postdoctoral fellows to be coupled into a Caltech K-12 outreach program. During each month of this pilot program, two Caltech CCNE students took their science to public schools in Los Angeles to expose and educate younger students on cancer nanotechnology.

The MIT-Harvard CCNE focused postdoctoral, graduate, and undergraduate training efforts on the interface between nanotechnology and "wet-bench" cancer biology, particularly *in vivo* mouse model studies. Consistent joint scientific group meetings enabled cross-disciplinary dissemination of research efforts and new knowledge. In addition, Dr. Angela Belcher's laboratory has developed a Nanotechnology Teaching Module for elementary school students to provide hands-on experience with nanotechnology called "Seeing the Nanoscale." The laboratory has been working with approximately 50 students around the country on projects as part of the FIRST LEGO League (FLL). FLL presents science and technology concepts to children ages 9 through 14, using real-world context and hands-on experimentation. About 30 students visited the Belcher laboratory. Dr. Belcher also met with students in Berkeley, California, to design projects involving nanotechnology to seek out cancer cells. Dr. Bhatia's lab has continued to host middle school girls in the lab through the MIT KEYs program. KEYs is a motivational program that brings 11-13 year old girls together with MIT female students to participate in workshops held periodically throughout the year. The goal of KEYs is to empower young women by promoting their self-confidence, increasing their self-esteem, and unveiling opportunities for their potential career paths.

Research and education at the graduate and postgraduate levels have been tightly linked at the Northwestern CCNE**.** Over the past year, 34 graduate students and 20 postdoctoral fellows were actively engaged in CCNE research. Recognizing the unique opportunity to provide undergraduate research opportunities in hands-on translational medical nanotechnology research, the Northwestern CCNE directed efforts over the past year to research, develop, and launch a Research Experience for Undergraduates (REU). The program effectively leverages two existing programs at Northwestern University: the NSF-sponsored REU program at the International Institute for Nanotechnology and the NCI-funded Emerging Technologies Continuing Umbrella of Research Experiences (ETCURE) Program at the Robert H. Lurie Comprehensive Cancer Center. The nine-week summer program included an intensive immersion in laboratory-based scientific research, which was augmented by research seminars, a field trip to Argonne National Laboratory (ANL), professional communication workshops, technical writing workshops, and access to and training on state-of-the-art instrumentation, social activities, a final symposium, and experience as a submitter and peer reviewer in *Nanoscape: The Journal of Undergraduate Research in Nanotechnology*.

The Washington University CCNE developed a nanotechnology course (Current Topics in Nanomedicine), which was taught through the Division of Biology and Biomedical Sciences (DBBS) and was cross-listed in the School of Engineering and Applied Sciences (SEAS). The course masters included Drs. Samuel Wickline, Michael Hughes, Patrick Winter, and Irfan Ahmad. The Washington University CCNE has successfully implemented a distance learning component for developed nanotechnology courses and students from the UC-Santa Barbara, UC-Berkeley, Emory University, and

Georgia Tech have enrolled, receiving credit through Washington University. The lectures have been videotaped and archived using a freeware program called Moodle, allowing access to other CCNE investigators from Washington University and other sites in the country. Additionally, this CCNE convened a nanomedicine seminar series and the annual Nanotechnology and the Life Sciences Workshop in collaboration with the National Heart, Lung, and Blood Institute Program of Excellence in nanotechnology at the Washington University in St. Louis.

The CCNE at University of North Carolina successfully applied to its graduate school for establishment of a Graduate Certificate in Nanotechnology, which supplemented the training of students and postdoctoral fellows in an interdisciplinary fashion, including a specialized course, research rotation, and course selections designed to foster cross-disciplinary training between the physical and biological sciences.

The Stanford CCNE organized a nanomagnetic biosensor and MEMS symposium at the 2006 International Magnetics Conference. Center researchers also hosted a monthly seminar titled "Integrated Cancer Biology Seminar" that focused on biocomputational problems requiring analysis of high-throughput molecular data. Dr. Robert Sinclair, principal investigator of the Stanford CCNE's nanotechnology characterization core, has been working with the International Association for Nanotechnology (IANANO) in providing training for their "Training the Trainer" series of workshops to educate science teachers and practicing scientists and engineers in nanomaterials characterization.

Webinars on Cancer Nanotechnology

In January 2010, the UCSD CCNE, working with NanoTecNexus and ONCR Staff, launched a webinar series on cancer nanotechnology titled, *"New Strategies and Innovations to Understand, Treat, and Monitor Disease."* This webinar series was geared toward physicians, pharmacists, nurses, therapists, health providers, technologists, and researchers in all areas of science. So far, two series have been given. A combined total of eleven sessions were delivered across the two series, which could be viewed separately or one could participate in the full series to get a comprehensive picture of how nanotechnology is changing cancer treatment. Each module was accredited by the Accreditation Council for Continuing Medical Education (ACCME®). Each session was designated for 1.0 *AMA PRA Category 1 Credits™* and physician and healthcare providers could get a maximum of 11 credits combined. Both series brought expertise from many fields within the ANC and was intended to demonstrate how translational nanotechnology can enhance the diagnosis, monitoring, and cutting-edge treatments of cancer. Several investigators from the ANC conducted a seminar session, including. Drs. Sadik Esener, Robert Mattrey, Mike Heller, Liangfang Zhang, Joseph DeSimone, Shad Thaxton, Greg Lanza, Shuming Nie, and Paul Mischel.

Diversity Training in Cancer Nanotechnology

The ANC and the NCI Center to Reduce Cancer Health Disparities (CRCHD) have partnered to provide training opportunities for high school and undergraduate students from underserved populations in nanotechnology. CRCHD provides a range of training opportunities for underrepresented and underserved groups (http://crchd.cancer.gov). Through mentoring, training, and different funding mechanisms, CRCHD provides opportunities necessary for trainees to launch careers as independent researchers. Called the Emerging Technologies Continuing Umbrella of Research Experiences (ETCURE) initiative, the overarching goals of this initiative are to:

- Create a pipeline of underserved students and investigators in the fields of emerging and advanced technologies;
- Increase the number of scientists from underserved populations with training in emerging technologies and cancer health disparities;
- Enhance the application of emerging technologies to cancer research through increased training and educational opportunities; and
- Foster academic, scientific, and multidisciplinary research excellence, culminating in the emergence of a mature investigator capable of securing competitive advanced research project funding.

