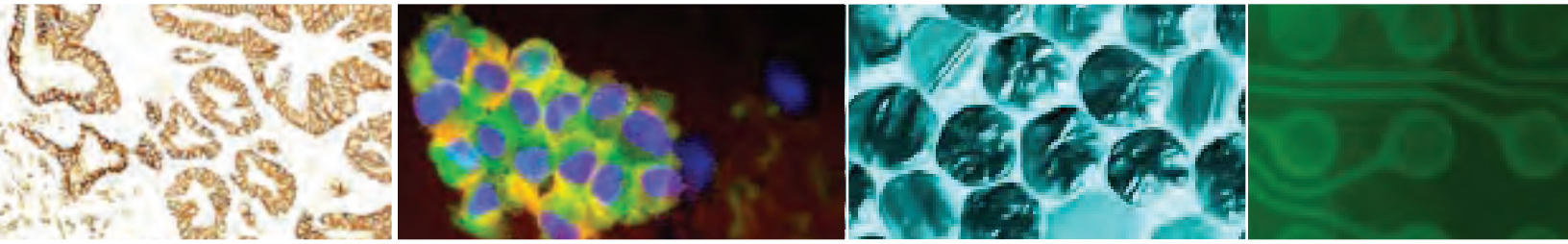
NCI Alliance for Nanotechnology in Cancer

BULLETIN

An NCI Alliance for Nanotechnology in Cancer publication to connect participants, partners, affiliates, and colleagues

2010: 5 Year Program Highlights



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Centers of Cancer Nanotechnology Excellence (CCNEs)

The CCNE programs serve as hubs of the NCI Alliance for Nanotechnology in Cancer to develop and apply nanotechnology and nanoscience solutions in diagnosis and treatment of cancer.

CAROLINA CENTER OF CANCER NANOTECHNOLOGY EXCELLENCE

University of North Carolina
Principal Investigator:
Rudolph Juliano, Ph.D.
Co- Principal Investigator:
Joseph DeSimone, Ph.D.

Highlights

The major goal of the Carolina CCNE has been the design of innovative, multifunctional nanodevices, which can be tested in mouse models of human cancer, with the intent of translation to the clinic. We have used breakthroughs in nanomaterials research to develop nanoscale tools for detection, diagnosis and treatment of cancer.

Our primary areas of focus have been development of:

- Smart nanoparticles for cancer therapy and imaging
- Carbon nanotube x-ray devices for *in vivo* cancer detection and treatment
- Hybrid nanomaterials for biomedical imaging and drug delivery
- Chemically patterned nanoscale surfaces for understanding the behavior of tumor cells
- Nanofluidic devices for rapid analysis of tumor cell signaling and migration

The C-CCNE has grown from a group of outstanding physical and biological scientists into an integrated multidisciplinary team, through a series of projects and cores that are working together to harness innovations in nanotechnology in the fight against cancer.

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Project and Core Highlights

PROJECT 1: Development of 'Smart' Nanoparticles
PI: Joseph DeSimone

Recent breakthroughs in the DeSimone laboratories using specifically-designed materials for imprint lithography have enabled an extremely versatile and flexible method for the direct fabrication and harvesting of monodisperse, shape-specific nano-biomaterials. The method, referred to as Particle Replication In Non-wetting Templates, or PRINT, allows for the fabrication of monodisperse particles with simultaneous control over structure (i.e. shape, size, composition) and function (i.e. cargo, surface structure).

Project 1 focused on the refinement of the PRINT technology to tailor the composition of particles and scale up their production. At the inception of the C-CCNE, nanoparticle production was done in small batches with engineered templates of a few centimeters in diameter. In conjunction with UNC spin-off Liquidia Technologies, particles are now produced from a continuous roll template offering production of significant quantities of particles.

Key studies in Project 1 involved refinement of the composition of particles based on materials and function. Particles are routinely fabricated from a variety of biocompatible materials, including PEG, PLGA and albumin and a range of cargos can be encapsulated including antibodies, chemotherapeutic agents and nucleic acids. In this project, the role of size, shape, and zeta potential on particle uptake by cell lines was examined. In this study, rod-shaped particles reminiscent of some bacteria were preferentially taken up by cells. Current efforts in this project include siRNA delivery via encapsulation and release from particles upon internalization into cells. These studies pave the way for production and delivery of precisely tailored nanoparticles.

PROJECT 2: Evaluation of the Applications of 'Smart' Nanoparticles to Cancer Therapy and Imaging
PI: Joseph DeSimone

In addition to tailoring size, shape and chemistry, PRINT particles can also be decorated with ligands that are specific to certain cell surface receptors in an effort to bind or to trigger receptor mediated endocytotic pathways in specific cell types. The surface of negatively charged PRINT nanoparticles can be decorated with specific ligands such as antibodies, small molecules, peptides and has proven useful for the selective targeting of certain cell types over other cell types. Once targeted with a cell specific ligand, the PRINT particle can deliver a cargo or be used for imaging. In this respect, PRINT particles promise great potential, since it is possible to utilize the ability to specifically target, be shape and size-specific, possess tunable matrices, as well as the ability to incorporate imaging contrast agents.

Studies under this project have examined the effects of targeting nanoparticles with the transferrin receptor, Herceptin, an engineered antibody that binds to the

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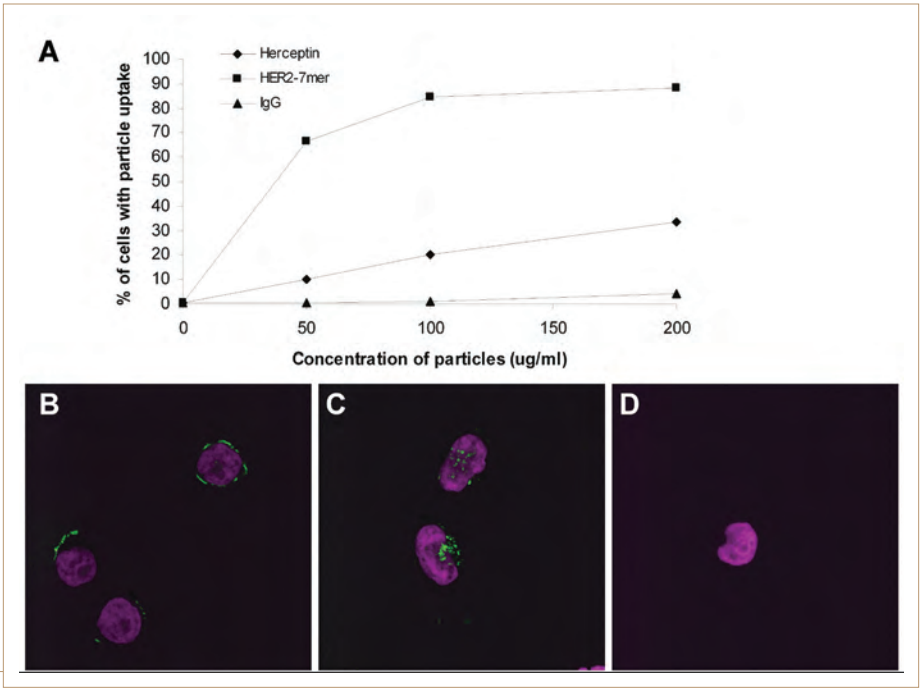

FIGURE 1. *(A) Internalization of functionalized particles into cells was examined using flow cytometry following 4h of incubation on BT474 cells. Particles incubated with HER2-7mer (squares) were significantly higher than Herceptin targeted (squares) and IgG control particles (triangles). Confocal images show particles associated with cells for both targeted Herceptin* ***(B)*** *and HER2-7mer* ***(C)*** *but not with the cell surface in the controls (IgG, D). Particles are seen by FITC fluorescence (green) and the nuclei are stained with TO-PRO-3 (magenta).*

oncogenic receptor HER2/neu, and a specialized HER2 affinity reagent created in the C-CCNE's Targeting Ligand Core. **Figure 1** shows the dramatically improved targeting of the nanoparticles with the C-CCNE developed HER2 affinity reagent. Additional studies have compared the response of a number of tumor cell lines to targeted nanoparticles, determining that sensitivity to the particles varies among the different cell lines. In animal studies, we are currently examining how particles accumulate in the tumors of mice with transplanted lung and ovarian tumors. This will help to understand how to target particles containing anti-cancer drugs to specific sites in the body, and avoiding toxic side effects on normal tissues.

PROJECT 3: Carbon Nanotube X-Ray
PI: Otto Zhou

The overall goal of this project has been to develop new x-ray computed tomography and radiotherapy instrumentation for cancer imaging and radiation therapy for both preclinical and clinical applications. The core technology is the carbon nanotube based field emission x-ray source technology that was pioneered by our team at UNC. The spatially distributed x-ray source technology has the potentially to fundamentally change how x-ray radiation is generated and utilized for cancer imaging and treatment. Instrumentation that is under development in the project includes a dynamic micro-computed tomography scanner for high resolution *in vivo* imaging of small

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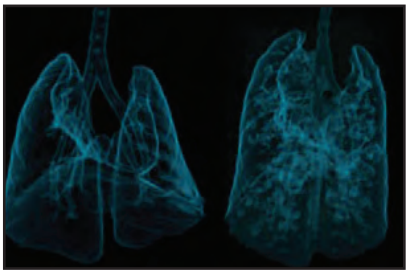

FIGURE 2. *Scientists, engineers and physicians at The University of North Carolina Hill work closely together on the applications of nanotechnology in the diagnosis, treatment and understanding of cancer. One application is the use of carbon nanotubes for improving the creation of x-rays to enable new techniques in the imaging and treatment of human and animal models of cancer. The rendered images above demonstrate a normal lung model (on the left) and multifocal lung tumor model (right) using the new imaging technique that is able to "freeze" biological motion. The murine lung tumor model not only demonstrates numerous tumors, but also the physiological changes in the shape of the lung because of the additional tumor burden. Images are courtesy of Drs. Otto Z. Zhou and Yueh Z. Lee. Murine models courtesy of Dr. William Y. Kim.*

animal models for cancer research, micro-radiotherapy system for preclinical radiation therapy research, and a stationary digital breast tomosynthesis scanner for early detection of human breast tumors.

The Project 3 team was successful in development of a small animal micro-CT system. A second scanner has been constructed and will be installed at the UNC Small Animal Imaging Core as a user facility. An additional scanner will be constructed and installed at University of Iowa.

Figure 2 shows an example of a micro-CT image of a mouse model of lung cancer, comparing a normal mouse with a mouse with multiple lung tumors. This system offers not only excellent resolution, but its digital control allows for cardiac and respiratory gating such that any movement of the animal from these functions does not distort the image.

Another achievement of Project 3 was installation of the first carbon nanotube-based stationary tomosynthesis image guidance system for radiation therapy in the newly opened North Carolina Cancer Hospital at UNC. The instrument was manufactured by XinRay Systems for Siemens Oncology. This instrument is being tested at UNC and will be integrated with the radiation therapy system for a clinical test which has been approved by the UNC Institutional Review Board.

PROJECT 4: Multimodal Nanoparticles For Imaging And Therapy
PI: Wenbin Lin

This project is developing new innovative approaches to cancer research, with significant implications in realizing early detection by magnetic resonance (MR) imaging and developing effective therapies for brain tumors. We are developing a new class of molecule-based nanomaterials for a number of biological and biomedical applications. In this emerging area of nanomedicine, we are designing nanoscale multimodal contrast agents for magnetic resonance imaging, optical imaging, and X-ray computed tomography and developing novel nanoparticles for targeted delivery of potent anticancer therapeutics.

We have 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 Gd chelates. We have also made further 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 the NMOF particles. These cargoes are released upon the degradation of the NMOF frameworks, and the rate of cargo release was controlled by coating the NMOF particles with a silica

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shell. Potential utility of the new NMOF-based nano-delivery vehicles for optical imaging and anticancer therapy were demonstrated *in vitro* using HT-29 human colon adenocarcinoma cells.

Another approach to nanoparticle-based diasnostics is using novel iodine-containing polymers. These particles are created to carry large payloads of iodine (~63 wt%) and have potential applications as a new class of contrast agents for computed tomography, adding additional power to this important diagnostic technique.

PROJECT 5: Nanofluidic Devices for Rapid Single Cell Analysis of Protein Expression and Activity
PI: J. Michael Ramsey

A critical problem in cancer care is the inability to provide rapid diagnostic or prognostic information for a patient using conveniently accessible sample materials. To address this issue, we are developing point-of-care microfluidics platforms that accept "as collected" patient samples and provide relevant biochemical information to the clinician within 30-60 min. We are presently utilizing whole blood samples collected from a finger stick, but also envision the use of needle biopsies in the case of solid tumor samples. The microfluidics devices will include sample processing to reduce complexity, a micro-flow cytometer for isolating targeted cell types, and nanotechnology-based biochemical assays to determine biochemical function in the isolated cells. The molecular data obtained from the patient's samples will provide biomolecular information relevant to disease diagnosis and the efficacy of therapeutic treatment.

Our initial efforts have concentrated on the analysis of finger stick whole blood samples for the enumeration of T-regulatory cells. We have demonstrated the ability to efficiently isolate leukocytes from whole blood samples and to then identify T-lymphocytes using an integrated flow cytometer. We have demonstrated the use of the micro-flow cytometry system for blood dosimetry applications using mouse models exposed to total body irradiation, using the microfluidic system to monitor lymphocyte depletion over time. Very small samples of less than 20 microliters of blood per animal were used, incubating the blood with fluorescently labeled antibodies for leukocytes of interest, and analyzing the blood directly on-chip. We have also demonstrated the use of this device to monitor blood monocyte levels after the injection of various doses of the chemotherapeutic agent Doxorubicin.

Once the cells are identified, specific cells can be lysed based upon the flow cytometry information, with two different approaches being explored for quantifying cellular proteins. These techniques integrate antibody recognition with other detection methods. In one example, target proteins are quantified using a fluorescence sandwich immunoassay. We are initially targeting the cellular protein FoxP3 for identification of T-regulatory cells, an important T-cell subset that presently cannot be identified by flow cytometric means using viable cells. Other cellular protein targets include phospho-ERK, Src, and Cdc42 concentrations — all targets of known relevance in cancer biology.

Another approach to molecular interrogation is the use of nanopores that can detect a single molecle. A new nanopore assembly has been constructed which incudes a reusable solid support in which a nanopore is simply mounted. The assembly is completely closed over the course of an experiment, protecting the sample from environmental effects. Chemically resistant materials have been employed to allow the use of various solvents. Our team is using DNA to test the system, and will expand to protein detection in the next phase of development.

PROJECT 6: Nanopatterned Surfaces
PI: Rudy Juliano

One of the major characteristics of cancer cells is their ability to invade locally and to metastasize to distant organs. This project uses a nanotechnology approach called nanopatterning to more closely investigate the invasive behavior of tumor cells. This allows us to see how the arrangements of molecules around the cancer cells affect their behavior, in hopes of discovering some of the key events in cancer cell invasion and metastasis.

We have combined two key technologies for a unique approach to the study of cancer cell motility and invasion. First, using nanopatterned surfaces that are regulated electrochemically allows real time alterations in the presentation of cell adhesive ligands.

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Surfaces can be patterned in a variety of shapes and molecular gradients can be generated. This challenges the cells with a changing environment, just as occurs *in vivo*. Second, the cells studied are engineered to express a variety of fluorescent biosensors that monitor the state of activation and subcellular localization of key signaling proteins such as Rho GTPases and tyrosine kinases. This allows a quantitative correlation of signal transduction process with changes in cell shape, migration and invasion.

Over the course of this project, the team has refined the method for creating modified surfaces, allowing a variety of molecules to be presented to cultured tumor cells and their response monitored. Both the location and the number of target molecules can be varied, allowing many options for studying the cellular responses.

Another approach to understanding the biology of tumor cells examines specific signaling molecules within the cells. The Rho family GTPases are proteins that modulate cell migration through the regulation of actin dynamics and adhesion. RhoA and RhoC are very similar in amino acid sequence, yet are associated with very different phenotypes in cancer. We hypothesized that these differences may be reflected in different spatio-temporal dynamics of activation indicative of different roles in morphodynamics and motility. To probe for these potential differences we generated a FRET-based biosensor for RhoC. This biosensor, together with our biosensor for RhoA, enabled us to compare the spatio-temporal dynamics of RhoA and RhoC activity in live mouse embryonic fibroblasts (MEFs). Our results revealed that these two very similar proteins correlated with different cellular activities, which helps to explain their association with different behavior of tumor cells. These studies are an important step to a better understanding of the properties of cancer cells, which will help to devise strategies to combat invasion and metastasis.

CORE 1: Animal Models Core
PI: Terry Van Dyke

The mission of the Animal Models Core is to provide mouse models to C-CCNE investigators as they progress toward milestones on their individual projects. For example, a mouse model of glioblastoma developed in Dr. Van Dyke's lab was used to test the image-enhancing nanoparticles developed in Project 4. Other tumor models are important to test the efficacy of nanoparticles containing chemotherapeutic drugs. The core lab also developed an important software package for mouse colony management.