The ETCURE program at the UCSD CCNE was developing a pipeline for training underserved investigators. Throughout Phase I of the ANC, there were seven undergraduates training in the ETCURE program, all of whom were either juniors or seniors. The curriculum for these students included several distinct features that were designed to prepare the students for a successful career in cancer research. The UCSD research training program had been led by Drs. Andrew Kummel and Tim Johnston. All students were active participants in nanotechnology research, weekly group lab meetings, and journal clubs. An integral program component was to provide the students with a research experience that enabled them to become independent competitive cancer researchers. It was paramount that the application of emerging technologies to cancer research through increased training and educational opportunities were being utilized to increase the number of underserved in the pool of future investigators.

In addition to the ETCURE program, the CRCHD together with the ANC utilized supplements to research grants to promote diversity in health-related research. This mechanism was used in several institutions funded through the ANC including UCSD, Stanford, Northwestern, and University of North Carolina.

Overall the ETCURE program provided a model for increasing diversity within the cadre of cancer research scientists within the field of emerging technologies and by extension cancer health disparities.

Outreach through Multimedia

The ANC Web site is a main venue for communication with the public and the broader scientific community. Since launching in 2004, the ANC Web site, http://www.nano.cancer.gov, provides immediate access to critical information and resources about cancer nanotechnology research funded through the ANC. The Web site provides content that is relevant to ANC members, researchers, and health professionals, as well as to cancer patients, their families, and the general public.

The site's information is logically arranged by topic, and a search function allows convenient text-word searching across all NCI Web pages. The Web site is a comprehensive resource that enables users to quickly find accurate and up-to-date information about nanotechnology-based drugs in clinical trials, ANC research programs, funding opportunities, and the ANC itself.

Major sections of the Web site include:

- *Learn About Nanotechnology* – with video, animations, visuals, glossary, and other educational resources for the public to understand the science of nanotechnology, its impact on cancer, and progress in this field of research
- *Collaborate* – with information to facilitate cross-disciplinary research, including funding, training, and data sharing resources
- *Alliance in Action* – with detailed ANC-funded program specifics, recent accomplishments, meetings and events, news, and published research

- *About the ANC* – with highlights of the ANC program mission and goals, areas of focus, and ONCR Staff.

The Web site also allows for frequent communication with the investigators involved in the ANC. The Nanotechnology Teaming Site, accessed through an Intranet portal, enables ANC investigators to engage in online discussions and develop relationships across the entire ANC. The password-protected component of the Intranet facilitates uploads of the files, presentations, and reports for easy sharing and collaboration.

To date, the ANC website has experienced over one million unique visitors from around the world and registered more than 40 million hits.

Multimedia Features enhance the ANC educational outreach efforts. Several ANC awardees have developed several multimedia presentations for the general public. For example, the UCSD CCNE produced a 3-minute video incorporating animation and short interviews with UCSD CCNE research staff on how the CCNE is using nanotechnology to advance cancer diagnosis and therapeutics. The Washington University CCNE also produced a 2-minute video incorporating animation and interviews with its researchers on nanotechnology and its potential applications in cancer detection, treatment, and imaging. The animation is featured on the Web site homepage (www.sccne.wustl.edu) and was also posted on YouTube.com. Both the UCSD and Washington University videos have been replicated onto DVDs that are disseminated to industry, local secondary schools, and local organizations.

The NCI Alliance for Nanotechnology in Cancer Bulletin is published quarterly and distributed to all ANC members and shared with the public through the ANC Web site. The Bulletin showcases the research, outreach programs, commercialization activities, researchers, announcements, and job opportunities of the ANC. The Bulletins can be found on the ANC Web site at: http://nano.cancer.gov/action/news/alliance_bulletin.asp

Nanotech News provides the latest developments in nanotechnology and cancer research. These short news articles are published monthly and posted to the ANC Web site (http://nano.cancer.gov/action/news/nanotech_news.asp) for continued reference and distributed to all ANC Web site subscribers through a monthly email. Several science news Web sites pick up and distribute many of the stories, achieving broad distribution of cancer nanotechnology news to both scientific and general public audiences.

ANC Calendar is a high-quality, 4-color hanging wall calendar showcasing the exciting work being conducted by ANC researchers through "nano art". The calendar was one of the first projects coordinated by the Communication and Integration Working Group. The calendar is used to communicate the work of the ANC to the lay community. Images are solicited from all members of the ANC. In many cases, the submitted images are manipulated via design software to enhance artistic expression. Final images and layperson-friendly captions are shared with the contributing researchers before the final images are included in the calendar. The calendar is distributed among the ANC members, partners, donors, and policymakers.

Image Library is an online resource of nanotechnology visuals, created by the ANC members to share images and multimedia presentations of their research for educational purposes. The images, submitted by ANC members, provide a compelling visual of nanotechnology at work. These images are available to members of the media, researchers, and students to broaden understanding of the application of nanotechnology to cancer research and care. The Library is accessible through the ANC Web site at http://nano.cancer.gov/learn/understanding/library.asp

CHAPTER 10

Conclusion

The mission of NCI and the ANC is to relieve the burden of cancer. The ANC's efforts created innovative solutions to disease prevention, diagnosis, and treatment as well as translated several of these research findings into improved clinical practices.

Over the last five years, the ANC program has not only produced strong scientific results, but also helped in establishing the field of cancer nanotechnology and the community of researchers from disparate field working in it. The ANC has demonstrated that the multidisciplinary approach catalyzes research developments and advances clinical translation in cancer nanotechnology. By forming NCL, NCI established a hub for nanomaterials characterization and standardization which supports the community and aids and moving technologies through translational pipeline.

The early discoveries from the ANC program laid a strong foundation and demonstrated the numerous opportunities presented by nanotechnology materials design. Additional developmental work moves these discoveries towards the clinic. Some of the important developments within the last five years include the clinical validation of *in vitro* diagnostic technologies developed in the Caltech and Northwestern CCNEs, the promising clinical trial results for siRNA delivery at Caltech CCNE, the preclinical development of chemotherapeutic nano-formulations in the MIT-Harvard CCNE and the intracellular delivery studies using PRINT nanoparticles in the UNC CCNE, 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 the infrastructure for the uniform validation of nanomaterials for medical use.

The ANC continues to built on its foundation, , as evidenced by the number and preliminary success of clinical trials launched by ANC researchers. Some examples include Dr. Mark Davis's siRNA-nanoparticle delivery trial (Caltech CCNE), development of polymer nanoparticles with very long circulation time by BIND Biosciences, and new, approaching IND approval of a diagnostic technique for colon cancer by Dr. Sanjiv Sam Gambhir (Stanford CCNE).

The OCNR Program efforts to foster a collaborative spirit in the last five years 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 post-doctoral researchers were able to use network connections formed at PI meetings to establish their next positions.

In re-issuing the ANC, NCI has signaled that it continues to have high expectations for cancer nanotechnology's impact on clinical practice. Therefore, the goal of the next phase is to broaden access to cancer nanotechnology research through greater clinical translation and outreach to the patient and clinical communities and to support the development of entirely new models of cancer care.

CHAPTER 10

Path Forward

The ANC has demonstrated that a multi-disciplinary 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 that failed clinical development as a result of toxicities relating to their systemic administration; nanoparticle delivery stands to improve the therapeutic index of these drugs and create new therapeutic opportunities relating to their use. Drugs and devices will converge in multi-functional 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, but 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.

Cancer prevention strategies have largely overlooked nanotechnology tools so far, so more research in needed in this area. 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 here as well.

The second phase of the ANC began 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 clinically-worthy technologies as well as on 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 investigators given 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

Selected Research Articles

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APPENDIX 2

Selected Intellectual Property Disclosures

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110. Lanza GM. *Cytotoxic Peptides on Nanoparticle Carriers for Therapy. CCNE - Washington University*
111. Lanza, GM, Senpan A, Pan D, Caruthers SD & Wickline SA. *Development of Colloidal Iron Oxide Contrast Agent for Magnetic Resonance Imaging (MRI) and Magnetic Particle Imaging (MPI). CCNE - Washington University*
112. Levi J, Gambhir SS & Keren De A. *New Methodology for Imaging Bioluminescence Resonance Energy Transfer (BRET) from Live Cells and Living Subjects. CCNE - Stanford*
113. Levi J, Gambhir SS & Keren S. *Novel Molecular Imaging Contrast Agents for Photoacoustic Imaging. CCNE - Stanford*
114. Li C, Lu W, Melancon M, Xiong CY & Stanford J. *Targeted Hollow Gold Nanoshells for Diagnostic and Therapeutic Applications. CNPP - U. of Texas*
115. Li W, Lin B & Tu LC. *Devices for Studying Cancer Invasion and Migration. CCNE - UNC*
116. Li, C., Lu, Wei, Xiong, C-Y., Zhang, J. Z. *Targeted Hollow Gold Nanostructures and Method of Use. CNPP - U. of Texas*
117. Lin W, Kim J, Rieter W & Taylor K. *Hybrid Nanomaterials as Multimodal Imaging Contrast Agents. CCNE - UNC*
118. Lu H & Cheng J. *Controlled Ring-Opening Polymerization of NCA Using Organosilicon Reagents. CCNE - Washington University*
119. Lu J, Lalush D, Yang G & Zhou O. *A Stationary X-ray Digital Tomosynthesis System for Breast Imaging. CCNE - UNC*
120. Lu J, Liu Z & Zhou O. *Micro-Focus Field Emission X-Ray Sources and Related Methods. CCNE - UNC*

121. Lu J, Zhang J, Lalush D & Zhou O. *Binary Multiplexing X-Ray Radiography. CCNE - UNC*
122. MacDonald R, Koynova R & Wang L. *Superior Lipofection Activity of Cationic Lipid Mixtures at the Gel-Liquid Crystalline Phase Transition. CCNE - Northwestern*
123. Manalis S. *Integrated Microsystem for Biomolecular Detection. CNPP - MIT*
124. Manalis S. *Measurement of Concentrations and Binding Kinetics* (patent application has been approved by the examiner). *CNPP - MIT*
125. Manalis S. *Suspended Microchannel Detectors* (US Patent No. 7,82,329). *CNPP - MIT*
126. McCarthy J, Jaffer F, Weissleder R. *Targeted Imaging and Therapy via a Fibrin Avid Peptide. CCNE - MIT-Harvard*
127. Meade T, Dravid V, Aslam M & Schultz Sikma E. *Novel T1-T2 Multimodal Contrast Agents for Magnetic Resonance Imaging. CCNE - Northwestern*
128. Meade T, Schultz Sikma E & Ulrich B. *T1/T2 MRI Contrast Agent with Cleavable Linker for Detection of Enzyme Activity. CCNE - Northwestern*
129. Meade T, Song Y & Kohlmeir E. *Macromolecular MRI Contrast Agents Through Copper(l) Catalyzed [3+2] Cycloaddition Reaction for Cellular Tracking and Fate Mapping. CCNE - Northwestern*
130. Meade T., Mirkin C. Song Y., Xu X. *Intracellular Delivery of MR Contrast Agents with DNA Modified Gold Nanoparticles. CCNE - Northwestern*
131. Mirkin C, Georganopoulou D, Xu X & Hill H. *In situ Detection of Nucleic Acids Based upon the Light Scattering Properties of Silver-Coated Nanoparticle Probes. CCNE - Northwestern*
132. Mirkin C, Giljohann D, Seferos D & Patel P. *Modulating Uptake of Olignucleotide-Modified Nanoparticles. CCNE - Northwestern*
133. Mirkin C, Han MS & Lytton-Jean A. *Chip-Based Detection of Duplex and Triplex DNA Binders with DNA Modified Gold Nanoparticles. CCNE - Northwestern*
134. Mirkin C, Han MS & Lytton-Jean A. *Colorimetric Screening of DNA Intercalators with Gold Nanoparticle Probes. CCNE - Northwestern*
135. Mirkin C, Hill H & Hurst S. *Polyvalent DNA-Au Nanoparticle Conjugates Exhibit Size-Dependent Cooperativity. CCNE - Northwestern*
136. Mirkin C, Huo F & Lytton-Jean A. *Asymmetric Functionalization of Nanoparticles Based on Thermally Addressable DNA Interconnects. CCNE - Northwestern*
137. Mirkin C, Huo F, Zheng A & Zheng G. *Multi-Scale Soft Pen Lithography. CCNE - Northwestern*
138. Mirkin C, Hurst S, Han MS & Lytton-Jean A. *Screening the Sequence Selectivity of DNA-Binding Molecules Using a Gold Nanoparticle Based Colorimetric Approach. CCNE - Northwestern*
139. Mirkin C, Lee JS & Seferos D. *Conjugation and Thermodynamically Controlled Separation of 2 nm Gold Nanoparticles Densely Functionalized with DNA. CCNE - Northwestern*
140. Mirkin C, Lee JS, Hill H & Elghanian R. *Scanometric Protein Array Detection with DNA-Functionalized Gold Nanoparticles. CCNE - Northwestern*
141. Mirkin C, Lee JS, Lytton-Jean A & Hurst S. *Silver Nanoparticle Oligonucleotide Conjugates Based on DNA with Triple Cyclic Disulfide Moieties. CCNE - Northwestern*
142. Mirkin C, Levine J, Xu X, Zhao Z, Qin L & Wei W. *A Fluorescence Recovery Assay for the Detection of Protein-DNA Binding. CCNE - Northwestern*
143. Mirkin C, Lytton-Jean A & Hurst S. *Maximizing DNA Loading on Gold Nanoparticles. CCNE - Northwestern*
144. Mirkin C, Min SH, Lytton-Jean A. *Colorimetric Screening of Triplex DNA Binders with Gold Nanoparticles Probes. CCNE - Northwestern*
145. Mirkin C, Patel P, Giljohann D, Seferos D & Daniel W. *Multifunctional Peptide and oligonucleotide Nanoparticles for Gene Regulation. CCNE - Northwestern*
146. Mirkin C, Seferos D & Gilijohann D. *Nanoparticles for Control of Drugs. CCNE - Northwestern*
147. Mirkin C, Seferos D & Giljohann D. Locked *Nucleic Acid-Nanoparticle Conjugates. CCNE - Northwestern*
148. Mirkin C, Seferos D & Giljohann D. *Particles for Detecting Intracellular Targets. CCNE - Northwestern*
149. Mirkin C, Taxton C, Rossi N, Giljohann D.*Gold Nanoparticles for Therapeutics. CCNE - Northwestern*
150. Mirkin C, Xu X & Han MS. *A Gold Nanoparticle-Based, Real-Time Colorimetric Screening Method for Endonuclease Activity and Inhibition. CCNE - Northwestern*
151. Mirkin C, Xu X & Rosi N. *Asymmetric Functionalization of Nanoparticles with Oligonucleotides. CCNE - Northwestern*
152. Mirkin C, Zheng G & Daniel W. *High Sensitivity Telomerase Detection Based upon*