CORE 2: Combinatorial Ligand Core
PI: Rihe Liu

The Combinatorial Ligand Core has used combinatorial library approaches to generate novel reagents having cell receptor selective binding abilities. Thus this Core develops the ligands that confer 'smartness' (biological recognition) to smart nanoparticles. In particular, Project 2 relies on affinity reagents, or ligands, to target nanoparticles to particular cells. The technologies of this

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Core allow tailored ligands to be made that selectively recognize a type of cancer cell, and not the surrounding tissue.

One example is the development of molecules called aptamers that tightly and specifically bind to pancreatic cancer cells but not normal pancreatic cells. Such aptamer selection relies on a newly developed method called cell-SELEX. This technique uses whole live cells as complex targets, to evolve multiple aptamers that specifically recognize multiple membrane-bound proteins of cancer cells. Counter selection against normal cells is introduced to screen out common molecules expressed on both cancer cells and normal cells. This method results in molecules that can specifically recognize tumors and function as "address labels" to deliver drugs or other cargo to cancer cells.

CORE 3: Small Animal Imaging
PI: Weili Lin

The Imaging Core is operated in conjunction with the UNC Biomedical Research Imaging Center and provides PET, SPECT, CT, optical imaging, and MR imaging capabilities. A total of 10 imaging devices are currently housed in the SAI core and we have created a user-friendly imaging suite together with animal holding space. Dr. Zhou of Project 3 is building a carbon nanotube based micro-CT (see **Figure 2**) which will add high quality *in vivo* CT imaging capabilities to this Core. These capabilities provide research support for monitoring effects of targeted nanoparticles and development support in evaluating new imaging contrast materials. Key interactions have occurred with Projects 1, 2, 3 and 4 over the course of the C-CCNE. The Small Animal Imaging Core is managed by Dr. Hong Yuan, who has extensive experience with PET imaging techniques and has coordinated discussion sessions between the investigators and technical faculty determining the best mode of imaging for their studies.

CORE 4: Pharmacokinetics, Biodistribution, and Biocompatibility
PI: Frank Szoka

A thorough understanding of the *in vivo* distribution of any injected substance is critical to its evaluation as a candidate for clinical use. The Pharmacokinetics, Biodistribution, and Biocompatibility Core (PK Core) is important for the stringent testing of the *in vivo* behavior of nanoparticles. It complements studies done at UNC-CH within Projects 2 and 4 and using the Small Animal Imaging Core, mapping the fate of nanoparticles that are injected in mice bearing tumors. Knowing the amount of particles reaching the tumor as compared to that reaching other parts of the body, and clearance from the body is important to developing nanoparticles for *in vivo* use. The PI, Dr. Szoka is a pioneer in development of liposomes, nanoparticles and dendrimers as drug carriers. This includes basic research as well as applied research and development leading to technology commercialization. He also has vast experience in the pharmacokinetic/pharmacodyanmic analysis of carrier systems.

CENTER FOR CANCER NANOTECHNOLOGY EXCELLENCE FOCUSED ON THERAPY RESPONSE

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

The goal of this grant is the development and validation of nanotechnology to eventually predict which patients will likely respond to a specific anti-cancer therapy and to monitor response to therapy. Through an integrated, cohesive five-year plan, we are pursuing both the use of ex vivo protein nanosensors and *in vivo* nanoparticles for molecular imaging. We are also expanding to new applications. The substantial progress of CCNE-TR (http://mips.stanford.edu/public/grants/ccne) has exceeded even our expectations and is briefly presented below. Our progress over the last 4 years, as summarized in **Figure 1 (page 12)**, supports the likelihood of continued growth and delivery on promises to cancer patients. Using nanotechnology-centric solutions developed through the CCNE-TR, we are also training the next generation of Cancer Nanotechnologists to further advance how we manage cancer.

Publications

One measure of the progress of our CCNE-TR is the publications that have resulted from the efforts of numerous students, post-doctoral fellows, and faculty. There have been more than 140 research publications from the period 2006-2009. Of these, 28 papers have been in very high impact journals (impact factor>7). These include **18 in the Nature series**, 5 in PNAS, and 5 in the PLOS series. It is important to note that many of the faculty had never before published with each other. In fact, the CCNE-TR has really helped to bring faculty from very different disciplines together as was the original intent of the NCI CCNE U54.

New Research Funding Attributed to the CCNE-TR

New funding that can be directly attributed to the CCNE-TR has been quite robust, includes a variety of funding mechanisms, and exceeds $150 million. We have received over $26.4 million in NIH funding, $13.5 million in non-NIH funding, $64.9 million in private funding, and $779 thousand from industrial grants. We have received **3 major** NCI grants during this last 3 years: (i) The Networks in Translational Research (NTR) (C. Contag PI; S.S. Gambhir project leader), which allows us to start clinical trials of colonoscopy using SERS gold based nanoparticles ($6M over 5 years). (ii) The Ovarian SPORE (N. Urban PI; S.S. Gambhir project leader, which allows us to do more work on ovarian cancer early detection, including imaging strategies, and provides us access to human blood/tissues for our clinical translational core ($10M over 5 years). (iii) The Physical Sciences in Oncology Center (PSOC, U54) (S.S. Gambhir, S. Quake, S. Wang investigators), which allows us to pursue nanotechnology based solutions to understand the origins of cancer (~$20M over 5 years). This is in addition to several other program projects which we directly benefit from, including the *In Vivo* Cellular and Molecular Imaging Center at Stanford (ICMIC@Stanford) P50 (S.S. Gambhir PI)

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($10M over 5 years), which has just been submitted for a competing renewal and has a new prospective trial, based on the CCNE-TR, combining PET imaging and magneto-nano sensor technology to monitor response to lung cancer treatment. We have also recently submitted an Early Detection Research Network (EDRN) grant for $5M over 5 years (S.S. Gambhir PI, S. Wang investigator) to combine our nanosensor technology with new molecular imaging technologies. Dr. Shan Wang (PI) and Dr. Andy Wyrobek (Co-PI, from LBNL but not associated with CCNE) have received a major contract proposal to Biomedical Advanced Research and Development Authority (BARDA) on rapid and accurate proteomic index dosimetry (RAPID) for $40M over five years. This grant uses the magneto-nano protein chip technology developed through the CCNE.

New Training Grants

An NCI R25T post-doctoral fellow training grant in molecular imaging, Stanford Molecular Imaging Scholars (SMIS) Program (http://mips.stanford.edu/smis/) to fund ~15 post-doctoral fellows (S.S. Gambhir PI), over 5 years, was also funded. The R25T mechanism is specifically designed to train post-doctoral fellows across a range of disciplines; SMIS mentors from across these disciplines comprise eight different R25T program areas available to fellows. Several of the post-doctoral fellows in this SMIS program are pursuing nanotechnology related research. For example Dr. Bryan Smith, a bioengineer, had his article on nanoparticle behavior in living subjects featured as the cover of Nano Letters in 2008. We have also obtained other types of training funds (e.g., NIH minority supplement, DOD fellowship, and K99/R00) to help our fellows transition from post-doctoral scholars to independent investigators. The key feature of our training programs is that they help bridge laboratories of CCNE faculty, thereby stimulating innovation and **training of the next generation of leaders**.

New Major Support from Stanford University

Stanford University has recently made **major new** investments fueled by the success of the CCNE U54 and the ICMIC P50. In particular, since we have been very successful in bringing together faculty from the Schools of Medicine, Engineering, and Humanities & Sciences, the Dean of the School of Medicine in conjunction with the other Deans has provided 8 new FTE faculty positions, their full startup packages, and 35,000 sq. ft. of new space for the Canary Center for Cancer Early Detection at Stanford. In addition a new 24,000 sq. ft. clinical translational research facility in the Stanford Hospital has been provided (finishing construction in late 2010) to help with clinical trials that will integrate *in vitro* and *in vivo* strategies. These resources are significant for any University and are providing substantial advantages to CCNE-TR faculty. For example, at least 3 of the 8 new FTE faculty positions will be at the interface of nanotechnology and early cancer detection and will be recruited with joint appointments across the three major Schools at Stanford. Likewise, the University has invested significantly in advanced instruments for the Stanford Nanocharacterization Laboratory (e.g., aberration-corrected TEM) and the Stanford Nanofabrication Facility (e.g., electron beam writer), approximately $10M over the past two years.

Infrastructure Funding

In addition to providing space and faculty billets, the School of Medicine, the Cancer Center and the University have continued to provide significant funds to help us with the growth of CCNE-TR/MIPS. These include new funding in 2009 ($1.2 million) to help upgrade small animal imaging equipment. In addition, new funds in 2009 in excess of $1,000,000 for more equipment for the radiochemistry facilities in Lucas Expansion have been provided. We have also received over $10,000,000 from the Stanford Hosptial to buy new equipment for our clinical Molecular Imaging facility, in addition to costs for the space that is being renovated in the hospital. We have also received financial support from the Departments of Radiology, Medicine, Radiation Oncology, and the Cancer Center towards infrastructure costs.

New Major Support from the Canary Foundation

The Canary Foundation, headed by Don Listwin, continues to play a major role in our growth. In 2008, the Foundation committed to over $15,000,000 in funding to Stanford University (S.S. Gambhir PI) for early cancer detection and helped us obtain the commitment for 8 new faculty billets and a new building, which is now home to the "Canary Center for Cancer Early Detection at Stanford." We have been

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allocated ~35,000 square feet in this new building and it will allow us to recruit many new faculty at the interface of molecular imaging and *in vitro* diagnostics specifically for cancer detection. The Canary Foundation supports a major effort on blood biomarker discovery and validation (well in excess of $25M in support to date) as it relates to early cancer detection for ovarian, lung, prostate, and pancreatic cancers throughout their network including Stanford and the Fred Hutch Cancer Center. In addition, Canary has helped us to develop relationships with three new cancer foundations. Furthermore, the Canary Foundation is helping us with significant outreach to the local and national communities where we continue to educate people about emerging technologies for cancer early detection including our *in vitro* nanosensors and *in vivo* nano-molecular imaging technologies. Just recently, the Canary Foundation has also committed significant additional funds ($6.5M over 5 years) specifically for our recently proposed CCNE-T initiative to NCI.

CCNE-TR Nanobiotechnology Seminar Series

We have been continuing with these monthly seminars to bring outstanding cancer nanotechnologists and oncologists to within the reach of the CCNE-TR team, as well as to the broader scientific/clinical community at large. These well-attended seminars (http://mips.stanford.edu/public/nanobiotech_seminar.adp) are free, open to the public, and webcasted. The exclusive focus of this seminar series is to bring investigators from the other funded CCNE's and the cancer nanotechnology relevant centers to speak about the latest research developments at those centers. All previous lectures can be accessed via the link.

Links to other CCNEs

We have had significant interactions (a total of 42) with other CCNEs and programs and continue to strengthen these further. For example, Dr. Wang collaborated through NCI Diagnostic *In Vitro* Sensor Working Group (DISWG) and shared hCG standard curve data with Caltech (J. Heath/R. Fan), Northwestern CCNE, and MIT (S. Manalis). Another example is that we have a 3-way collaboration among Stanford CCNE-TR (A. Wu lab), Caltech/UCLA Nanosystems Biology Cancer Center (NSBCC) (M. Phelps) and North Carolina CCNE (J. DeSimone) on dynamic imaging and biodistribution of nanoparticles. Stanford was a key player in the *in vitro* diagnostics working group that also involved CCNEs at Northwestern and Caltech and MIT Nano-platform program successfully evaluating nanosensing platforms across the CCNEs.

Some specific examples of our achievements within the past 4 years of grant are given below.

A. Scientific achievements:

1. Femtolmolar sensitivity, a six log dynamic range and matrix insensitivity for our multiplex magnetonanosensor chip developed within Project 1 (Wang).

2. In Project 2, libraries of amphiphilic surfactants have been prepared and studied with single wall carbon nanotubes (SWNT), based upon biocompatible PEGylated alkyl and aryl carbon chains, such as octadecene, maleic anhydride, phospholipids and fluorescein. Allowing the tailoring of SWNT length, biocompatibility, dispersity, and number and type of functional groups. Moreover, the PEGylation has been optimized to reduce biofouling and non-specific interactions in complex systems.

3. Limits of detection and dynamic ranges from the femtomolar to the nanomolar (~ 9-orders of magnitude) have been demonstrated with SWNT for complex samples including cell lysates, conditioned cell media, analyte-spiked sera, and ex vivo sera from mouse models without significant contribution of non-specific binding. Isotopically-labeled SWNT Raman tags have been employed for up to five-analyte simultaneous detection, allowing a high degree of multiplexing.

4. Project 4 has identified a set of putative serum and cell surface biomarkers of gefitinib/cetuximab therapeutic response from *in vitro* tissue culture studies, verified that these markers distinguish between lung cancer models of varying gefitinib sensitivity *in vitro* and *in vivo*. Affinity reagents to these markers are currently being made.

5. Project 5 synthesised activatable probes that can produce 10-fold increase in signals after activation. Our probe can produce up to 80-fold upon MMP-2 treatment, and displayed great sensitivity for imaging MMP-2 *in vivo*. We have also made the QD-BRET sensors for detecting MMP-7 activity in mouse serum.

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6. Project 6 tested the *in vivo* sensitivity of the SWNT-ICG particles and found it to be 170 pM. This is a significant improvement of over 2 orders of magnitude as compared with plain SWNTs. We have tested the new particles and were able to observe specific targeting of the nanoparticles in tumors upon intravenous injection to tumor-bearing mice.

7. Non-invasive imaging with Raman spectroscopy in Project 6 continues to prove itself as a powerful tool to evaluate nanoparticle delivery in preclinical models. Its pM sensitivity and multiplexing capabilities are unsurpassed. We have currently been able to multiplex up to 10 unique surface enhanced Raman scattering (SERS) nanoparticles in a living mouse.

8. We learned by Intravital microspy that is under development in Project 6 that RGD-SWNTs bind neovasculature (unlike controls), extravasate from U87MG vasculature (unlike SKOV-3 vasculature), and bind to individual tumor cells once extravasated (in greater quantity than controls, and binding lasts for weeks, unlike the more temporary association with controls). This work systematically chronicles the microscale behavior of targeted SWNTs in tumor from injection through extravasation, binding, and clearance using complementary fluorescent and Raman techniques.

9. In collaboration with project 4, Project 6 has identified 7 cell surface proteins that change differentially between the Iressa/Tarceva sensitive and resistant cell lines. Studies are on-going to confirm that we can measure the change in expression level of the cell surface proteins by flow cytometry following treatment with Iressa/Tarceva. This is done to validate the cell surface markers before moving to *in vivo* imaging studies utilizing the established mouse models.

10. Our Core 3, continue to support a node of the caNanoLab application as a part of the federated network of nodes supplying access to nanoparticle characterization data through caBIG.

11. A new kind of quantum dot that is self-illuminating has been developed based on Bioluminescence Resonance Energy Transfer (BRET) technology developed in the Gambhir Lab. This approach leads to marked gains in sensitivity for small animal imaging.

12. Proteomics based discovery of blood based biomarkers and risk factors for lung cancer among women smokers and non smokers is underway.

B. Translational achievements:

The CCNE-TR is committed to clinical translation of our nanotechnologies by leveraging our large network of clinical trials and patient samples at USC, FHCRC, UCLA, Cedars Sinai Medical Center, and Stanford. Towards this, we are working on clinical translation of our technologies via our research and developments in ***In Vitro* Proteomic Nanosensors** and ***In Vivo* Molecular Imaging**.

1. Project 6 is currently in the process of talking with the FDA to seek approval of our inert gold Raman nanoparticles for local administration in the human colon to evaluate tumor targeting potential and ultra sensitive detection with our prototype Raman endoscope.

2. In collaboration with GE, Project 6 has demonstrated whole-body, real-time preclinical imaging of QDot fluorescence with time-gated detection and further development of this platform is underway.

3. Analysis of several clinical samples is now underway.

3.1. **Phase II trial of Docetaxel and Erlotinib (multi-center) in patients with Prostate Cancer:** Patients with progressive adenocarcinoma of the prostate enrolled in this study, involving. Normal patient population samples, patient and control pre treatment, during treatment, post study serum samples were collected and currently these are being analyzed.

3.2. **Phase II clinical study in patients with non-Hodgkin's lymphoma:** Nanoscale proteomic analysis of biologic response to atorvastatin in lymphoma patients is the subject of this trial. Single agent Atorvastatin for patients with Low Grade or Refractory NHL. NIA Detected Decrease in phospho-STAT3/5 in cells from lymphoma patient treated with statin. Magnetoanosensor distinguished subtle changes in patient plasma cytokines.

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3.3. **Prospective trial (at Fred Hutch) for monitoring advanced non-small cell lung cancer patients treated with Erlotinib:** Primary Objective of this trial is to determine if early changes in specific plasma proteins are predictive of response/benefit of treatment as measured by progression free survival, overall survival and radiographic response. Pre, during, post-treatment patient blood samples are being analyzed.