Nanoparticle Conjugates with Barcode Amplification. CCNE - Northwestern

153. Mirkin C., Giljohann D., Seferos D., Prigodich A., Patel P. *Gene Regulation with Polyvalent siRNA Nanoparticle Conjugates. CCNE - Northwestern*
154. Mirkin C., Hill H., Hurst S. *Curvature-Induced Base Pair Slipping Effects in DNA-Nanoparticle Hybridization. CCNE - Northwestern*
155. Mirkin C., Kim D., Weston D. *Multiplexed Scanometric Immunoassay for Protein Cancer Markers. CCNE - Northwestern*
156. Mirkin C., Massich M., Giljohann D., Seferos D. *Controlling Immune Response Using the Polyvalent Nanoarchitecture of DNA Gold Conjugates. CCNE - Northwestern*
157. Mirkin C., Reed OC., Thaxton S., Giljohann D. *Localized Delivery of Gold Nanoparticles for Therapeutic and Diagnostic Applications. CCNE - Northwestern*
158. Mirkin C., Seferos D., Prigodich A., Giljohann D. *Methods for Stabilizing Nucleic Acids. CCNE - Northwestern*
159. Mirkin C., Seferos D., Prigodich A., Giljohann D. *Polyvalent DNA Nanoparticle Conjugates Stabilize Nucleic Acids. CCNE - Northwestern*
160. Mirkin C., Wang Y., Wei W., Maspoch D. Wu J. *Superparamagnetic Sub-5 nm Fe@C Nanoparticles: Isolation, Structure, Magnetic Properties and Directed Assembly. CCNE - Northwestern*
161. Mirkin, C, Thaxton S & Giljohann D. *Nanoparticle Supported Lipid Bi-Layer Bio-Mimetic Structures. CCNE - Northwestern*
162. Moon D, Kim SJ. Han J. *Nanofluidic Preconcentration Device in an Open Environment. CNPP - MIT*
163. Nie S. *Development of Paclitoxel-ScFvEGFR-heparin for Treatment of Cancer. CCNE - Emory/GT*
164. Nie S. et al. *Porous Materials Embedded with nanospecies, methods of fabrication thereof, and methods of use thereof. CCNE - Emory/GT*
165. Nie S. *Hydroxyl-Derivatized Surface Coatings for Minimizing Nonspecific Binding of Nanoparticles. CCNE - Emory/GT*
166. Nie S. *Multi-Functional Au-PEG-Doxorubicin-Folate Drug Nanocarrier for in vitro Cellular Targeting and Drug Efficacy Study. CCNE - Emory/GT*
167. Nie S. *New and Improved Methods for Synthesis of Heparin-Taxol-Folic Acid Ternary Conjugate. CCNE - Emory/GT*
168. Nolan G et al. *Application of Optical Polarization for Raman Peak Signal Variation to Allow Better Deconvolution of Multiple Spectra. CCNE - Stanford*
169. O'Halloran T, Nguyen S & Lee SM. *Polymer-Caged Lipsomes: a pH-Responsive Delivery System with High Stability. CCNE - Northwestern*
170. Olson J, Zhang M, Veiseh M, Gabikian P, Bahrami B. Ellenbogen R, Sze R & Veiseh O. *Fluorescent Chlorotoxin Conjugate for Intra-Operative Visualization of Cancer. CNPP - U. of Washington*
171. Pan D, Lanza GM & Wickline SA. *Particles for Imaging (Soft Particle Filing). CCNE - Washington University*
172. Pan D, Lanza, GM & Wickline SA. *Water Soluble Nano-Bialys: A Vascularly Constrained, Slow Releasing Nano-carrier for Therapeutic Imaging. CCNE - Washington University*
173. Pandey RK et al. *Highly Efficient PAA-Based Multifunctional NPs for Tumor Imaging and Therapy. CNPP - SUNY Buffalo*
174. Pandey RK et al. *PAA NPs Enhances the Tumor Imaging Potential of Certain Fluorescence and PET Imaging Agents. CNPP - SUNY Buffalo*
175. Pandey RK, Goswami LN, Morgan J, Bergey EJ, Prasad PN & Oseroff AR. *Post-Loaded Photosensitizing and Imaging Agents in silica Nanoparticles for Multivalent Actions. CNPP - SUNY Buffalo*
176. Pandey RK, Goswami LN, Morgan J, Ohulchanskyy T, Roy I, Bergey EJ, Prasad PN & Oseroff AR. *Organically Modified in silica Nanoparticles with Covalently Incorporated Photosensitizers for Drug Delivery in Photodynamic Therapy. CNPP - SUNY Buffalo*
177. Paras NP, Law WC, Yong KT & Roy I. *One-Pot Aqueous Phase Synthesis of Highly Luminescence Core/ Shell Quantum Dots. CNPP - SUNY Buffalo*
178. Paras NP, Yong KT & Roy I. *A Method to Produce Water-Dispersible Highly Luminescent CdSe/CdS/ZnS Quantum Dots for Biomedical Applications. CNPP - SUNY Buffalo*
179. Parrott M, Gratton S & DeSimone JM. *Degradable di-Alkyl Silane Cross Linkers for Particle Replication in Non-Wetting Templates (PRINT). CCNE - UNC*
180. Pourmand, S & Wang, Y. *Modification of Surface for Biomolecule Immobilization. CCNE - Stanford*
181. Prasad PN, Knight PR, Wallace PK, Burzinski R, Kachynski A, Kuzmin A, Yong KT, Roy I. Advanced Fiberoptic Based Flow Cytometer for Ultrasensitive and Multiplexed *in vitro*