3.4. **Blood Biomarkers of Tumor Response for Her-Kinase Axis Treatment:** From 16 candidate blood biomarkers 7 were picked for further study. Validation of these biomarkers using mouse tumor models is in progress.

3.5. **Detection of MMP-7 and MMP-2 from Ovarian Cancer Patient Serum Samples:** Detection of MMP activity vs. MMP amount is the topic of this investigation. MMPs exist in both pro (enzymatically inactive) and active forms. ELISA assay generally measures the total MMP amount (including both pro and active forms). Whether active MMPs may be a more accurate predictive biomarker than the total MMP amount is currently under investigation using our self-illuminated QD-BRET platform. This platform offers several advantages over existing detection technologies such as it is amenable to multiplexing, no excitation source is needed, high signal to noise ratio detection signals, can be performed directly in serum samples and it is ratiometric in that it is independent of variations in the sensor concentrations.

3.6. **Additional studies** with samples from clinical trails are also being initiated for Earlier Ovarian Cancer Detection and Advanced Lung cancer treatment

4. Evaluation of clinical translation potential of our Photoacoustic Molecular Imaging platform is also currently underway.

C. Commercial achievements:

1. **MagArray Inc.** was formed as a spinoff of Project 1 (Wang) in which the company received a Phase II SBIR contract in 2009 for nanotechnology translation of our Magneto Nano Chip. This is to pursue commercialization of cancer diagnostics. The company received **Phase II SBIR contract** in 2009 for nanotechnology translation.

2. Project 5 spin-off company, **ImaginAB**, secured an SBIR grant to continue working on the development of cys-diabodies for cancer imaging purposes.

3. **Zymera**, a privately held nanobiotechnology company, is a spinoff of Project 5 and 6. It is pioneering the commercialization of self-illuminating red to near infrared emitting Quantum Dot technologies for preclinical *in vivo* imaging and as an analytical platform for molecular detection.

4. **Endra Inc,** derived from Project 6, is commercializing a breakthrough medical imaging technology that combines the properties of optical imaging with ultrasound to enable advanced medical imaging. Based on the principles of photoacoustics, Endra's equipment utilizes ultrasound to detect the miniscule amount of heating caused by laser light deep within tissue, providing high contrast imaging at depths and spatial resolution far exceeding existing techniques.

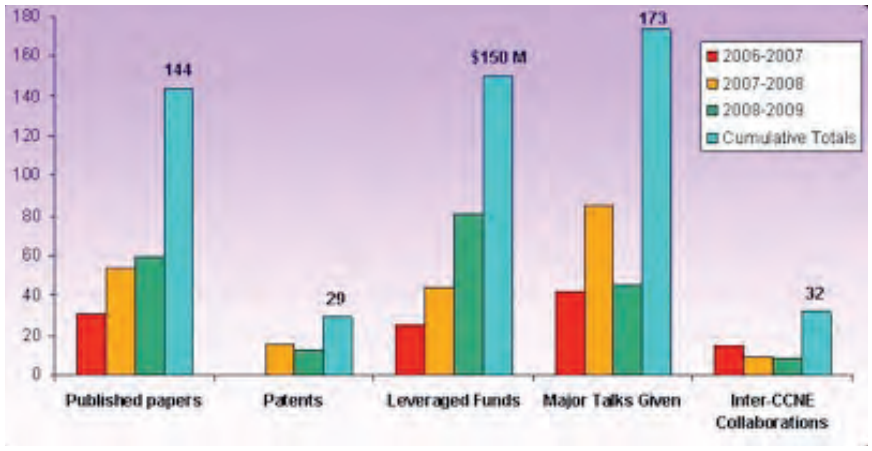

FIGURE 1. *A brief summary of overall achievements of CCNE-TR within the last 4 years.*

CENTER OF NANOTECHNOLOGY FOR TREATMENT, UNDERSTANDING, AND MONITORING OF CANCER (NANO-TUMOR)

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

The UCSD Nanotumor Center has been investigating the potentials of nanotechnology to fight cancer. Our research effort has led to several recent accomplishments in the following areas

Heller, Esener: On chip Junk DNA isolation and analysis for cancer detection.

In cancer research, it is a significant challenge to separate and identify low levels of clinically relevant biomarkers such as specific DNA molecules (DNA biomarkers) in complex samples like blood, saliva and urine. Existing state of the art analytical methods are slow, relatively expensive and lead to significant degradation and loss of the desired DNA molecules. Additionally, DNA biomarker detection is often limited by sample size; i.e., only a relatively small amount of blood may be drawn from very ill patients, the elderly, and infants. Thus, sample preparation processes that are inefficient or require high dilution of the original sample often fail or are unreliable for isolating cancer and other disease related biomarkers at lower concentration ranges. This is in particular a significant problem for early detection of cancer and for residual disease monitoring.

It has been hypothesized that as a result of fast cancer cell turn over, the blood of cancer patients should contain both higher amounts and larger size fragments of DNA biomarkers (nanoparticulates) when compared to blood from healthy donors. However, a rapid, low cost and accurate point of care (POC) solution has not been possible because of the overall low levels of DNA nanoparticulate biomarkers in blood samples. Now, using a novel high conductance dielectrophoretic (DEP) device at a low AC frequency we are able to separate DNA nanoparticulates directly from whole blood samples of leukemia patients without the need for prior sample preparation. This demonstrates the potential of the DEP technology for truly "Seamless Sample to Answer Cancer Diagnostics." Using a DEP microarray device, the DNA nanoparticulates in the blood sample become concentrated in the high field regions around circular microelectrodes, while larger entities such as blood cells concentrate into low field regions, making their removal possible by a simple wash procedure. The detection of high levels of fluorescent stained DNA nanoparticulates from the blood of a Chronic Lymphocytic Leukemia (CLL) patient compared to blood

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from a healthy control is shown below in **Figure 1**. A new company "Biological Dynamics" has recently been formed and funded by Angel Investors to pursue the development and commercialization of this technology for early cancer detection and chemotherapy/residual disease monitoring. This work resulted in four published articles, one patent application and the licensing of the technology by the UCSD Tech Transfer Office to Biological Dynamics.

Krishnan R, Dehlinger DA, Gemmen GJ, Mifflin RL, Esener S and Heller MJ, "Interaction of nanoparticles at the DEP microelectrode interface under high conductance conditions", Electrochemical Communications, V11, #8, 1661-1666, 2009.

Krishnan R and Heller MJ, "An AC electrokinetic method for the enhanced detection of DNA nanoparticles", J. Biophotonics, V2, #4, pp. 253-261, 2009.

Krishnan R, Sullivan BD, Mifflin RL, Esener SC, and Heller MJ, Alternating current electrokinetic separation and detection of DNA nanoparticles in high-conductance solutions, Electrophoresis, v. 29, #9, pp. 1765-1774, May 2008.

Ruoslahti: CendR peptides that overcome transport limitations within tumors.

One of the main problems in cancer treatment is the poor penetration of drugs into tumor tissue. Drugs only penetrate the distance of a few cell diameters into the tumor tissue from blood vessels. This poor penetration appears to arise from two main factors: First, tumor vessels are poorly perfused with blood, which limits the delivery of blood-borne compounds to tumors. Second, tumors have a high interior pressure, which is thought to result from poor lymph drainage causing tissue fluid to flow out of the tumor. This prevents efficient entry of drugs from the blood vessels into the tumor tissue. Tumor blood vessels are leaky, which partially makes up for the poor penetration of drugs (the so called enhanced permeability and retention — EPR-effect), but this process is not very effective. It is size-dependent and varies greatly from tumor to tumor. Interstitial fibrosis can further retard the

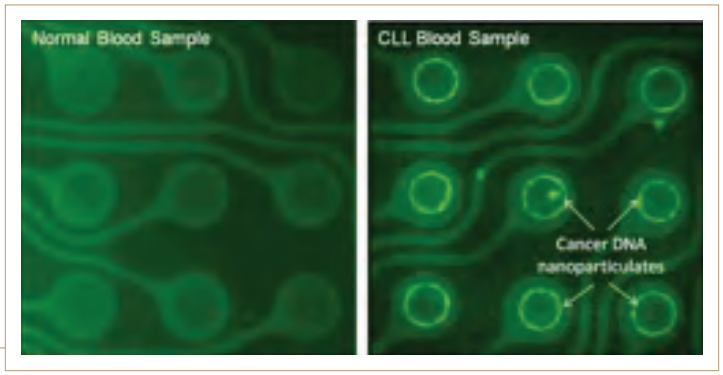

FIGURE 1. *Left side shows microarray after DEP of normal blood sample, with no DNA nanoparticulates detected. Right side shows microarray after DEP of CLL patient blood with large amounts of fluorescent stained DNA nanoparticulates detected. Analysis time 15 minutes. (CLL samples from Dr. Thomas Kipps, UCSD Moores Cancer Center)*

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diffusion of drugs and other molecules through tumors. Nanoparticles, which are promising for drug delivery as they can lower side effects, are even less likely because of their size to penetrate into tumors than small molecular weight drugs. We have discovered a tumor-specific tissue penetration peptide that solves this problem, allowing us to deliver nanoparticles and any other payload deep into the tumor tissue. The payload does not even have to be coupled to the peptide; the peptide activates a transport system in the tumor cells that sweeps bystander molecules, such as therapeutic agents, along with the peptide, into the tumor tissue.

This work has yielded several publications, is patented, licensed to two companies, tested in human tissue, and starting clinical trials.

Sugahara, K.N., et al., *Coadministration of a Tumor-Penetrating Peptide Enhances the Efficacy of Cancer Drugs*. Science, 2010.

Sugahara, K.N., et al., *Tissue-penetrating delivery of compounds and nanoparticles into tumors*. Cancer Cell, 2009. **16**(6): p. 510-20.

Teesalu, T., et al., *C-end rule peptides mediate neuropilin-1-dependent cell, vascular, and tissue penetration*. Proc Natl Acad Sci U S A, 2009. **106**(38): p. 16157-62.

Trogler, Mattrey: Color Doppler imaging (CDI) of nanoparticles.

This effort focused on the use of monodisperse hollow porous nanoparticles filled with fluorocarbon gas to indicate location of breast tumors during surgery by ultrasound imaging. Nanoparticles have great potential to assist in the color Doppler ultrasound intra-operative imaging (CDI) of breast tumors. This technology would replace the use of radioactive seeds which are painful to implant and can only be employed for localization of single tumors in the breast while retaining the far lower rate of second surgeries with active intraoperative locations. Previous attempts to use ultrasound imaging to detect nanoparticles utilized solid silica 100 nm nanospheres to enhance B-mode tissue imaging. Hollow porous silica nano shells were prepared in the 200 nm to 2 mm range and filled with fluorocarbon gas. These nanoparticles were injected into the tumor area in human tissue and even 50 microliter injections could be readily imaged by CDI. One of the unique features of CDI is that it has extraordinary sensitivity for the detection of bubbles using stimulated acoustic emission (SAE), a characteristic

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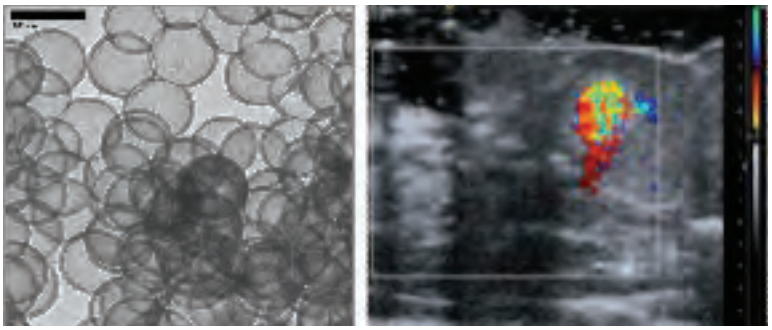
used routinely in the clinic. Using iron doping, a method to incorporate small amounts of iron into the silica, we have also developed a unique 200 nm gas-filled biodegradable silica nanoshell which can be imaged by CDI. These particles are small enough to accumulate in even very small millimeter-sized ovarian tumors by EPR (enhanced penetration and retention) and be readily taken up by tumor cells. Some of the potential advantages of these nanoparticles are that their surface chemistry allows the attachment of many different kinds of molecules (antibodies vs. peptides) that might direct them to the tumors and increase their uptake by the tumor cells. The fact that the nanoshells can be destroyed locally in the tumor by high power ultrasound enables multiple independent images of the same region by nanoshells targeted to the tumor using different targeting ligands or stimulators for uptake.

This work has yielded 1 publication, a UCSD Disclosure, the results are validated in human tissue, and is at the pre-clinical stage.



CDI of Gas Filled NanoShells (Left) Electron microscope image of the nanoshells. (Right) A 100 microliter injection in human breast tissue. The colored area is the CDI signal from the microshells (5 mm diameter) while the grey area is the breast tissue.

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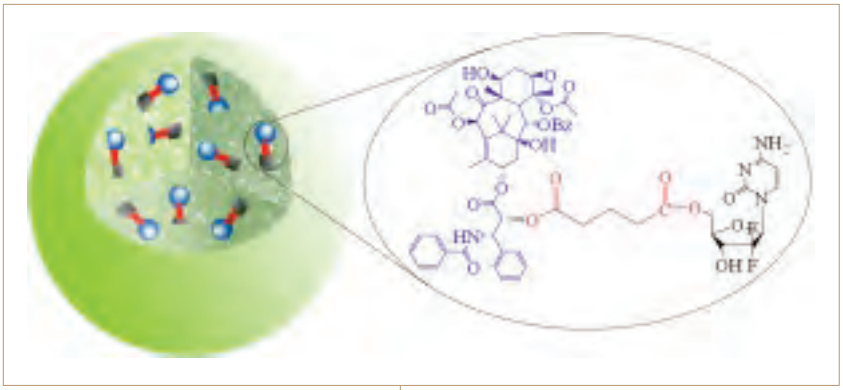
Zhang and Esener: Dual drug PLGA particles with capability to load two drugs in a precise ratio in a single particle.



Combinatorial therapy includes loading two drugs onto the same drug delivery nanocarrier for precise control of the dosage and scheduling of the drugs.

A recent editorial in Science magazine (*Science* 2010, 328, 137) welcomes the recent announcement by the US Food and Drug Administration (FDA) of its plan to draft new guidelines for testing and approving the use of multidrug treatments of diseases. This change of FDA's Regulations is expected to expedite the development of novel drug combinations and spur more academia-industry collaborations to translate the combinatorial therapies to practice. For cancer therapy, advanced understanding of cancer biology and clinical outcomes have validated that combined therapy of two or more drugs provides a promising strategy to suppress cancer drug resistance as different drugs may attack cancer cells at varying stages of their growth cycles. However, one major challenge of combinatorial therapy is to unify the stability, distribution, and cellular uptake of various drug molecules, to allow precise control of the dosage and scheduling of the multiple drugs, thereby maximizing the combinatorial effects. To solve this problem, we have developed a unique approach to loading two drugs onto the same drug delivery nanocarrier in a precisely controllable manner. This is achieved by binding two drugs to one another using cleavable linkers to form a so called drug conjugate before incorporation into the nanocarriers. In contrast to loading individual types of drugs separately, this drug conjugate strategy enables one to load multiple drugs onto the same drug carrier with a pre-defined stoichiometric ratio. The cleavable linkers allow the individual drugs to resume the therapeutic activity after the drug conjugates are delivered into the target cells and unloaded from the delivery vehicle.

Future direction of this project is to further optimize this new nanoparticle-assisted combinatorial drug delivery approach and to pursue clinical evaluation of the combination therapy after finishing proper *in vivo* tests.

This work has yielded 1 publication, 1 patent, and is currently at the preclinical trial stage.

Aryal, S.; Hu, C-M.; Zhang, L. Combinatorial drug conjugation enabled nanoparticle dual drug delivery, *Small* 2010

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Kummel, Blair: Automated imaging of breast tumor margins.

Breast conservation therapy (BCT) or partial mastectomy has been proven to have equal survival efficacy compared to full mastectomy through multiple studies with greater than 10 years of follow-up and is currently considered the standard of care. Positive margin rates in BCT continue to range from 25-50%; when the tumor is removed, the block of tissue is examined after the operation to determine if there are cancer cells at the edge (margin) of the excised tissue. When cancer cells are present at the margin, a second surgery is usually performed. Second surgeries are stressful for patients and carry surgery related risks. Thus, it is desirable to avoid second surgeries and maintain the level of diagnostic accuracy.

With that goal, we have developed a process to characterize margins during the first surgery that should allow us to spare the need for second surgery. We have demonstrated the first fully automated analysis including autofocus and cellular debris filtering of intraoperative touch preparation evaluation of surgical margins from breast conservation therapy. In touch prep, the tumor is removed by a surgeon and pressed onto a coated slide to remove cells from the surface of the tissue. Our automated microscopy technique is used to determine if the cells detached from the tissue surface are cancerous. Touch preps were prospectively performed on 47 patients undergoing breast conservation or breast reduction surgery. A fast (2 min/slide) algorithm was developed to automate discrimination between true epithelial cells and cellular debris. The accuracy of the automated fluorescence analysis was 95% for identifying invasive cancers compared against final pathologic diagnosis. Accuracy in detecting ductal carcinoma in-situ was 78%. The overall specificity was 100% (there were no false positives). The sensitivity for ductal carcinoma in situ was 75% and invasive cancer was 90%. Overall sensitivity was 87% which is comparable to the best reported results from manual examination of intra-operative touch preparations. The same software is being developed for automated analysis of permanent breast tumor sections.

This work has resulted in 2 publications, a third publication in preparation, UCSD Disclosure, is validated in a clinical trial, a 2nd clinical trial is in progress, and discussions are ongoing on commercial development of the developed software.

1. M. J. Cortes, D. Martin, S. Sandoval, M. Ruidiaz, D. Messmer, J. Wang-Rodruguez, W. Trogler, A. C. Kummel, S. Blair, "Automated Microscopy to Evaluate Surgical Margins via Touch Prep in Breast Cancer" Annals of Surgery **16(3)**, 709-20 (2009)

2. S. L. Blair, J. Wang-Rodriguez, M. J. Cortes, D. Messmer, A. C. Kummel, W. Trogler, "Enhanced touch preps to improve the ease of interpretation of intra-operative breast cancer margins." *The American Surgeon*, **73**, 973 (2007)

EMORY-GEORGIA TECH CCNE ACCOMPLISHMENT HIGHLIGHTS:

Emory University and Georgia Institute of Technology
Principal Investigators: Shuming Nie, Ph.D.

The Emory-Georgia Tech CCNE has made a number of exciting advancements in translational cancer nanotechnology over the period of the center award. These advancements span a wide range of research areas, from fundamental studies and nanoparticle development to clinical translation for diagnostics and treatment. In particular, our center has made substantial progress in 4 key areas:

- *In vivo* and intraoperative imaging
- Multiplexed molecular diagnostics
- Targeted cancer therapy and theranostics
- Cancer bioinformatics and biocomputing tools.

1. *In Vivo* and Intraoperative Cancer Imaging

We have made significant progress in the development of novel nanoparticles for use in cancer applications, including gold nanoparticle Raman tags for *in vivo* imaging (1). These probes have a biocompatible coating and a unique spectroscopic signature, making them ideal for use in deep tissue animal imaging. We have shown that coupling the nanoparticles to cancer targeting moieties results in accumulation at the tumor site, enabling highly sensitive tumor imaging *in vivo* (**Figure 1**).

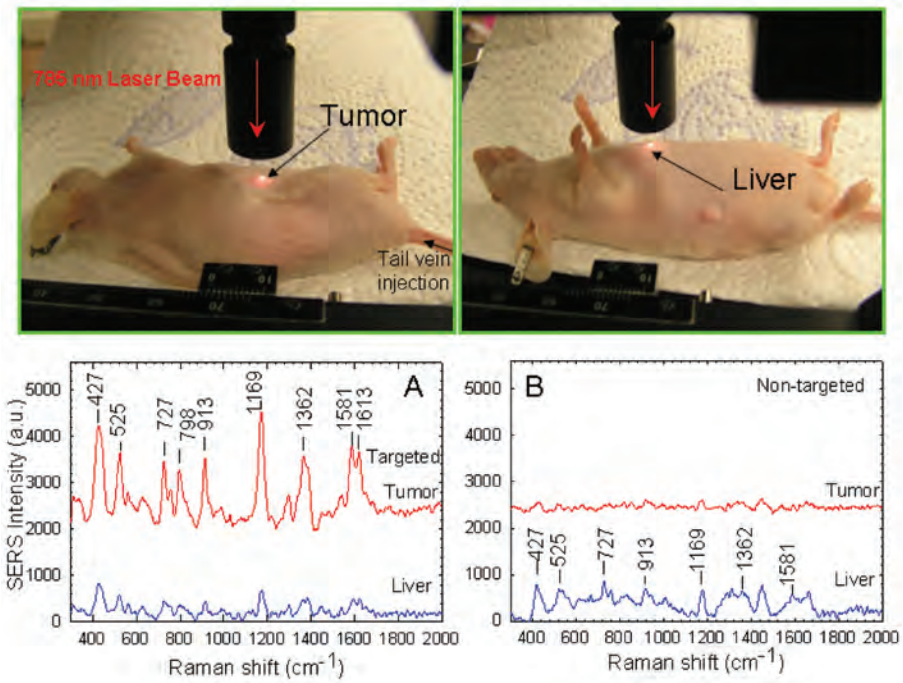

FIGURE 1. *SERS spectra obtained from the tumor and the liver locations by using targeted (**a**) and nontargeted (**b**) nanoparticles. Two nude mice bearing human head-and-neck squamous cell carcinoma (Tu686) xenograft tumor (3-mm diameter) received ScFv EGFR-conjugated SERS tags or pegylated SERS tags. The particles were administered via tail vein single injection. SERS spectra were taken 5 h after injection. In vivo SERS spectra were obtained from the tumor site (red) and the liver site (blue) with 2-s signal integration and at 785 nm excitation. The spectra were background subtracted and shifted for better visualization. The Raman reporter molecule is malachite green, with distinct spectral signatures as labeled in **a** and **b**.*

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We have also developed a new class of quantum dots (QDs) with light emission spectra that are tunable by lattice strain (2). These nanocrystals are lattice-mismatched heterostructures grown by epitaxial deposition of a compressive shell (e.g., ZnSe) onto a soft and small nanocrystalline core (e.g., CdTe). This combination of a "squeezed" core and an "expanded" shell (coupled straining) causes dramatic changes in both the conduction and valence band energies, resulting in nanoparticles with highly tunable fluorescence emission over a wide range of visible and near-infrared wavelengths (500 nm to 1050 nm). This control is critically important in the design of QDs for *in vivo* applications, where light penetration through tissue is a limiting factor (3). In addition, the strain-tuned dots are much less toxic in comparison with traditional cadmium-containing QDs because the overall cadmium contents are reduced by 10-20 fold.

Furthermore, we have recently developed an integrated imaging and spectroscopy system for intraoperative detection of tumor margins (patent filed, early startup company in progress). Surgery cures approximately 45% of all patients with cancer and provides a dramatic survival advantage in comparison to chemotherapy and radiation therapy, which cures only 5% of patients. A complete resection is the single most important predictor of patient survival for almost all solid tumors, providing a three to five fold improvement in survival compared to a partial or incomplete resection. We have shown that nanoparticles can be designed to specifically target tumors, enabling real-time imaging of cancer *in vivo*. However, sensitive instrumentation is needed to detect the nanoparticles during surgery and clearly define tumor margins to assist surgeons in achieving a complete resection. By combining a handheld spectrometer (pen-sized fiber-optic probe for wavelength resolved fluorescence and Raman measurements) with a wide-field color imaging system, real-time detection of tumor cells can be performed intraoperatively (**Figure 2**).

This system provides spectroscopic information about tumor presence (measured by contrast agent accumulation) with a laser channel indicating the laser beam location and a color video channel for visualizing the surgical field. A near-infrared (NIR) channel allows the surgeon to visualize the contrast agent and accurately locate the tumor margins during surgery.

2. Multiplexed Molecular Diagnostics

In the field of biodiagnostics, we have had a number of key achievements in translating nanoparticle technology to clinical applications. In collaboration with clinicians and engineers, we have developed robust protocols for multiplexed and

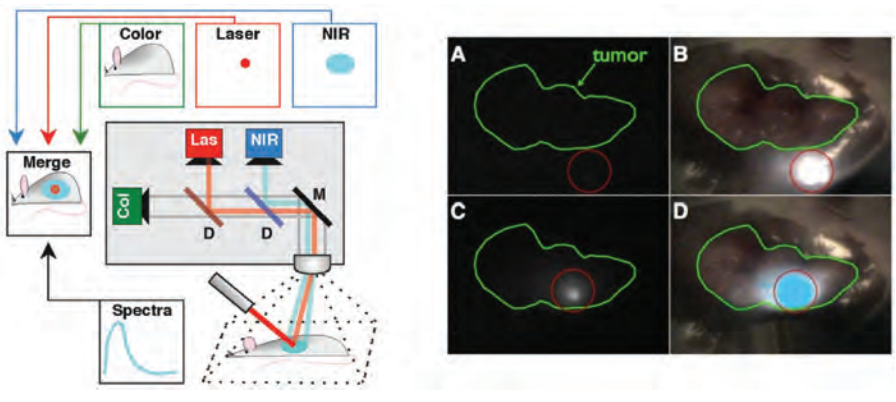

FIGURE 2. *(Left) Schematic diagram showing the integrated spectroscopy and imaging system for cancer surgery. The spectroscopic channel (provided by the SpectroPen) allows ultrasensitive detection of tumor cells at specific locations. With two dichroic beamsplitters one after another (Las and NIR), the imaging system allows simultaneous viewing of the NIR laser beam spot, the NIR fluorescence/Raman signals, and the visible color image. (Right) While no NIR signal (ICG) is visible when the SpectroPen laser is focused off a tumor after intravenous contrast agent administration (**a**, **b**), the signal dramatically increases when the SpectroPen laser is passed over the tumor (**c**, **d**).*

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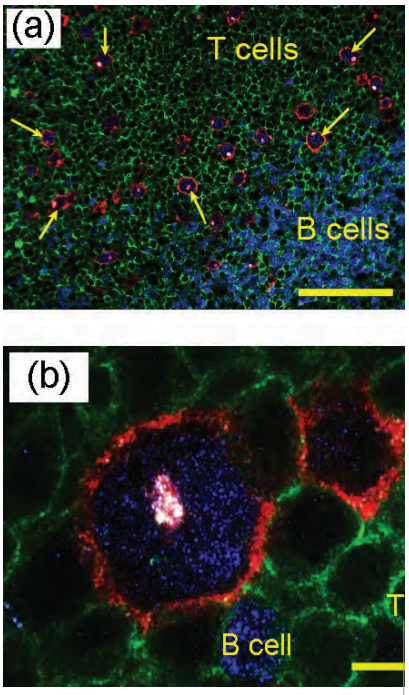

FIGURE 3. *Multiplexed QD staining images of HRS malignant cells and infiltrating immune cells on lymph node tissue specimens of a Hodgkin's lymphoma patient. (**a**) Malignant HRS cells (red membrane, blue nuclear, and read/whitish Golgi) are identified by a unique multiplexed staining pattern of CD30 positive (membrane staining), CD15 positive (Golgi staining), Pax5 positive (nuclear staining), and CD45 negative. They are differentiated from infiltrating B cells (blue nuclear staining) and T cells (green membrane staining). A few prominent HRS cells are indicated with arrows. Scale bar: 10 µm. (**b**) Detailed view showing the distinct staining patterns of HRS cells, B cells, and T cells. Scale bar: 100 µm.*

quantitative immunohistochemistry using bioconjugated QDs (4). These protocols have been further refined to address many clinical diagnostic problems, including the use of multiplexed quantum dots (QDs) and wavelength-resolved spectral imaging for molecular mapping of tumor heterogeneity on human prostate cancer tissue specimens (5) and Hodgkin's Lymphoma specimens. Without physically removing any cells from heterogeneous tissue sections, this nanotechnology approach allows the molecular profiles and morphological features to be "digitally" extracted from individual cells, cellular clusters, glands, and complex histopathological loci. Based on a panel of just four protein biomarkers (E-cadherin, high-molecular-weight cytokeratin, p63, and alpha methylacyl CoA racemase), we have shown that structurally distinct prostate glands and single cancer cells can be detected and characterized within the complex microenvironments of radical prostatectomy and needle biopsy tissue specimens. The results reveal extensive tumor heterogeneity at the molecular, cellular, and architectural levels, allowing direct visualization of human prostate glands undergoing structural transitions from a double layer of basal and luminal cells to a single layer of malignant cells. For clinical diagnostic applications, multiplexed QD mapping provides correlated molecular and morphological information that is not available from traditional tissue staining and molecular profiling methods. It also enables the sensitive detection of low-abundant tumor cells, such as Hodgkin's and Reed-Sternberg cells. We have shown that a panel of 4 protein biomarkers allows accurate detection of these cells, which comprise only 1% of the heterogeneous infiltrating cells in lymph node tissues. The results obtained from multiplexed QD imaging indicate that QD staining can provide a more sensitive diagnostic analysis and differentiate specimens that are ambiguous using standard methods. This increased sensitivity could have important implications for the diagnosis of Hodgkin's Lymphoma as well as other cancers where low-abundant cells are important. (**Figure 3**)

3. Targeted Cancer Therapy and Theranostics

We have made considerable progress in several key areas including (i) synthesis and characterization of self-assembled mothership nanoparticles by using branched polyglycerols as a biocompatible and nontoxic scaffold; (ii) demonstration of nanoparticle accumulation in solid tumors and clearance from the RES organs; (iii) active versus passive tumor targeting; and (iv) improved *in vivo* treatment efficacies. Nie (biomedical engineer), Shin (physician-scientist), Yang (imaging scientist), and Chen (cancer biologist) have co-authored a publication in Nature Biotechnology describing the targeted detection of head and neck tumors *in vivo* using surface-enhanced Raman (SER) nanoparticles. We also have a manuscript recently published in ACS Nano reporting on the

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development of Heparin-folic acid (FA)-Taxol™ nanoparticles and their evaluation as antitumor agents using xenograft models.

We have designed theranostic nanoparticles by using Poly-glycerol (PG) as a multifunctional platform, paclitaxel (TX) as a therapeutic drug, folic acid (FA) as a tumor targeting ligand, together with a near infrared dye (Cy5.5) for fluorescence imaging. The folate receptor is overexpressed in many types of human tumors, including metastatic breast and lung cancers. Driven by hydrophobic interactions between the attached TX molecules, these conjugates self-assemble into uniform nanoparticles, dramatically increasing the water solubility of the drug (10 mg/mL injected dose). While the self-assembly of amphiphilic block copolymers in water is well known, the self-assembly of randomly modified homopolymers has been less studied and presents interesting properties and possibilities. We believe that the nanoparticles formed by the conjugates have a structure resembling that of hydrogel nanoparticles. A major difference is that these nanoparticles are physically "crosslinked" by hydrophobic interactions, not by covalent chemical bonds. When the self-assembled nanoparticles are spread on a glass surface, single particles can be directly visualized by using standard fluorescence microscopy, allowing us to monitor the "unloading" process of these theranostic particles. With the same optical setup, the unloaded cargos (that is, individual polymer molecules) are not visible because of their much weaker fluorescence signals.

The PG-TX theranostic nanoparticles with (GFT) and without (GT) folate targeting have been used to study nanoparticle accumulation in tumors and clearance from RES organs. By *in vivo* optical imaging, both of the GT and GFT nanoparticles showed strong accumulation at the tumor sites with the signal intensity continuously increasing over 48 hours. Monitoring up to one week showed persistent retention of nanoparticles in the tumor, with tumor accumulation data nearly identifical for both GT and GFT. This result is consistent with that of Davis, Park and their co-workers who have also shown that targeted and non-targeted nanoparticles exhibit similar biodistribution and tumor localization. A major conclusion from these studies is that the amount of nanoparticles delivered to large tumors is primarily determined by extravasation though the leaky vasculatures and does not directly involve binding to tumor cells. In addition, the lack of functional lymphatic vessels in large tumors prevents the nanoparticles from being drained out and is responsible for their persistent accumulation in tumors. Importantly, this class of PG-based theranostic nanoparticles is able to dissociate and unload its molecular cargos in blood, producing small polymer molecules that are rapidly cleared from the liver, spleen and other RES organs.

By using small tumors and micrometastases, we have also obtained conclusive evidence demonstrating that theranostic nanoparticles can be actively targeted to solid tumors. Selective accumulation of FA-targeted nanoparticles (GFT) were detected in these small tumors (2-3 mm) while non-targeted nanoparticles showed only very little signal above the background. It is well-known that angiogenesis starts after tumor reach certain critical sizes (about 1mm) (6). The small tumors in this study should have developed some vascular structures, and have larger surface area-to-volume ratios when compared with large tumors (7). Therefore, in small tumors and micrometastasis, receptor binding and endocytosis of FA-targeted nanoparticles lead to their tumor accumulation and retention, whereas non-targeted nanoparticles are not taken by the tumor cells and are eventually drained away by lymphatic vessels that are still functional at the tumor peripheries.(7)

Finally, we have shown improved anti-tumor activity of these nanoparticles *in vivo* in comparison with free drug. Both targeted and nontargeted nanoparticles resulted in significant antitumor effects in large tumors, whereas the free drug showed little effect on tumor inhibition, consistent with the *in vivo* targeting results. Histological analysis of tumor sections from different groups also showed that the injection of nanoparticle drugs reduced the expression level of Ki67, a cell proliferation biomarker; and H&E staining showed apoptosis of tumor cells and the presence of fibrotic tissues, whereas the free drug led to little difference compared to the control untreated group. The therapeutic outcome also can be correlated to the imaging results in small tumor groups. The targeted nanoparticles quickly induced tumor regression after the first injection

whereas the non-targeted nanoparticles and free drug showed a clear delayed response for treatment. Improved *in vivo* efficacy has also been demonstrated in xenograft models by using heparin-based nanoparticles with trapped paclitaxel. As shown in **Figure 4**, the growth curve of tumor xenografts showed that all the treatment groups including free TX, heparin-TX (HT-T), and heparin-folate-TX (HFT-T) significantly inhibited the growth of tumor as compared with the control ($p<0.0001$ for each treatment). Although the tumor volumes were similar in HT-T and free TX-treated groups ($p=0.608$), FR-targeted HFT-T more potently reduced tumor growth than free TX ($p<0.0001$). After 5 injections, the average tumor volumes in TX, HT-T, and HFT-T groups were 1670.3 ± 286.1 mm3, 1211.3 ± 448.1 mm3, and 92.9 ± 78.2 mm3, respectively. Importantly, there was little or no toxicity as judged by animal weight loss or organ pathology.