Diagnosis Using Quantum Dot-Based Probes. *CNPP - SUNY Buffalo*

182. Qian XM & Nie SM. *Polymer Coated SERS Nanoparticle Tags and Their Use Thereof. CCNE - Emory/GT*
183. Radu CG, Witte ON, Nair-Gill E, Satyamurthy N, Shu CJ & Czernin J. *Positron Emission Tomography Probes for Imaging Immune Activation and Selected Cancers. CCNE - Caltech*
184. Radu, CG, Ribas T, Witte ON, Kwong G & Heath GR. *Nucleic Acid Tetramers for High Efficiency Multiplexed Cell Sorting. CCNE - Caltech*
185. Rameshwer S, Thommey PT & Baker JR. *Targeted Delivery of Imaging Agents/Drugs to Cancer Cells. CNPP - U. of Michigan*
186. Rao J, Gambhir SS, So M, Xu C & Loeing AM. *Self-Illuminating Quantum Dot Conjugates For imaging in living Cells or Animals. CCNE - Stanford*
187. Reiter RE & Wu AM. *Engineered Anti-Prostate Stem Cell Antigen (PSCA) Antibodies for Cancer Targeting. CCNE - Stanford*
188. Riley & Lanza G. *Chelating ligands based on nitrogen-containing ring systems, specifically Tc-99m. CCNE - Washington University*
189. Ruoslahti E & Jarvinen T. *Methods and Compositions Related to Targeting Wounds, Tissue, and Tumors New* (PCT/US2007/086627). *CCNE - UCSD*
190. Ruoslahti E & Jarvinen T. *Molecular Changes in the Vasculature of Injured Tissues. CCNE - UCSD*
191. Ruoslahti E *Methods and Compositions Related to Terminal Arginine Peptides. CCNE - UCSD*
192. Ruoslahti E, Lee BH & Kim IS. *Bladder Tumor-Targeting Peptide and Use Thereof. CCNE - UCSD*
193. Ruoslahti E. *CendR Peptide (iRGD) Enhancement of dmg and Nanoparticle Tissue Penetration. CCNE - UCSD*
194. Ruoslahti E. *Methods and Compositions Related to Internalizing RGD Peptides. CCNE - UCSD*
195. Sailor M. *Luminescent Porous Silicon Nanoparticles, Methods of Making and Using Same. CCNE - UCSD*
196. Sailor M. *Method for Preparation of Micellar Hybrid Nanoparticles for Combined Therapeutic and Diagnostic Medical Applications. CCNE - UCSD*
197. Sailor M. *Preparation of Magnetic Iron Oxide Nanoworms for in vivo Tumor Targeting. CCNE - UCSD*
198. Sailor MJ, Orosco, Pacholski & Miskelly. *Optical Biosensor for Protease Activity Using Protein-Coated Porous Silicon Phototonic Crystals. CCNE - UCSD*
199. Sailor MJ, Segal E, Vecchio KS, Bhatia SN, Park J & Derfus AM. *Method for Heating of Discrete Droplets Using Amphiphilic Magnetic Particles Derived from Porous Silicon. CCNE - UCSD*
200. Sathe T & Nie SM. *Mesoporous Silica Beads Embedded with Semiconductor Quantum Dots and Iron Oxide Nanocrystals: Dual-Function Microcarriers for Optical Encoding and Magnetic Separation. CCNE - Emory/GT*
201. Segal E, Perelman LA & Sailor MJ. *Method to Control the Phase Transition of a Polymer/ Hydrogel by Confinement in an Electrochemically-Prepared Porous Nanostructure. CCNE - UCSD*
202. Shi X, Wang S & Baker JR. *Dendrimer Based Compositions and Methods of Using the Same. CNPP - U. of Michigan*
203. Sokolov K, Milner T, Aaron J, Oh J, Ji X & Li C. *Methods and Compositions Related to Hybrid Nanoparticles, CNPP - U. of Texas*
204. Stupp S, Lee HK & Soukasene S. *Liposome-Encapsulated Peptide Amphiphile Nanostructures. CCNE - Northwestern*
205. Thaxton S., Mirkin C., Giljohann D., Weston D. *Templated Sherical High Density Lipoprotein Nanoparticles. CCNE - Northwestern*
206. Tian, Yan, Ma, Hood, Foltz, Madan *Use of gene expression signatures to determine cancer grade. CCNE - Caltech*
207. Trogler WC, Yang J, Ulrik J, Esener SC & Messmer D. *Hollow Silica Nanospheres and Methods of Making Same. CCNE - UCSD*
208. Tseng HR et al. *3D Nanostructured Substrates for Highly Efficient Capture of Circulating Tumor Cells (CTCs). CCNE - Caltech*
209. Tseng HR et al. *Supramolecular Approach for Production of Size-Controllable Nanoparticles. CCNE - Caltech*
210. van Dam M, Heath JR & Elizarov A. *Methods and Devices for Interfacing with a Microflidic Chip. CCNE - Caltech*
211. van Duyne R, Schatz G, Zhao J, Zhang X, Das A & Sligar S. *Resonance Surface Plasmon Spectroscopy: Low Molecular Weight Substrate Binding to P450. CCNE - Northwestern*
212. Van Duyne RW, Hall P, Anker J, Modica J, Mrksich M & Lin Y. *Method and Materials for Detection of Calcium Modulated Protein Conformational Switching with High Resolution*

Localized Surface Plasmon Resonance (LSPR) Nanosensors. CCNE - Northwestern

213. Wang M. *BioNano-lnformatics for Personalized Medicine. CCNE - Emory/GT*
214. Wang SX, Osterfeld SJ, Yu H, Pourmand N & White RL. *Quantitative Magneto-Nano Biosensors with Real-Time Proximity Detection Capability. CCNE - Stanford*
215. Wang SX, Pourmand N & White RL. *Mesoporous Silica Beads Embedded with Semiconductor Quantum Dots and Iron Oxide Nanocrystals: Dual-Function Microcarriers for Optical Encoding and Magnetic Separation. CCNE - Stanford*
216. Wang SX, Wilson RJ & Hu W. *Direct Physical Fabrication of Multilayer Nanoparticles. CCNE - Stanford*
217. Wang SX, Wilson RJ & Hu W. *Magnetic Sifter. CCNE - Stanford*
218. Weissleder R, Josephson L, Cima M, Hong R. *Magnetic Resonance Based Viscometer._CCNE - MIT-Harvard*
219. Weissleder R, Josephson L, Taktak S. *NMR based method of measuring ions using magnetic particles. CCNE - MIT-Harvard*
220. Weissleder R, Lee H, Ham D, Liu Y, Sun N. *Chip-NMR Biosensor for Detection and Molecular Analysis of Cells and CMOS Mini NMR System and its Application for Biomolecular Sensing. CCNE - MIT-Harvard*
221. Wickline S, Lanza G & Hughes M. *Application of Information-Theoretic Signal Receivers to detection of Nanoparticle coated Surfaces in vivo. CCNE - Washington University*
222. Wickline S, Lanza G & Pan. *Novel Contrast Agents for Spectral CT Imaging. CCNE - Washington University*
223. Wickline S, Ramchandra & Schlesinger. *Selective delivery of membrane permeabilizing peptides to cells with molecularly targeted nanoparticles for therapeutic benefit. CCNE - Washington University*
224. Wickline S, Solin S, Hughes M & Wallace. *Multifunctional Nanoscopy for Imaging Living Cells. CCNE - Washington University*
225. Wickline SA & Solin SA. *EXX Nano Arrays for the Study of Cancer Cells. CCNE - Washington University*
226. Woloschak G & Paunesku T. *Use of 32P Radiolabeled Oligonucleotides and TiO2-nucleic Acid Nanocomposites in Tandem to Accomplish Localized Nucleic Acid Scission. CCNE - Northwestern*
227. Woloschak G, Wu A & Paunesku T. *An Approach to Computed Tomography and Magnetic Resonance Imaging Using Same Nanomaterials. CCNE - Northwestern*
228. Woloschak G, Wu A & Paunesku T. *An Approach to Magnetic Resonance Imaging Based on Magnetic Nanocrystals. CCNE - Northwestern*
229. Woloschak G, Wu A & Paunesku T. *Preparation of Core-Corona-Shell Nanocrystal Materials and the Formation Process of Nanocomposite Materials. CCNE - Northwestern*
230. Woloschak G, Wu A & Paunesku T. *Preparation of Core-Shell Nanocrystal Materials Based on Metal Components and the Formation Process of Nanocomposite Materials Including Metal Elements. CCNE - Northwestern*
231. Woloschak G, Wu A & Paunesku T. *Preparation of Magnetic Nanocrystal Materials and the Formation Process of Magnetic Nanocomposite Materials. CCNE - Northwestern*
232. Woloschak GE & Wu A. A *Relocatable Method for Various Microscopes. CCNE - Northwestern*
233. Wu AM, Olafsen T & Raubitschek AA. *Engineered Anti-CD20 Antibody Fragments for in vivo Targeting. CCNE - Stanford*
234. Wu AM, Reiter RE, Lepin EJ, Marks JD & Zhou Y. *High Affinity Anti-PSCA Antibodies for Cancer Targeting. CCNE - Stanford*
235. Yamakawa M, Wang L, Sun L, Chan S, Zhu J & Nolan G. *Method to Enhance Analysis of Raman/SERS Based Molecular Markers through Raman Optical Activities. CCNE - Stanford*
236. Yang L, Nie S et al. *Targeted Multifunctional Nanoparticles for Cancer Imaging and Treatment. CCNE - Emory/GT*
237. Yang L, Nie S, et al. *Nanostructures, Methods of Synthesizing Thereof, and Methods of Use Thereof. CCNE - Emory/GT*
238. You, J. & Li, C. *Near-infrared light triggered drug release from hollow gold nanospheres (HAuNS) and HAuNS-Loaded Microspheres. CNPP - U. of Texas*
239. Yu H, Wang SX & Pourmand N. *Biomolecule Immobilization on Biosensors. CCNE - Stanford*
240. Zhang M, Gunn J & Yee C. *Specific T Cell Labeling for Cancer Immunotherapy. CNPP - U. of Washington*
241. Zhang M, Kohler N & Gunn JA. *Bifunctional Poly(ethylene glycol) Silane Immobilized on Metallic Oxide-Based Nanoparticles for Conjugation with Cell Targeting Agent. CNPP - U. of Washington*
242. Zhang M, Olson J, Sze R, Ellenbogen R, Veiseh O, Sun C & Gunn J. *Chlorotoxinlabeled nanoparticle compositions and methods for*

targeting primary brain tumors. *CNPP - U. of Washington*

243. Zhang M, Sun C, Veiseh O & Bhattarai N. *Specific Targeting of Brain Tumors with an Optical/MR Imaging Nanoprobe Across the Blood Brain Barrier*. *CNPP - U. of Washington*
244. Zhang M, Sun C, Veiseh O, Bhattarai N. *Specific Targeting of Brain Tumors with an Optical/MR Imaging Nanoprobe Across the Blood Brain Barrier*. *CNPP - U. of Washington*
245. Zhang M, Wang S, Kohler N, Lin Y & Sun C. *Magnetic Nanoparticle-Conjugates as Contrast Agents or Drug Carriers for Cancer Diagnosis and Therapeutics*. *CNPP - U. of Washington*
246. Zhou O, Lu J, Lee Y, Cheng Y, Zhang J, Yang G & Qiu Q. *Methods, Systems, and Computer Program Products for Multiplexing Computed Tomography*. *CCNE - UNC*
247. Zhou O. & Chang S. *A compact microbeam radiotherapy system for clinical and preclinical use*. *CCNE – UNC*