4. Cancer Bioinformatics and Biocomputing Tools

Our center is at the forefront of biocomputing and the integration of nanotechnology with cancer bioinformatics tools. These tools include (1) new algorithm and software tools for cancer biomarker discovery and validation; (2) digital processing of multiplexed QD, surface-enhanced Raman scattering (SERS), and cellular/tissue imaging data for fundamental studies and cancer diagnostics; (3) real time video tracking for intra-operative cancer imaging in surgical oncology; and (4) interfacing with NCI cancer Biomedical Informatics Grid (caBIG) and caNanolab. We have recently received NCI caBIG certification for silver-level compatibility for two of our bioinformatics tools (caCORRECT and omniBiomarker), which are now available for use by the larger research community. These software tools have been recently used by clinicians for analyzing complex microarray data to select diagnostic biomarkers for renal cell carcinoma (8).

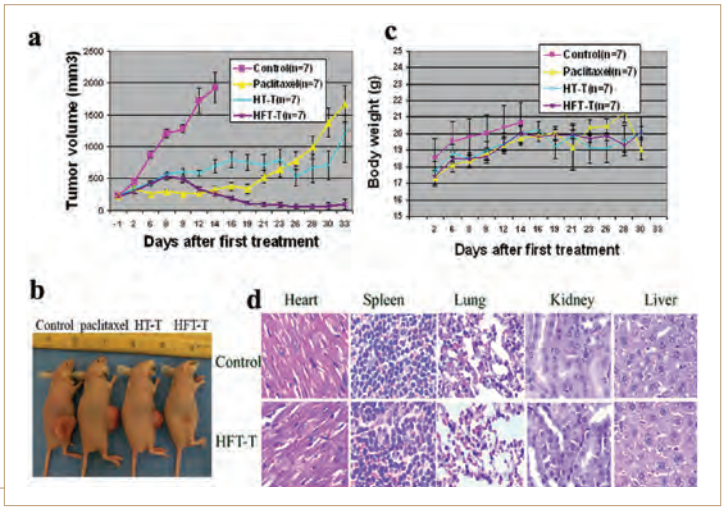

FIGURE 4. *In vivo therapeutic efficacy of TXl trapped in self-assembled heparin nanoparticles. (**a**) Tumor volume data for standard TX and different nanoparticle formulations. (**b**) Photograph of animals from different treatment groups. (**c**) Body weights of the mice in all groups. (**d**) Micrographs of H&E organ staining from control and HFT-T-treated mice. Note that the chemically modified heparins show little or no anticoagulation activity (less than 1% of the original value).*

Table 1: List of Emory-GT CCNE Bioinformatics and Biocomputing Tools and Software Packages

Bioinformatics Solutions		Data Management Features			Community Integration Features		Knowledge Curation
Tool	Description	Datasets	Quality Assurance	Need for Integration	Quality Assurance	Knowledge Provided	Quality Assurance
caCORRECT	*Chip artifact CORRECTion [1]*	*Renal Breast Prostate datasets*	*Reads from caBIG caArray*	*Yes*	*caBIG Silver certified*	*Clean microarray data / gene expression*	*Cleaned microarray data stored in ArrayWiki*
omni Biomarker	*Biomarker identification [2]*	*Renal, breast, ovarian, and lung cancer*	*Yes, passed through caCORRECT*	*Yes*	*caBIG Silver certified*	*Gene expression biomarkers*	*Validated in wet lab, results stored in ArrayWiki*
QD IHC	*QD immunohisto-chemistry image analysis [3]*	*Prostate Renal cell carcinoma, Breast cancer*	*QD quality control*	*No*	*Research prototype finished, evolving into OmniSpect grid service*	*Quantification of biomarkers for traditional IHC and multiplexed QDs*	*QDWiki*
omniSpect	*Multispectral quantification and image analysis*	*QD, SERS, Imaging Mass Spectrometry*	*QD quality control*	*Yes*	*Research prototype finished in preparation for caBIG silver review*	*Yes*	*SERSWiki*
3D-VIE	*3D QD imaging*	*Prostate cancer*	*QD quality control*	*No*	*Research prototype in development*	*No*	*QDWiki*
omniClinic	*All-in-one storage of datasets and analytical results*	*Prostate Renal Head and Neck cancer*	*No*	*Yes*	*No*	*Yes*	*No*
ArrayWiki	*Quality microarray repository and knowledge [6]*	*>1500+ experiments, >30000 microarray chips*	*Processed by caCORRECT*	*Yes*	*Research prototype finished*	*Microarray meta-data*	*Community curated*
TissueWiki	*Quality tissue microarray repository and knowledge*	*Human Protein Atlas*	*Algorithmic checking of cancer grades*	*Yes*	*Research prototype finished*	*Tissue microarray meta-data*	*Community curated*

In addition to the research highlighted above, the success of our center has resulted in many key achievements, including the formation of three startup companies, the establishment of unique and cross-disciplinary collaborations, and a number of leveraged funding awards for our investigators to continue this exciting research.

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8. Osunkoya A, Yin-Goen Q, Phan J, Moffitt R, Stokes T, Wang M, et al. Diagnostic biomarkers for renal cell carcinoma: selection using novel bioinformatics systems for microarray data analysis. Human pathology. 2009.

MIT-HARVARD CENTER OF CANCER NANOTECHNOLOGY EXCELLENCE

MIT and Harvard University,
Massachusetts General Hospital
Principal Investigators: Robert Langer, Ph.D., and Ralph Weissleder, M.D., Ph.D.

Giant Steps in Cancer Research, Thanks to Tiny Technologies

The Boston-based Center for Cancer Nanotechnology Excellence (CCNE) unites some of the world's leading researchers from academic institutions such as Massachusetts Institute of Technology and Harvard Medical School, and clinical institutions such as Massachusetts General Hospital, Brigham and Women's Hospital, and Beth Israel Deaconess Medical Center. Through collaborations among biologists, engineers, chemists, materials scientists, and physicians, the quest for innovative new treatments and tools for detecting, diagnosing, and monitoring cancer in patients is advancing along multiple fronts.

From the start of this initiative in 2005 through the first six months of 2009, CCNE investigators working on one or more of five discrete projects published more than 300 publications, issued approximately 30 patent applications, trained more than 130 biomedical professionals, and started five companies to commercialize their discoveries. Here are just a few of CCNE's accomplishments to date.

PROJECT 1: Targeted Polymeric Nanoparticles for Cancer Therapeutic Applications

Robert Langer, Sc.D.;
Omid Farokhzad, M.D.

The ability to one day treat cancer patients by implanting therapeutic nanoparticles will make possible more efficient and effective drug delivery that not only releases drugs directly to targeted cells, but at the same time reduces toxicity by not spreading the drug where it's not needed. CCNE investigators envision a further evolution of this idea: the development of a nanorobot that carries drugs to a tumor, images it, and then reports back when its payload has been delivered. Implanting a theranostic nanorobot and viewing in real time the reduction of tumor cells is, investigators believe, how medicine will be practiced a generation from now.

At the same time, ensuring that a targeted nanoparticle is successful in its mission is a daunting challenge. The nanoparticles must be designed with molecular specificity in order to evade the immune system, be absorbed by the target cells — and only those cells — and release the drug at the right time and over the desired period of time, whether hours or days. Using combinatorial strategies, CCNE researchers developed a library of more than 3,000 targeted nanoparticle formulations with

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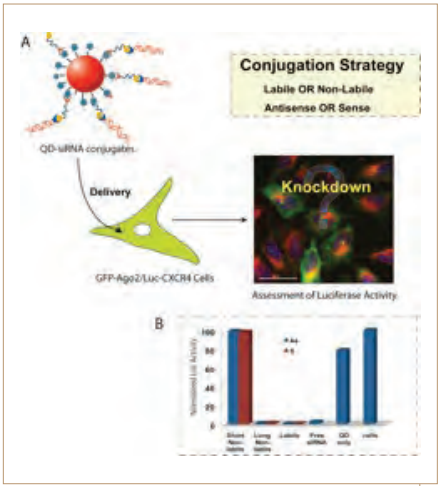

FIGURE 1: *(A) Schematic for probing the effect of conjugation strategy on gene silencing by QD-siRNA conjugates. (B) Knockdown of luciferase by QD-siRNA conjugates with antisense (As) or sense (S) strand of siLuc conjugated with labile and non-labile cross-linkers of varying length.*

important and intentional variations in size, chemical structure, charge, and density of surface molecules. This has facilitated the development of nanoparticles with precise properties that have been successfully tested in mouse, rat, and monkey models, and will be introduced in a human trial later in 2010.

PROJECT 2: Treatment of Cancer with siRNA Delivered by Nanoparticles

Phillip Sharp, Ph.D.;
Sangeeta Bhatia, M.D., Ph.D.

Silencing or knocking down the expression of an oncogene through RNA interference (RNAi) provides the means to halt a tumor's growth at the source. One method of effecting RNAi is by introducing short interfering RNAs (siRNAs) into the targeted cells. Successfully delivering siRNA is difficult, however, because it must be able to survive degradation in the body, travel to the surface of the target cell, and cross the cell membrane. *In vivo*, free siRNA is cleared too rapidly through the kidneys so a variety of carriers have been explored, with mixed results. CCNE investigators conducted a systematic evaluation of coupling strategies to judge how siRNA should best be attached to nanoparticles in order to achieve effective silencing. They found that labile bonds are most effective in enabling the free release of siRNA into the cell cytoplasm. If non-labile bonds are used, tethers should be employed to ensure that the siRNA hits its mark.

In another study, investigators proved the effectiveness of a novel biomaterial called a lipidoid (because the fat-like molecule resembles a lipid) as a delivery vehicle for siRNAs. As siRNAs are negatively charged, they can't cross cells' fatty outer membrane by themselves, but lipidoids have proven successful in mouse models. In fact, not only can they carry a specific siRNA to a target gene, payloads as high as 20 distinct siRNA strands may be possible. Because many cancers result from multiple malfunctioning genes, that could be a huge advantage. Researchers have developed methods to rapidly produce, assemble, and screen a wide variety of lipidoids in order to select the most effective ones for specific applications. In studies with mice, they were able to deliver five doses of siRNA, which silenced multiple genes in the mice's livers (**Figure 1**).

PROJECT 3: Development of Clinical-Grade, Targeted Magnetic Nanoparticles

Ralph Weissleder, M.D., Ph.D.

This project alone has resulted in more than 60 manuscripts, 10 patent applications, four grant applications, two start-up companies, and one FDA approval. One of its goals is to develop novel magnetic nanoparticles (MNPs) to enhance detection sensitivity for magnetic resonance imaging and cancer diagnostics in blood samples. With an iron core and ferrite shell, and containing cancer-seeking ligands, the MNPs are ideal

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FIGURE 2: *Schematic of the DMR system. The system consists of an array of microcoils for NMR measurements, microfluidic networks for sample handling and mixing, embedded NMR electronics, and a permanent magnet for portable operation. The whole setup can be packaged as a hand-held device for portable operation.*

contrast agents. Researchers found that by modifying the shell's composition, they could fine-tune the physical properties of the MNPs. More than 500 preparations were screened and synthesized, and 50 were tested *in vivo* in mouse models. Not only were they effective, they also are biodegradable, with the iron ending up as hemoglobin. CCNE researchers recently developed a polymer coat for the MNPs that helps to stabilize the surface and provides "handles" for attaching molecules and dyes.

Because early detection is directly linked to more favorable outcomes, investigators also sought to test the efficacy of targeted MNPs for detecting early prostate cancer. Having already successfully developed the first clinically used nanoparticle for nodal cancer staging, they decided to explore attaching peptides for novel cancer targets and image them. They were able to demonstrate that non-invasive imaging correlated with both prostate cancer grade and cell migration capability, providing information that could help to stratify patients and ensure the most optimal treatment (see **Figure 2**).

PROJECT 4: Discrete Sensor Devices for Determining Cancer Targets

Michael Cima, Ph.D.

Diagnosing cancer is typically done through surgical removal of a tissue sample. 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 implant a sensor during the biopsy that can monitor the tumor for weeks or months to track how it is responding to treatment. CCNE researchers designed an implantable sensor over the course of a month that successfully tracked human tumors that had been transplanted into mice. The sensor, which can be picked up on MRI, contained magnetic nanoparticles coated with antibodies specific to the target molecules (in this case, human chorionic gonadotropin, a hormone produced by human tumor cells). These devices could be tailored to monitor drugs to ensure they are reaching the tumors, and could also be designed to measure pH (acidity) or dissolved oxygen levels, which are indicators of tumor status.

The essence of research is that each answer found for a particular question gives rise to new questions. For example, given that this implantable sensor works, how can it be made easier, faster, and more convenient to use? Researchers found the answer is replacing huge immovable MRI machines with a handheld magnetic resonance detector that can interrogate the implanted sensor anytime, anywhere. With the use of inductively coupled sensors, the handheld model enables tumor monitoring even when it is impossible for the patient to physically be in a hospital setting.

PROJECT 5: Novel Semiconducting Nanocrystals for Cancer Detection

Angela Belcher, Ph.D.;
Moungi Bawendi, Ph.D.

Semiconducting nanocrystals (known as "quantum dots") have a range of properties that make them attractive fluorophores for biological imaging; however, these products of inorganic chemistry must be specially configured to make them biologically compatible. CCNE investigators have been collaborating with other institutions in creating design rules to optimize quantum dots for *in vivo* applications. This includes defining size, morphology, charge, and ability to release drug cargos with precision. Applying these parameters, they were able to show that the quantum dots cleared a mouse model's kidneys within a day — this is important because quantum dots are not biodegradable, so they must be small enough to be cleared by the patient's system (a threshold size of approximately 5.5 nanometers was established).

Researchers are also developing multiuse quantum dots that can target, image, and treat tumors at the same time with a single particle. This requires assembling individual capabilities as needed for a given application and experimenting with different coatings to ensure the desired targets are reached, the correct drugs are released at the proper time by the right triggers, and the drugs are able to penetrate the cell membrane. Ten different materials have been tested in mouse models. Additionally, researchers have been able to image the whole mouse, which is important for identifying metastases and which will be essential for diagnosing and treating cancer in humans.

The Road Ahead

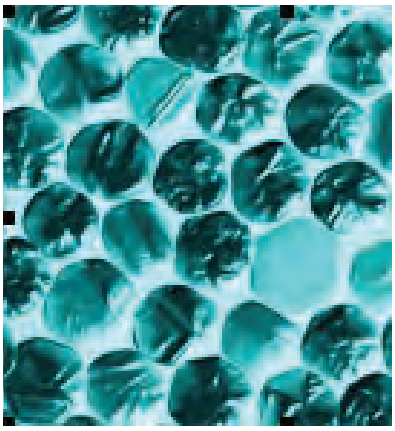
Research is never an end in itself, only a means to an end. Leveraging the collaborations that CCNE researchers have formed and the discoveries they've made to date, their work continues, bolstered by the knowledge that progress is being made — not only in the lab but also on the all-important road that leads from the lab to the marketplace. The ultimate goal, after all, remains to find more effective treatments for human cancer patients and to one day control and curtail the spread of this insidious disease.

NANOMATERIALS FOR CANCER DIAGNOSTICS AND THERAPEUTICS

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

PROGRAM SUMMARY

The Northwestern University Center of Cancer Nanotechnology Excellence (NU-CCNE), under the leadership of Professor Chad A. Mirkin, represents a strongly integrated partnership between the NU Robert H. Lurie Comprehensive Cancer Center (RHLCCC) and the NU International Institute for Nanotechnology (IIN). Established with funding from the National Cancer Institute of the NIH in 2005, the NU-CCNE includes a multidisciplinary group of nano-scientists, cancer biologists, engineers, and clinicians with the primary research goal of designing and testing nanomaterials and nanodevices for their translational application into the clinic. Researchers from the University of Chicago and Yonsei University, South Korea are partnering with the NU-CCNE in this effort. The NU-CCNE research is organized into six multidisciplinary research teams. Project teams include faculty researchers with expertise spanning nanomaterial fabrication and characterization; cancer and cell biology; translational and clinical cancer diagnostics; and detection and therapeutics. In addition to the primary projects, development funds from the university enable the Center to support pilot projects each year in new and emerging areas (11 supported to date). The Center also supports two Cores: the Dissemination and Administration Core and the Nanofabrication Facility Core. Operating primarily within the framework of a single university permits the NU-CCNE to optimize the intensive level of integration and collaboration required to create an accelerated pathway from conception to clinical trial for nanomaterials and nanodevices to overcome cancer. Some of the significant advances achieved over the past five years are described below.



NU-CCNE Project #1 researchers have used nanotechnology to develop highly sensitive diagnostic systems for cancer. The image above shows DNA functionalized gold nanoparticles assembled into a two-dimensional superlattice. Gold nanoparticles have proven very effective as highly sensitive biodiagnostic agents.

PROJECT #1: Development of Barcode Assays for the Detection of Prostate Cancer Recurrence

Chad A. Mirkin, Ph.D. (leader); Anthony Schaeffer, M.D.; Richard P. Van Duyne, Ph.D.; Shad Thaxton, Ph.D., M.D.; Chang Liu, Ph.D.

Project #1 represents a significant collaborative success in translational nanotechnology. After early comparative evaluation of different nanoparticle-enabled biosensor technologies, gold nanoparticle-based PSA bio-barcode immunoassays with over 300 times the sensitivity of conventional tests were used to perform both clinical pilot (n=18 patients) and large retrospective (n=417 patients) studies to assess the potential benefit of enhanced biomolecule detection sensitivity in the context of prostate cancer recurrence. Data demonstrated that increased sensitivity enables: 1) The definition of PSA values consistent with disease cure, 2) A diagnostic lead time of PSA recurrence, and 3) A quantitative assessment of the PSA

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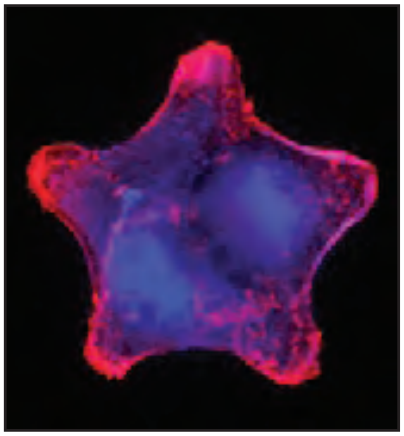

NU-CCNE Project #2 researchers have developed biological assays to understand the ways in which cancer cells migrate from existing tumors to create new, metastatic tumors in different regions of the body. These are used to characterize/quantify cell-based assays for population analysis of metastatic cells and to screen antimetastatic drug candidates.

response to secondary treatment(s). Each of these capabilities may lead to significant benefits with regard to patient outcomes and healthcare cost savings — topics for prospective study. Building upon these successes, it became clear that the unique properties of nucleic acid functionalized nanostructures, similar to those used as extracellular diagnostic probes, directly result in universal cell uptake and deep tissue penetration. Accordingly, significant progress has been made with regard to evaluating nucleic acid functionalized gold nanoparticles as highly potent therapeutic and theranostic agents for cancer.

PROJECT #2: Deconstructing Directional Cell Motility in Metastasis Through Nanopatterning

Milan Mrksich, Ph.D. (University of Chicago, leader); Bartosz A. Grzybowski, Ph.D.; Chad A. Mirkin, Ph.D.; Steven T. Rosen, M.D; Jill Pelling, Ph.D.

Surfaces micropatterned with cell adhesive/non-adhesive regions allow for precise control of 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 opaque, adhesion-resistant background. ASoMic is compatible with several important imaging modalities (e.g., wide-field, digital 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.

Researchers in Project #2 demonstrated that 3D 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. 2) In cells on polygonal islands, the analysis of microtubule growth trajectories establishes a novel mechanism for microtubule targeting to focal adhesions and helps identify new targets for antimotility drugs. 3) 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. 4) Finally, cells on asymmetric patterns ("ratchets") can be made to migrate in desired directions — this property allows for the geometry-based sorting of cancerous and noncancerous cells. Both types of systems — islands and tracks — constitute new types of microassays in which properties of cancerous cells can be studied in quantitative detail and using minimal numbers of cells.

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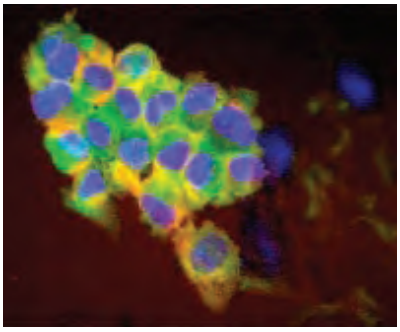

Researchers in the NU-CCNE Project #3 are developing new imaging probes. In the image above, exaggerated levels of TGF-β and NCAM-1 surface receptors are detected in medulloblastoma cells imaged by dual fluorophores. The nuclei of the cells are stained blue (DAPI), TGF-β is red, and NCAM-1 is green, resulting in an orange tint around the cells.

PROJECT #3: Bioactivated Nanoprobes for Molecular Imaging of Cancer

Thomas J. Meade, Ph.D. (leader); Jinwoo Cheon, Ph.D. (Yonsei University, South Korea); Steven T. Rosen, M.D.; Vinayak P. Dravid, Ph.D.

Project #3 researchers are developing new imaging probes that have improved sensitivity and specificity for early stages of cancer. They have developed ultrasensitive MR contrast agents that are based on the establishment of nanoscaling laws for the magnetic nanoparticle in terms of particle size, shape, and composition. Various kinds of multimodal imaging probes based on these magnetic nanoparticles have been realized including: optical imaging probes, targeting moieties, and drug and gene delivery systems. Two classes of magnetic nanostructures (MNS) conjugated separately with antibodies for receptor targets have been utilized for high specificity localization at medulloblastoma pediatric tumor cells *in vitro*. Besides providing high T_2 contrast and fluorescence signal, these MNS demonstrated therapeutic capabilities using thermal activation therapy, establishing a role as promising "theranostic" probes. A new class of dual contrast T_1/T_2 probes containing a cleavable linker for imaging in both T_1- and T_2-weighted MR modes has been created. These agents have the potential to deliver large payloads of Gd(III)-based complexes using a nanoparticle diagnostic platform for improved *in vivo* imaging. The nanoparticle probes developed in this project can be readily extended to various cancer cell lines, providing a strategy for effective early diagnosis and treatment of cancer.

PROJECT #4: Nanoscale Encasement and Targeted Delivery of Multifunctional Therapeutic Agents for Hematological Cancer and Solid Tumors

Thomas V. O'Halloran, Ph.D. (leader); SonBinh T. Nguyen, Ph.D.; Steven T. Rosen, M.D.; George C. Schatz, Ph.D.; Vincent Cryns, M.D.

Project #4 has developed and demonstrated the efficacy of two novel nanoscale drug delivery platforms. Two lead agents are now poised to enter IND-enabling studies (i.e., the final hurdle before human trials). The arsenic trioxide loaded Nanobin is effective in two murine tumor models of human cancer (triple negative breast cancer and mantle cell lymphoma) and shows superior stability in the NCI Nanotechnology Characterization Laboratory (NCL) assays. Pharmacokinetic analysis by NCL reveals that this nanoscale agent works in part by increasing plasma exposure by 300-fold compared to free arsenic trioxide. A second lead Nanobin that is capable of combination chemotherapy with two proven agents, cisplatin and arsenic trioxide, is highly effective at inhibiting triple negative breast cancer growth *in vivo*. These nanoparticulate platforms are safe at the effective dose and furthermore demonstrate efficacy with far less adverse effect on fertility than the parent drug, leading to their designation

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as fertio-protective agents. The group has also developed a second platform, the polymer caged Nanobin (PCN), that is more stable and pH responsive than the parent Nanobin. Platinum and Doxorubicin loaded PCN reveal strong synergy and facile click-chemistry modification with magnetic resonance contrast agents and targeting ligands.

PROJECT #5: TiO_2 Nanocomposites for Targeted Treatment and Imaging of Prostate Cancer

Gayle Woloschak, Ph.D. (leader); Chung Lee, Ph.D.; Raymond Bergan, M.D.; Mark Ratner, Ph.D.; Vincent Cryns, M.D.; Tatjana Paunesku, Ph.D.; Thomas Meade, Ph.D.

Project #5 has developed a series of titanium dioxide nanocomposites (TiNCs) with formulations as simple as pure TiO_2 nanoparticles, to core-shell and core-corona-shell nanocomposites with internal layers suitable for magnetic resonance imaging (MRI), to nanoparticles with surface conjugated MRI contrast agents based gadolinium. Surface conjugation of optically fluorescent dyes was also accomplished allowing a simple approach for nanocomposite monitoring in cells and tissues. Photocatalytic activity of these nanocomposites was tested and found to surpass photocatalytic properties of pure TiO_2 nanoparticles; charge separation and reactive oxygen species produced by photoactivated TiNCs were damaging to *in vitro* and cellular DNA. Lastly, small molecules, nucleic acids and peptides were used for functionalization of nanocomposites — resultant nanoconjugates were used for targeting prostate cancers in a transgenic mouse model, and papilloma virus carrying cells in a rabbit model or liver carcinoma. MRI of these two model animals was performed at time points as late as 24 hours post-injection of nanoconjugates.

PROJECT #6: Multifunctional Nanostructures for Therapeutic Targeting of Breast Cancer

Samuel Stupp, Ph.D. (leader); SonBinh Nguyen, Ph.D.; Karl Scheidt, Ph.D.; William Gradishar, M.D.; Vincent Cryns, M.D.

The key achievements of this project have been the demonstration of peptide amphiphiles (PAs) and amphiphilic block copolymers as novel platforms to assemble nanoscale targeted multifunctional cancer therapies. PAs have been assembled into nanofibers that display bioactive tumor-targeting or cell killing epitopes at the periphery. One specific example of a key success of this platform is KLAK PA, which uses a cytotoxic peptide sequence to kill cancer cells. Unlike other nano-delivery vehicles, KLAK PA does not require the addition of chemotherapy. KLAK PA is membrane-lytic but is less cytotoxic to untransformed cells, indicating a degree of tumor selectivity. In addition, KLAK PA promotes caspase-independent cell death, suggesting that these nanostructures may be effective against breast tumors with apoptotic defects. The therapeutic flexibility of the PA platform is exemplified by the encapsulation of drugs such as camptothecin (CPT) in the hydrophobic nanofiber core. A4G3E3 PA-encapsulated CPT forms nanofibers in aqueous solution and is cytotoxic to cancer cells *in vitro* as well as *in vivo* using an orthotopic xenograft breast cancer model. Amphiphilic block copolymers have also been assembled into polymer nanoparticles (PNPs) with high drug-loading capacities, narrow particle size distributions, and targeting capabilities that allow for the specific delivery of high drug doses to cancer cells. The versatility of these drug-loaded, multimodal PNPs has been demonstrated via conjugation to a variety of targeting-capable bioactive ligands and gold nanoparticles. The degree of surface functionalization can be adjusted at will, allowing for the tuning of targeting ability to correlate to different types of cancer.

CORE #1: Dissemination and Administration

Chad A. Mirkin, Ph.D. (leader); Steven T. Rosen, M.D.; Gayle Woloschak, Ph.D.; Warren Kibbe, Ph.D.; Alfred Rademaker, Ph.D.; Nanjang Hou, Ph.D.; Kathleen Cook, MBA; Matthew Ruchin; Debra Chandler; Anne Ehlinger; Rebecca McNaughton, Ph.D.; Margaret Connolly

The NU-CCNE Dissemination and Administration Core conceives and implements effective community building

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Professor Thomas Meade speaking at the NU-CCNE Nano Boot Camp for Clinicians 2009.

and cross center fertilization; conceives and implements effective educational and outreach programs; develops and implements effective dissemination, technology transfer, and commercialization plans; provides cross-center bioinformatics support; and provides on-going oversight of financial, programmatic, and scientific programs. Some of the achievements over the past five years are listed below.

Community Building & Cross-Center Fertilization — organized monthly Executive Committee teleconferences, quarterly project meetings, annual site visits, and annual review meetings with members of the Technology Council and the Center Steering Committee; extensive involvement with the annual NCI Alliance PI Meeting (hosted 2008 meeting); launched Alliance Calendar project in 2007 (the 2011 calendar is currently in production); served as lead editor for the Alliance Best Practices Operations Manual.

Integrative Training — supported 31 graduate students and 26 postdoctoral fellows actively engaged in Center research; organized Nano Boot Camp for Clinicians in 2009 providing clinicians and medical students with an understanding of nanotechnology, current advances in the field, and the prospects for translating these advances to the clinic (>150 registrants), organized 39 Frontiers in Nanotechnology Seminar Series drawing over 100 attendees each; organized annual symposium (>630 attendees annually) featuring world renowned external speakers; launched summer research experience for undergraduates program in 2007 (28 supported to date).

Technology transfer and Commercialization — launched five new companies commercializing NU-CCNE developed technologies (Nanotope, 2005, S. Stupp, Utilizes self-assembling amphiphilic molecules for drug discovery and novel therapeutic tissue regeneration; PreDx, 2006, T. Meade, creating bioactivatable contrast agents for imaging diagnostics; American Bio-optics, V. Backman, detection of cancerous cells with LED probes; SAMDITech, 2007, M. Mrksich, high-throughput biomarker screening for *in vitro* nanodiagnostics; Aurasense, 2009, C. Mirkin and C. S. Thaxton, developing novel nano-therapeutics). Seven companies with overlapping NU-CCNE research interests are members of the IIN Nanotechnology Corporate Partners (NCP) program (Abbott Laboratories, Agilent Technologies, Applied Biosystems, Arryx, Inc. (subsidiary of Haemonetics), NanoInk, Inc., Nanosphere, Inc., Ohmx Corporation).

Public outreach programs offered by this NU-CCNE Core provide the general public an opportunity to learn about the fundamentals of nanotechnology and to explore the larger questions about the smallest technology. These include

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managing the production of the NCI Alliance Calendar, hosting and organizing free events such as the Nanotechnology Town Hall Meeting series, Science Café, Science Saturdays, and All Scout Nano Day

Metrics to date: Leveraged funding >$67 million, 195 journal articles published, 64 patents filed, 43 CCNE funded seminars/ symposia, 267 presentations given around the world by NU-CCNE researchers.

CORE #2: Nanofabrication Facility

Vinayak P. Dravid, Ph.D. (Co-Director), Teri W. Odom, Ph. D. (Co-Director)

The Nanofabrication Core provides advanced analytical tools, techniques, and expertise for nanoscale characterization of hard and soft materials to all NU-CCNE researchers, which has been vital to the advancement of the research. The analytical and characterization instrumentation, capabilities and expertise in electron microscopy, scanning probe microscopy, and surface science have been extensively used by the NU-CCNE (and others, including interactions with Stanford CCNE) for *every* CCNE project. Several dozen CCNE researchers have benefited from access, advice, and assistance of Core personnel and affiliates for characterizing biological cells, sub-cellular architecture, and nanostructure-cell interactions of relevance to cancer. Also, the Core has been successful at scaling and providing standardized nanoparticle (NP) probes and nanoscale patterned biological surfaces. Core personnel have developed well-characterized and consistent nanopatterned substrates (lipids, protein and DNA arrays) that were used by CCNE researchers to understand cell-pattern interactions, cell growth, migration, as well as sub-cellular architecture (e.g., actin filaments, microtubules) of particular significance for translational cancer research. In one example of platform development, magnetic oxide nanostructures were synthesized and optimized for imaging, diagnostics, and therapeutics of localized cancer. These multifunctional probes are a promising technology for several applications in cancer research and translation, ranging from magnetic cell sorting to high contrast MRI and RF thermal activation.

The Nanofabrication Core provides advanced analytical tools, techniques, and expertise for nanoscale characterization of hard and soft materials to all NU-CCNE researchers, which has been vital to the advancement of the research.

NANOSYSTEMS BIOLOGY CANCER CENTER (NSBCC)

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

The Nanosystems Biology Cancer Center (NSBCC) at California Institute of Technology was created as a collaborative effort among Caltech, the Institute for Systems Biology (ISB), UCLA Geffen School of Medicine, and the Jonsson Comprehensive Cancer Center. Caltech represented the field of chemistry, materials, and physics of nanotechnology science and engineering. ISB brought knowledge in systems biology. Finally, UCLA Geffen School of Medicine and the Jonsson Comprehensive Cancer Center focused on the science, technology, and clinical applications of cancer biology to validate newly developed nanotechnology-based therapeutics, diagnostics, and imaging agents in the clinical setting.