APPENDIX 3

Companies Associated with Alliance

Company (Year Founded) Web Site	ANC Affiliation Investigator(s)	Technology
Calando Pharmaceuticals (2005) http://www.calandopharma.com/	Caltech CCNE Mark Davis	**Therapeutics** Cyclodextrin-based polymer nanoparticles for targeted delivery of siRNA
Calhoun Vision (1997) http://www.calhounvision.com	Caltech CCNE Robert Grubbs	**Materials** Light-inducible intraocular lenses based on a nanomaterial whose shape can be adjusted via light exposure
Cell Fluidics (2008) http://www.momentum-biosciences.com/	Caltech CCNE Hsian-Rong Tseng, Paul Mischel, James Heath	**Diagnostics & Therapeutics** Platform technology for personalized medicine in cancer diagnosis and therapy that integrates microfluidics with high resolution analysis of tumor cells isolated from clinical samples
Homestead Clinical http://www.homesteadclinical.com/	Caltech CCNE James Heath	**Diagnostics** DNA-Encoded Antibody Library (DEAL) technology and nanosensors for high-throughput screening of thousands of proteins to predict and prevent disease and to predict how an individual patient will respond to a specific therapy
Insert Therapeutics (2000) http://www.insertt.com/	Caltech CCNE Mark Davis	**Therapeutics** Cyclodextrin-based polymer nanoparticles for targeted delivery of small molecule therapeutic products
Integrated Diagnostics (2008)	Caltech CCNE Leroy Hood, James Heath	**Diagnostics** Organ-specific blood molecular protein fingerprints for diagnosis and the assessment of drugs in personal medicine model
Materia (1998) http://www.materia-inc.com/	Caltech CCNE Robert Grubbs	**Materials** Synthesis of new materials for the rapid construction of new nanotechnology-based pharmaceuticals and for use in microfluidic devices for the synthesis of radiopharmaceuticals
Molecular Biomarkers	Caltech CCNE James Heath, Michael Phelps, Hsian-Rong Tseng	**Technology Incubator** Identify early stage technologies developed at the Caltech CCNE and technology transfer to nanotechnology startup companies
Sofie Biosciences (2008)	Caltech CCNE Caius Radu, Owen Witte, Michael Phelps	**Diagnostics** Development of *in vivo* PET-based molecular imaging probes using microfluidics

Endra, Inc. (2009)	Stanford CCNE Sanjiv Sam Gambhir	**Diagnostics** Photoacoustic imaging to monitor the therapeutic efficacy of oncology drugs on internal organs
Enlight Biosciences (2008) http://www.enlightbio.com/	Stanford CCNE Sanjiv Sam Gambhir	**Technology Incubator** Development of transformational enabling technologies in areas of highest potential impact within the drug discovery process. Emphasis is on technologies that strengthen the connection between preclinical research, clinical research, and point-of-care
GE Global Research http://www.ge.com/research/	Stanford CCNE	**Diagnostics** Superparamagnetic iron oxide (SPIO) nanoparticles with high-saturation magnetization and high permeability for use in magneto nano-sensors
ImaginAb (2007) http://www.momentum-biosciences.com/	Stanford CCNE Robert Reiter, Anna Wu	**Diagnostics** Engineered antibody-based diagnostic imaging agents for improved diagnostic imaging
MagArray (2005) http://www.magarray.com/	Stanford CCNE Shan Wang	**Diagnostics** Molecular detection system based on magnetic nanotags (nanoparticles) and spin valve sensor arrays for rapid and portable DNA and protein fingerprinting
Nodality http://www.nodalityinc.com/	Stanford CCNE Garry Nolan	**Diagnostics & Therapeutics** Technology platform for drug development including biomarker identification and analysis, drug discovery research and development, patient stratification, and monitoring the pharmacodynamics of therapeutics
Visual Sonics (1999) http://www.visualsonics.com/	Stanford CCNE Sanjiv Sam Gambhir	**Diagnostics** High-resolution *in vivo* micro imaging systems devised specifically for non-invasive small animal research
Zymera (2007) http://www.zymera.com/	Stanford CCNE Jianghong Rao, Sangeeta Bhatia, Sanjiv Sam Gambhir	**Diagnostics** Luminescent nanocrystal for clinical diagnostic applications
Alnylam Pharmaceuticals (2002) http://www.alnylam.com/	MIT-Harvard CCNE Philip Sharp	**Therapeutics** Novel therapeutics using liposomal-mediated delivery of siRNA and microRNA
BIND Biosciences (2006) http://www.bindbio.com/	MIT-Harvard CCNE Robert Langer, Omid Farokhzad	**Therapeutics** Therapeutic nanoparticles for the targeted intracellular delivery of large payloads of small molecules, nucleic acids, peptides and proteins
Lumicell Diagnostics, Inc. (2008)	MIT-Harvard CCNE Moungi Bawandi, David Lee Ralph Weissleder	**Diagnostics** Developing intraoperative *in vivo* pathology approaches for improved cancer margin resection.

MicroCHIPS, Inc. (2000) http://www.mchips.com/	MIT-Harvard CCNE Michael Cima, Robert Langer	**Diagnostics & Therapeutics** "Smart" implantable devices possessing controlled-release drug capabilities to create sophisticated therapy and monitoring systems
T2 Biosystems (2006) http://www.t2biosystems.com/	MIT-Harvard CCNE Robert Weissleder, Robert Langer, Tyler Jacks	**Diagnostics** Magnetic nanoparticle assay technology for *in vitro* diagnostics
Tempo Pharmaceuticals (2009) http://www.tempopharmaceuticals.com/	MIT-Harvard CCNE Robert Langer	**Therapeutics** Multi-compartmental, nanoparticle-based therapeutics for varied release rates within a single nanoparticle
VisEn Medicinal (2000) http://www.visenmedical.com/	MIT-Harvard CCNE Ralph Weissleder	**Diagnostics** Nanoparticles for fluorescence imaging applications
American Bio-Optics (2006) http://www.americanbiooptics.com/	Northwestern CCNE Vadim Backman	**Diagnostics** Minimally-invasive optical diagnostics test using coherent backscattering spectroscopy to analyze how the reflected light interacts with the lining of the colon allowing for nanoscale characterization of cell architecture in order to identify patients at high risk for colon cancer
Grzybowski Scientific Inventions http://www.cellensemble.com/	Northwestern CCNE Bartosz Grzybowski	**Materials** Combination of micropatterned substrates and surface chemistry to custom engineer cell shape or constrain cell motions to predetermined geometries for future anti-metastatic drug screening
NanoInk http://www.nanoink.net	Northwestern CCNE Chad Mirkin	**Materials** Nanoscale manufacturing and application development for the life science and semiconductor industries using Dip Pen Nanolithography® and high-resolution NanoEncryption™ technology
Nanosphere (2000) http://www.nanosphere-inc.com/	Northwestern CCNE Chad Mirkin	**Diagnostics** Nanotechnology-based molecular diagnostics capable of ultra-sensitive detection of nucleic acid and protein biomarkers using biobarcode technology
Nanotope (2007) http://www.nanotope.com/	Northwestern CCNE Samuel Stupp	**Therapeutics** Developing a customizable chemical matrix of nanofibers that provides three-dimensional bioactive scaffolding in which cells and tissues may grow and differentiate and actively directs surviving cells to re-grow damaged tissue. Two primary features of the matrix are its customizable bioactivity and controlled gelation that result from engineered small individual molecules that self-assemble into nanofibers under physiological conditions.