The concept behind the NSBCC was that the measurement and analysis needs of systems approaches to cancer, ranging from cancer biology to clinical oncology, should drive the nature and direction of the advances in nanotechnology science and engineering. The CCNE built a community of cancer scientists/clinicians, technologists, and biologists through the execution of six projects and the associated development of core facilities. The projects were aimed at developing and validating tools for the early detection and stratification of cancer through rapid and quantitative measurements of panels of serum and tissue-based biomarkers. Based upon the rapid evaluation of small tissue and serum samples, another project concentrated on the evaluation of the efficacy of molecular therapeutics for cancer. The CCNE developed new approaches in cancer immunotherapy to detect rare circulating tumor cells. In parallel, the immunotherapy was improved by fabricating the molecular imaging probes through the combination of chemistry and a chip-based technology. The major cancers that were targeted were glioblastoma (GBM), prostate cancer, and ovarian cancer. The CCNE established partnerships with three NCI-funded SPOREs, in addition to several other NCI-funded programs.

The projects conducted in this CCNE, were designed for the development of biomarker panels and technologies that can enable the early detection of cancer (and hence their cures through conventional therapies), the stratification of cancers, the ability to follow cancer progression, the ability to stratify patients as responders or non-responders to therapy, and the ability to monitor *in vivo* cancer biology and therapeutic responses. These projects were also building an oncology community that is receptive to co-developing and taking advantage of state-of-the-art technologies that can provide tools for the fight against cancer.

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The biggest achievements of the last five years of the program is the translation of developed technologies into the clinic, and this is happening in multiple ways with melanoma and glioblastoma (GBM) patients. For example, Dr. James R. Heath (Caltech) has made significant advances in *in vitro* diagnostic assay technology. He has developed synthetic antibodies which are cheaper and more stable than the natural versions, and tested them in the clinic with the Integrated Blood-Barcode Chip (IBBC) which he engineered. The IBBC is capable of multiplexed detection of proteins in whole blood samples. Additionally, while working with Dr. Paul Mischel of UCLA, Dr. Heath used his IBBC for the molecular and functional analysis of glioblastoma tumors, to help identify patients with the greatest potential for positive response to Avastin therapy. This is a step toward personalized cancer care.

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

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

The biggest achievements of the last five years of the program is the translation of developed technologies into the clinic, and this is happening in multiple ways with melanoma and glioblastoma (GBM) patients.

THE SITEMAN CENTER OF CANCER NANOTECHNOLOGY EXCELLENCE

Washington University
Principal Investigator:
Samuel Wickline, M.D.

The Siteman Center of Cancer Nanotechnology Excellence (SCCNE) at Washington University, in collaboration with the University of Illinois at Urbana-Champaign and the Alvin J. Siteman Cancer Center, provides a research and clinical resource in the Midwest for both the fundamental exploration of nanotechnologies applied to cancer and also their translation, commercialization and application in the clinical environment. Over the past 5-years, the SCCNE's major highlights have been:

Development of novel nanoparticle-based agents/methods

- Angiogenesis MR imaging agents
  - Gd-PFC-integrin targeted NP
    - GMP production established
    - Australian clinical trial initiated but on hold to allow 19F trial to procede
  - ^{19}F-NP at 3T
    - FDA approved trial of angiogenesis imaging to be held at Wash U upon completion of IRB approval: anticipated start 6 months
    - Numerous technical 19F pulse sequence and hardware advances (coil designs) reported in collaboration with Philips HealthCare
  - Spectral CT
    - Unique "multicolor" CT imaging hardware combined with targeted nanoparticle agents for K-edge imaging: first in class
    - Demonstration experimental hardware/software completed and resident at Wash U: design paradigm for planned clinical unit established
  - Ultrasound: Novel information theoretic detectors deployed and transferred to other labs
  - Mn and iron oxide colloidal NP for T1weighted reported and patents submitted for MRI
- Angiogenesis ultrasound imaging agents: first in class NP for targeted molecular imaging
  - Integrin targeted NP demonstrated in numerous cancer examples at clinical and lab frequencies
  - New high-sensitivity, real-time information theoretic receivers described, demonstrated and deployed.
- Multiplexed targeted diagnostic and imaging structures for clinical development
  - Cytotoxic peptides (melittin)—in preclinical testing at NCL and Kereos
    - Targeting/imaging demonstrated *in vivo* with numerous tumor types
    - Agent accepted by NCL for preclinical development
  - Melittin peptide mutants as therapeutics and universal linkers
    - Fusion peptides for therapy demonstrated *in vitro*
    - Cell labeling and tracking demonstrated *in vitro*
  - siRNA delivery demonstrated *in vitro*
    - Unique lipid raft delivery mechanism directed to cytoplasmic compartment

Movement of EXX nanoarrays into commercial sector

- Semiconductor nanoarrays for molecular diagnostics and *in vivo* sensing
  - Major technical roadblocks eliminated
  - High content/high throughput screening focus
    - 1st product: new "E-field "sensor: first of kind for cancer diagnostics, excitable cells, etc.
  - New startup formed (PixelEXX) with funding from:
    - Biogenerator (STL) seed funding secured
    - Series A syndicate being sponsored by ArchVentures (Chicago) and series A term sheet signed

NanoOntology and NanoPK

- Modules transferred to either public domain or to caNano via project 4
  - Options for future work with FDA in process

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Cancer Nanotechnology Platform Partnerships (CNPPs)

The CNPP programs engage in directed, product-focused research that aims to translate cutting-edge science and technology into the next generation of diagnostic and therapeutic tools. These platforms serve as the core technologies for a wide array of specific applications that will ultimately benefit cancer patients.

The 12 CNPPs that receive funding apply strong interdisciplinary technical and oncology expertise to six key platform categories in nanotechnology:

- *Molecular imaging and early detection*
- *In vivo nanotechnology imaging systems*
- *Reporters of efficacy*
- *Multifunctional therapeutics*
- *Prevention and control*
- *Research enablers*

Project Summary

DNA-LINKED DENDRIMER NANOPARTICLE SYSTEMS FOR CANCER DIAGNOSIS AND TREATMENT

University of Michigan
Principal Investigator: James Baker Jr., M.D.

Names of each faculty investigator and senior participant and their discipline/department:

James R. Baker Jr., MD, Principal Investigator, Professor of Internal Medicine, Director, ***M****NIMBS*

Istvan Majoros, PhD, Research Assistant Professor of Internal Medicine, ***M****NIMBS*

Yuehua Zhang, PhD, Research Assistant Professor of Internal Medicine, ***M****NIMBS*

Seok-Ki Choi, PhD, Research Assistant Professor of Internal Medicine, ***M****NIMBS*

Thommey P. Thomas, PhD, Research Assistant Professor of Internal Medicine, ***M****NIMBS*

Jolanta Kukowska-Latallo, PhD, Research Investigator of Internal Medicine, ***M****NIMBS*

Mark M. Banaszak Holl, PhD, Professor of Chemistry and Macromolecular Science and Engineering, ***M****NIMBS*

Bradford G. Orr, PhD, Professor of Physics, Associate Director, ***M****NIMBS*

Theodore Norris, Ph.D., Professor of Electrical Engineering & Computer Science, ***M****NIMBS*

Douglas G. Mullen, Graduate Student Macromolecular Science and Engineering, ***M****NIMBS*

Daniel Q. McNerny, Graduate Student Chemical Engineering, ***M****NIMBS*

Yunbo Guo, Graduate Student Electrical Engineering & Computer Science, ***M****NIMBS*

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Development of technique to separate dendrimers functionalized with a defined number of ligands. Significant progress has been made towards precisely controlled material using gold nanoparticles. A number of strategies exist in the literature to synthesize and/or isolate milligram quantities of gold nanoparticles with a single functional group and up to 95% purity has been achieved. Gold nanoparticles with 0-5 conjugated ligands have also been isolated in sub milligram quantities using either gel electrophoresis or ion exchange chromatography. This level of control has not been attained for other nanoparticle-based systems. In fact, with few exceptions, the analytical techniques commonly used to characterize these systems (NMR, HPLC, GPC, MALDI-TOF, UV-Vis) have been unable to identify the distribution of nanoparticle-ligand components. Under this project, we have developed the ability to resolve the distribution of components using HPLC for poly(amidoamine) (PAMAM) dendrimer samples conjugated with 3-(4-(prop-2-ynyloxy)phenyl)propanoic acid (Alkyne Ligand). The results of these studies revealed that dendrimer-ligand distributions are heterogeneous (a sample with a ligand mean of 5.7 was composed of 18 dendrimer-ligand components), are poorly represented by the arithmetic mean, and are sensitive to pre-existing distributions of conjugation sites on the parent dendrimer. After resolving the dendrimer-ligand distributions, we employed semi-preparative HPLC to successfully isolate 9 different dendrimer components with precise numbers of ligands. Generation 5 (G5) PAMAM dendrimer was conjugated with (3-(4-(2-azidoethoxy)phenyl)propanoic acid) (Azide Ligand) to produce dendrimer with a mean of 4.3 ligands. Dendrimer samples with 0, 1, 2, 3, 4, 5, 6, 7 and 8 Azide Ligands were isolated from the dendrimer conjugate 3 and characterized by 1H NMR and analytical HPLC. Levels of purity for these samples were found to be greater than 80%. This approach shows great promise to overcome batch reproducibility challenges in dendrimer-based systems, and has enabled us to separate constructs with a defined number of ligands.

Development of targeted gadolinium-loaded dendrimer nanoparticles for tumor-specific magnetic resonance contrast enhancement. We synthesized and tested a target-specific MRI contrast agent for *in vivo* imaging of xenograft tumors. To do this, we G5 PAMAM dendrimers were functionalized with FA a bifunctional NCS-DOTA chelator that forms complexes with gadolinium (Gd III). After the addition of GdCl3, the targeted gadolinium-loaded dendrimer nanoparticles Gd(III)-DOTA-G5-FA were injected into immunodeficient (SCID) mice bearing xenograft tumors expressing folate receptor (FAR). The 3D MRI results showed specific and statistically significant signal enhancement in tumors generated with targeted Gd(III)-DOTA-F5-FA compared with signal generated by non-targeted G5(III)-DOTA-G5 (**Figure 1 – page 41**). Additional targeted imaging conjugates are being synthesized by Clicking together dendrimers functionalized with DOTA and a variety of targeting ligands.

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Development of an ultrahigh-sensitivity optical biosensing platform for label-free biomolecular detections. We have successfully developed a novel optical biosensor for real-time, label-free biomolecular detections. Its ultrahigh sensitivity was characterized with bulk solvent refrative index change, and we also have demonstrated its capacity for small molecule detection with biotin-streptavidin parters. The binding of the smallest molecule D-Biotin, with a molecular weight of 244 Da, was experimentally observed at a higher signal-to-noise ratio than what was obtained using the state-of-the-art surface plasmon resonance (SPR) based system. Based on our optical biosensor prototype, we constructed a second instrument that utilizes a more advanced fluidics system. This instrument is currently being calibrated and will be used to measure the binding of a targeted Click conjugate (G5-FA Clicked to G5-MTX), and screening for potential new small-molecule targets.

Click-chemistry was successfully employed to generate a two-function dendrimer-based imaging agent for use in a large-scale animal study.

Synthesis of binary dendrimers (FA and 6TAMRA) using Click chemistry

The two-function imaging agent was sythesized by coupling together two dendrimer-based modules. The first module was composed of a folic acid conjugated dendrimer. 39.2 mg of this module was generated. In addition to the folic acid molecules conjugated to the dendrimer, this dendrimer module also posessed a linker molecule with a terminal alkyne. The second module was a dendrimer with 6TAMRA. 26.3 mg of the 6TAMRA conjugated dendrimer was generated. This dendrimer also posessed a linker molecule with a terminal azide. The two modules were coupled via the 1,3-dipolar cycloaddition reaction ('click' chemistry) between the alkyne moiety on the first dendrimer and the azide moiety on the second dendrimer. 24.6 mg of this compound was generated.

The FA-6TAMRA dendrimer-based modular platform was evaluated *in vitro* with a human epithelial cancer cell line (KB) and found to specifically target the over-expressed folic acid receptor. This material is currently being used for an animal study.

Isolation of dendrimer with exact numbers of functional groups

Methods that are commonly used to functionalize dendrimer with targeting, imaging, and theraputic molecules result in a distribution of dendrimer species, each with a different number of conjugated groups. This population distribution is, in-fact, common for many different types of nanoparticles that are being developed for theraputic use. Recently, using semi-prep HPLC, a dendrimer conjugate with an average of 0.45 azide linker molecules was separated and isolated into the individual dendrimer species that give rise to the average number for the material. ^{1}H NMR was then used to confirm the number of linker molecules per dendrimer for each of the isolated species. This is the first time that dendrimer with exact numbers of functional groups have been isolated and dramatically

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enhances our ability to control the synthesis of functional dendrimer platforms. Because the isolated dendrimer samples have exact numbers of 'click' functional groups (alkyne or azide), a number of different targeting, drug, dye, dendrimers, and dendrons can be conjugated to the isolated dendrimer. This development has the ability to bring substantial increases to the biological activity of the dendrimer platform due to the material purity that can now be achieved.

Synthesis of bifunctional dendrons (RGD and Methotrexate) using Click chemistry

The targeted dendron, alkyne-G3(COOH)(RGD), was used as a scaffold for the attachment of an additional functional moiety (**Figure 1**). The functionalized PAMAM dendrons maintain the binding specificity and multivalency of previous dendritic models while creating an orthogonally coupled scaffold more suitable for personalized medicine and applications.

Binary devices were synthesized through a unique alkyne group at the dendron focal point. The alkyne is reacted with an azide-functionalized dye, biotin, therapeutic molecule or a second dye-conjugated dendron in a copper-catalyzed 1,3-dipolar cycloaddition. This marks the first time two functionalized PAMAM dendrons have been coupled together. Extensive *in vitro* and *in vivo* testing was performed for the targeted therapeutic for uptake, efficacy and toxicity. (**Figure 2 – page 42**).

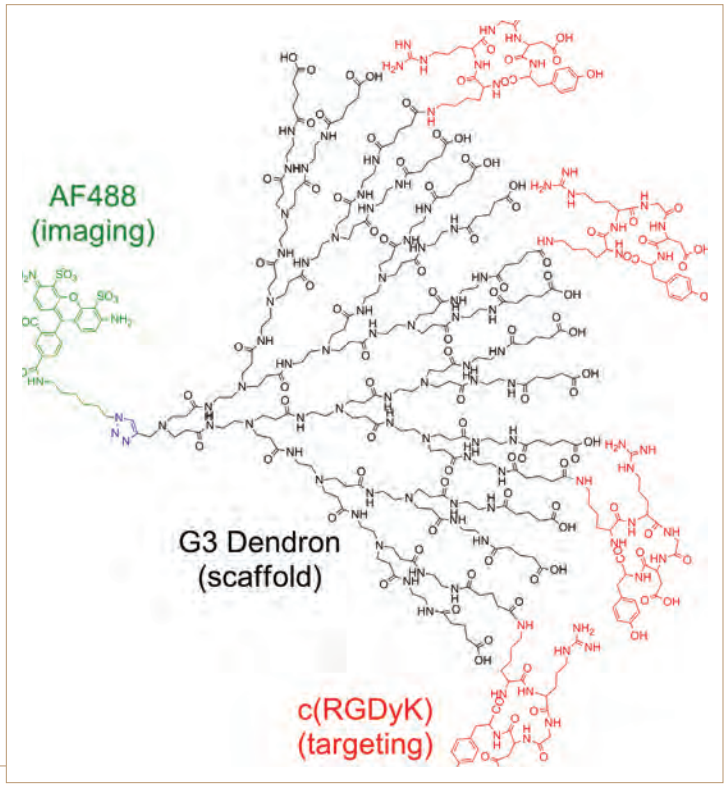

FIGURE 1. *Dendron conjugate AF-G3(COOH)11.9(RGD)4.1* ***(4)*** *where Alexa Fluor 488 is conjugated to the dendron focal point via a 1,3-dipolar cycloaddition. The targeting moiety, c(RGDyK) peptide, isconjugated to the carboxylated surface of the dendron.*

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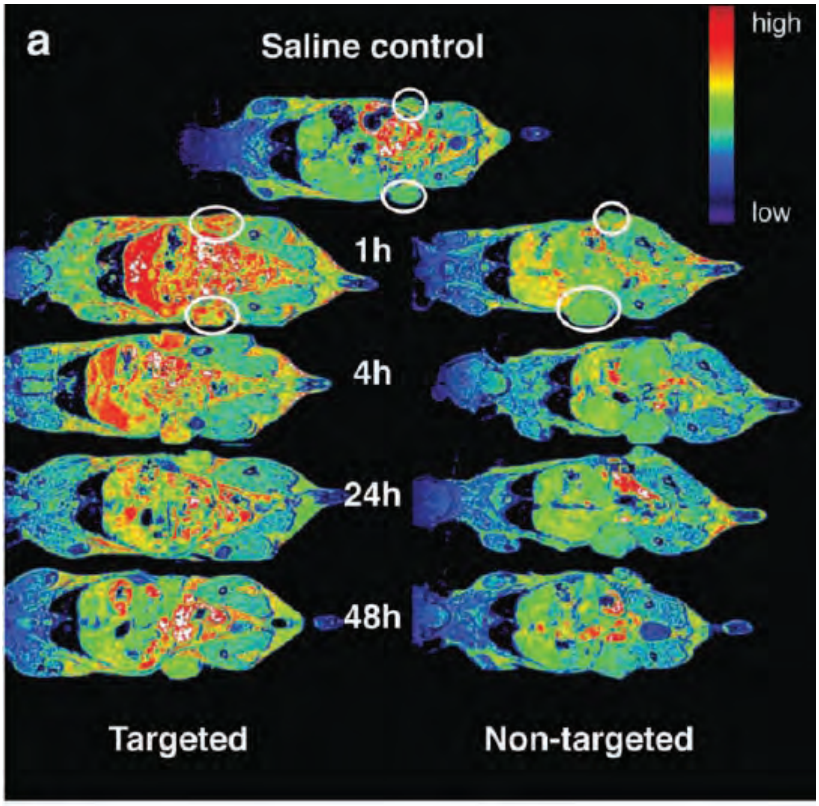

FIGURE 2. *MR imaged uptake of targeted and non-targeted contrast nanoparticles delivered at 0.029 mmol/kg of Gd. The top image is of a control mouse injected with saline and imaged at 1hour* ***(a)****. The series of images on the left from the mouse injected with targeted contrast Gd(III) DOTA-G5-FA and on the right from the mouse injected with the non-targeted contrast agent Gd(III)-DOTA-G5 were collected at 1, 4, 24, and 48 hours post-injection* ***(a)****. The circles are drawn around the tumor to guide the eye. Signal enhancement is created by increased water proton relaxation due to the presence of the contrast agent. Normalized histograms of the voxel intensities were created for the whole tumors on both flanks of mouse, for the two mice injected with targeted and non-targeted contrast presented on the series of images* ***(a)****. Comparison of targeted* ***(b)*** *and non-targeted contrast* ***(c)*** *showed statistically significant difference (KS test, $p < 0.05$) between the signals collected at 1, 4, and 24 hours.*

METALLOFULLERENE NANOPLATFORM FOR IMAGING AND TREATING INFILTRATIVE TUMOR

Virginia Commonwealth University
Principal Investigator:
Panos Fatouros, Ph.D.