Ohmx (2005) http://www.ohmxbio.com/	Northwestern CCNE Thomas Meade	**Diagnostics** Protein detection micro-chips for a handheld point-of-care device
Pharocore (Recent)	Northwestern CCNE Chad Mirkin	**Diagnostics & Therapeutics** Nanoscale prisms for multiplex tagging of molecular entities and for use in diagnostic and therapeutic applications
PreDx (2006)	Northwestern CCNE Thomas Meade	**Diagnostics & Therapeutics** Bio-activatable magnetic resonance nanoparticle-based contrast agents for dual molecular imaging & targeted therapeutic applications
SAMDITech	Northwestern CCNE Milan Mrksich	**Diagnostics** High-throughput biomarker screening for *in vitro* nanodiagnostics using self-assembled monolayers that present biological functionality against a non-interacting background and matrix-assisted laser desorption-ionization mass spectroscopy
B3 Biosciences (2007)	UNC & Stanford Bruce Sullenger (UNC), Andy Ellington (Stanford)	**Therapeutics** Development of targeting strategies for siRNA therapeutics using aptamer-based moieties
Liquidia (2004) http://www.liquidia.com/	UNC CCNE Joseph DeSimone	**Therapeutics** Nanoparticles with precisely engineered shape, size, surface functionalization, and deformability for the delivery, controlled release, and enhanced efficacy of nanotechnology-based therapeutics
XinRay Systems (2007) http://www.xinraysystems.com/	UNC CCNE Otto Zhou	**Diagnostics** Joint venture between Siemens Medical Solutions and Xintek, Inc. to develop a carbon nanotube based medical CT scanner
Xintek, Inc. (2000) http://www.xintek.com/	UNC CCNE Otto Zhou	**Diagnostics** Carbon nanotube-based field emission technologies and products for diagnostic medical imaging, homeland security and IT display
Kereos (1999) http://www.kereos.com/	Washington U CCNE Samuel Wickline, Gregory Lanza	**Diagnostics & Therapeutics** Ligand targeted emulsions containing perfluorocarbon nanoparticle cores for molecular imaging and targeted therapeutic delivery
Philips http://www.usa.philips.com/	Washington U CCNE	**Diagnostics** High intensity focused ultrasound (HIFU) instrumentation for imaging of tumor angiogenesis and evaluation of anti-angiogenic therapies
PixelEXX Systems (2008) http://www.pixelexx.com/	Washington U CCNE Samuel Wickline, Stuart Solin	**Diagnostics** Photoacoustic nanosensors to generate 3-dimensional maps of individual cancer cells for drug screening

DiagNano (2006) http://www.nanohc.com/	Emory-GT CCNE Shuming Nie	**Diagnostics** DiagNano's core technology, nanoparticle surface coatings allows quantum dots and other metal nanoparticles to be used in a number of biological applications, including *in vitro* diagnostics.
Nanovici http://www.nanovici.com/	Emory-GT CCNE Shuming Nie	**Therapeutics** Folate-targeted nanoparticles for therapeutic applications
Oxonica (2002) http://www.oxonica.com/	Emory-GT CCNE Michael Natan	**Diagnostics** Manganese-doped titanium dioxide nanoparticle-based biomarker detection platform for ultrasensitive and simultaneous detection of multiple disease biomarkers
Vivonetics (2002) http://www.vivonetics.com/	Emory-GT CCNE Gang Bao	**Diagnostics** Development of Fluorescence Resonance Energy Transfer (FRET) molecular beacons for *in vitro* nanodiagnostics
Cellular Bioengineering (2003) http://www.cellularbioengineering.com/	UCSD CCNE Michael Sailor	**Diagnostics** Spectrally barcoded microparticles containing nanostructures that act as robust, non-toxic tags for high-throughput screening and encoded bead-based assays
CytomX Therapeutics (2005) http://www.cytomx.com/	UCSD CCNE Patrick Daugherty	**Diagnostics & Therapeutics** Precise control of affinity, specificity and stability of peptide-based therapeutics, diagnostics, and reagents
Gensign (2007)	UCSD CCNE Dennis Carson, Yu-Tsueng Liu, Sadik Esener, Vineet Bafna	**Diagnostics** Nanodroplet reactor assay technology for *in vitro* diagnostics
Nanogen (1993) http://www.nanogen.com/	UCSD CCNE Sadik Esener	**Diagnostics** DNA hybridization arrays and molecular diagnostic kits and reagents for diagnostic applications
Affinity Biosensors (2006)	MIT CNPP Scott Manalis	**Diagnostics** *In vitro* nanodiagnostics using a suspended microchannel resonator (SMR) to perform analysis on living individual cells
Avidimer Therapeutics (2003) http://www.avidimer.com/	U Michigan CNPP James Baker	**Therapeutics** Dual attachment anti-cancer drug and targeting moiety to the same nanoparticle via dendrimers for targeted drug delivery
Carestream Health (2000) http://www.carestreamhealth.com	U Texas CNPP Chun Li	**Diagnostics** Fluorescently-tagged nanoparticles for *in vitro* and *in vivo* imaging, including deep near infrared (NIR) penetration imaging *in vivo*

Nanoparticle Biochem, Inc. (Recent) http://nanoparticlebiochem.com/	U Missouri CNPP Kattesh Katti	**Diagnostics & Therapeutics** Biocompatible nanoparticle production for tumor-specific diagnostic and therapeutic agents and antimicrobial agents
Nemucore Medical Innovations (2008) http://www.nemucore.com/	Northeastern CNPP Mansoor Amiji & Vladimir Torchilin	**Therapeutics** Reformulation of existing oncology drugs (generics) using Spontaneous Self Assembling Nanoparticles (SSANs) to develop generational improvements to widely used drugs and facilitate delivery of poorly soluble drugs