Work performed over the last five years focused on the synthesis, characterization, functionalization and *in vivo* application of a metallofullerene nanoplatform, $Gd_3N@C_{80}$, in a mouse brain tumor model of glioblastoma. The following key steps were accomplished:

1. We successfully produced sufficient quantities of $Gd_3N@C_{80}$ nanoparticles via the Kratschmer-Huffman generator. We have developed several functionalization protocols to optimize magnetic resonance imaging (MRI) relaxivity. We have also demonstrated extremely high ^1H MR relaxivities of water-soluble PEG functionalized and hydroxylated derivatives $Gd_3N@C_{80}$[DiPEG(OH_x] at different molecular weights (350-5000 Da) and at different magnetic field strengths, 0.35, 2.4 and 9.4 T. The measured relaxivities represent some of the highest values reported for commercial or investigational MRI contrast agents. Similar results were obtained with hydroxylated and carboxylated gadofullerenes. Luna Innovations has licensed this technology from Virginia Tech and they have submitted related $Gd_3N@C_{80}$ nanoparticles for evaluation at the Nanotechnology Characterization Laboratory (NRL). During the last year, we have worked closely with Luna personnel (exchange of data and samples) to help bring these exciting new diagnostic agents to commercial fruition.

2. We have successfully incorporated the β-emitter ^{177}Lu into the endohedral metallofullerene $^{177}Lu_3N@C_{80}$ cage using a modified Kratschmer-Huffman apparatus that allows remote preparation and extraction. This achievement makes possible the synthesis of multimodal nanoprobes for use as diagnostic (with encapsulated Gd) and therapeutic agents (with encapsulated ^{177}Lu) for a variety of applications.

3. We successfully conjugated the carboxylated endohedral gadolinium and radiolabelled fullerenes with a tumor specific IL-13 peptide that binds specifically to the IL-13Rα2 receptor which is over-expressed in glioblastomas.

4. There is an increase in glioma cell uptake of the IL-13 targeted carboxylated endohedral gadolinium fullerenes *in vitro* when compared to non-targeted carboxylated endohedral gadolinium fullerenes.

5. We have shown increased tumor retention with the targeting vs. the non-targeted nanoparticles in our *in vivo* mouse model and some normal tissue accumulation, primarily in liver, kidney and spleen.

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6. Through state of the art theoretical investigations, we showed that the $Gd_3N@C_{80}$ has a spin magnetic moment of 21 bohr magneton. Further, the Gd_3N has a distorted tetrahedral configuration where the barrier for N to move across the plane of Gd atoms is low. This should allow local heating of the cage through low frequency radiation allowing treatment of the cancer via hyperthermia. We also illustrated the role of OH groups attached to the surface of the cage. In particular, for odd number of OH groups, one finds a spin density on the cage which might affect the relaxation of the proton. (**Figure 3**)

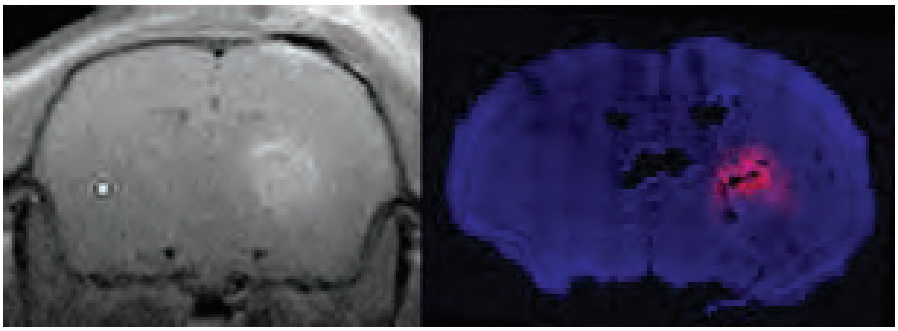

FIGURE 3: *(left) T1 weighted MR image obtained 1 day post intratumoral infusion by convection enhanced delivery of TAMRA-IL13 peptide targeted $Gd_3N@C_{80}$. The bright contrast due to the Gd clearly indicates the distribution and location of the infused nanoplatform. (right) Confocal image of a 50 μm section taken through the tumor 1 day post infusion. The fluorescent signal from the TAMRA label (pink) is similar in size and distribution to that shown on the MR image, suggesting that the peptide-fullerene conjugate remains intact to this time point.*

MULTIFUNCTIONAL NANOPARTICLES IN DIAGNOSIS AND THERAPY OF PANCREATIC CANCER

State University of New York
Principal Investigator: Paras Prasad, Ph.D.

This CNPP grant from SUNY Buffalo (PI: Paras Prasad) has focused on the development of two types of fluorescent nanoparticles, namely quantum dots and dye-doped ORMOSIL nanoparticles, as multifunctional probes for the diagnosis and therapy of pancreatic cancer. During the course of this grant, we have used a number of quantum dots and quantum rods, which include Cadmium based, Indium based, and most recently Silicon based ones. We have also developed various formulations of dye and QD-doped ORMOSIL nanoparticles, along with formulations of metallic nanoparticles for surface plasmon resonance (SPR) imaging, and zinc oxide nanocrystals for non-linear optical imaging. The following is a summary of the major highlights of this grant:

1. **Multiplexed labeling of cancer cells using quantum rods (QRs):** We have demonstrated the use of CdSe/CdS/ZnS QRs, emitting at two different (orange and red) colors, for multiplex labeling of human pancreatic cancer cells. This was carried out by conjugating QRs with the monoclonal antibodies anti-claudin-4 and anti-mesothelin, which are known to be overexpressed in this cancer. As a control, we have conjugated the non-pancreatic cancer specific antibody IgG with the QRs. Using confocal microscopy, we have shown in a multiplexed manner the preferential cellular uptake of the multicolored QRs conjugated with the specific antibodies, over that of the QRs conjugated with the non-specific antibody IgG (Yong et al. *Advanced Materials*. 2008, March 20, 1-6).

2. **Multimodal nanoparticles:** We have developed a multifunctional nanoprobe by co-encapsulating quantum dots (QDs) and magnetite (Fe3O4) nanoparticles within an ORMOSIL shell. The resulting nanoparticles exhibited both optical and magnetic properties. Upon placing a bar magnet at one side of the vial containing these nanoparticles, it can be clearly seen that the nanoparticles were drawn to the 'magnet side' of the vial, indicating that both the QDs and the magnetite nanoparticles were successfully co-localized within the ORMOSIL matrix. Further, these nanoparticles could be targeted to cancer cells *in vitro* using magnetic guidance (Law et al. *Journal of Physical Chemistry C*. 2008, 112(21) 7972-7977).

3. **Targeted delivery to pancreatic cancer using RGD conjugated NIR emitting QDs:** We have prepared near-infrared (NIR) emitting alloyed CdTeSe/CdS QDs as efficient optical probes for high contrast *in vivo* imaging of tumors. For tumor-specific delivery *in vivo*, the micelle-encapsulated QDs were conjugated with the cyclic arginine-glycine-aspartic acid (cRGD) peptide, which targets the αvβ3 integrins overexpressed in the endothelial cells during tumor angiogenesis. Using *in*

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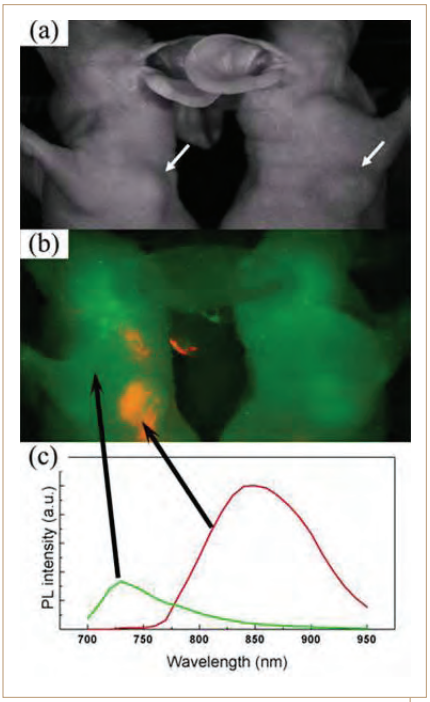

*In vivo transmission **(a)** and the corresponding luminescence **(b)** images of tumor-bearing mice, intravenously injected with either RGD-conjugated (left) or unconjugated (right) NIR QDs, with white arrows indicating the tumors. The localized spectra of the signals from the background (coded in green) and NIR QDs (coded in red) are indicated by arrows **(c)**.*

vivo NIR optical imaging of mice bearing pancreatic cancer xenografts, we have demonstrated that systemically-delivered cRGD conjugated QDs, but not the non-bioconjugated QDs, can efficiently target and label the tumors with a high signal-to-noise ratio (Yong et al. *Small*. 2009, 5(17), 1997-2004).

4. **Heavy metal free silicon QDs for targeted delivery to pancreatic cancer sites:** We have synthesized highly luminescent and stable silicon QDs using a unique methodology combining 'bottom-up' and 'top-down' approaches, which can be successfully dispersed in aqueous systems without compromising their optical properties. These heavy metal free QDs could be targeted to pancreatic cancer cells and tissues, both *in vitro* and *in vivo*, following bioconjugation with the RGD peptide. We did not observe any ill effects of these QDs on systemically treated animals, using both physical monitoring and histopathological analyses. These observations underscore the development of new generation of highly luminescent and heavy metal free QDs as targeted nanoprobes for cancer diagnosis and therapy (Erogbogbo et al. *ACS Nano*. 2008, 2(5), 873-876.

5. **Zinc oxide nanocrystals for nonlinear optical bioimaging:** We showed that biocompatible zinc oxide (ZnO) nanocrystals (NCs) having a noncentrosymmetric structure can be used as nonlinear optical probes for bioimaging applications *in vitro*. Sum frequency, second harmonic, and nonresonant four wave mixing nonlinear signals from this stable dispersion of ZnO NCs, targeted to the live tumor (KB) cells, were used for imaging. Robust intracellular accumulation of the folic acid targeted ZnO nanocrystals could be observed without any indication of cytotoxicity (Kachynski et al. *Journal of Physical Chemistry C*. 2008, 112 (29) 10721-10724).

NANOTECHNOLOGY PLATFORM FOR PEDIATRIC BRAIN CANCER IMAGING AND THERAPY

University of Washington
Principal Investigator: Miqin Zhang, Ph.D.

Miqin Zhang, Principle Investigator, Dept of Materials Science and Engineering, University of Washington

Richard Ellenbogen, Investigator, Neurological Surgery, UW, Harborview Medical Center

Raymond Sze, Investigator, Clinical Radiology, Children's National Medical Center (CNMC)

Donghoon Lee, Investigator, MR Imaging Physics, Dept of Radiology, UW

The long-term objective of this project is to help eliminate the suffering and death of children with brain cancer. Brain cancer is one of the the most deadly and intractable deseases. Treating malignant brain tumors has not shown much progress due to the difficulty in differentiating between tumor and healthy brain tissue, the sensitivity of normal brain tissue to current therapies, and the blood brain barrier's ability to prevent the passage of medicinal substances including drugs and contrast agents. In this project, we have developed a multifunctional nanoparticle platform serving as both imaging contrast agents and drug carriers that can pass biological barriers for non-invasive diagnosis, staging, treatment, and treatment response monitoring of brain tumors. The core components of this platform comprise a superparamagnetic iron oxide nanoparticle and a PEG polymer coating which is subsequently functionalized with tumor targeting chlorotoxin (CTX) and a near infrared fluorescing molecule (NIRF). 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 to normal brain tissue. Unlike other ligands which 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 not only provides a linkage between nanoparticles and functional coatings (targeting ligands, therapeutic molecules), but also prevents nanoparticles from aggregation, increases nanoparticle blood circulation time and biostability, improves cellular penetration of the nanoparticles, and assists nanoparticles to cross the blood brain barrier (BBB). Use of NIRF molecules minimizes autofluorescence interference from healthy brain tissue and allows visualization of tissues millimeters in depth due to the efficient penetration of photons in the near infrared range. Our results showed significantly preferential uptake of the nanoparticles by glioma cells over control nanoparticles. The specific targeting has been successfully demonstrated by comparing the uptake of the nanoparticle conjugates by glioma and by control cells without MMP-2 receptors.

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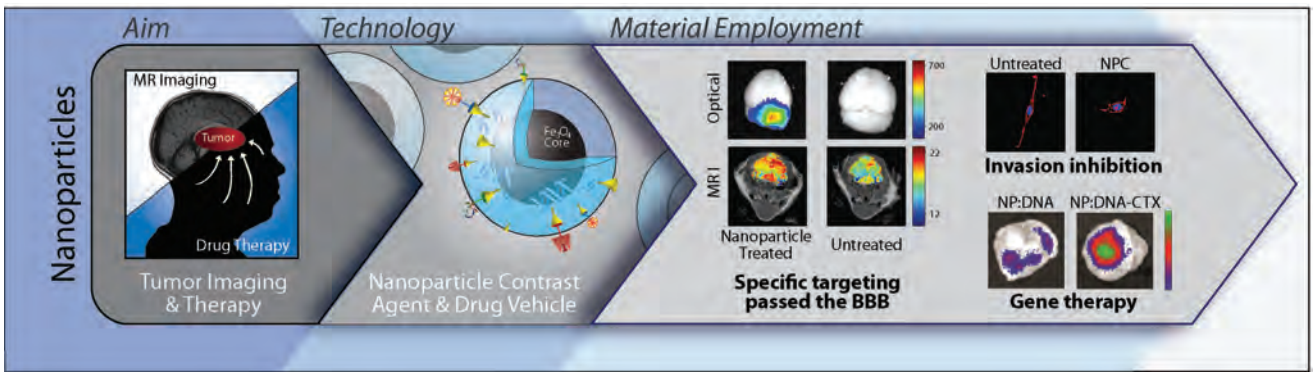
Our *in vivo* results demonstrated that the nanoparticle conjugates passed the BBB and bound to genetically engineered intracranial medulloblastoma in mice that bear an intact blood brain barrier. This multifunctional nanoparticle system can be used to detect tumors for diagnosis by MRI, illuminate and 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. On this nanoparticle platform, we have developed various nanoparticle systems carrying different target ligands and therapeutic payloads for tumor imaging and treatment, including T cell, lymphoma, and liver cancer imaging, and chemo-, gene-, immuno- therapies. Based on the knowledge and techniques developed in this project, we have published ~25 peer-reviewed journal articles . Our research has been highlighted in The Wall Street Journal, National Geographic News, MIT Technology Review, Nature Nanotechnology, NCI Alliance for Nanotechnology, King5 news, the Seattle Post-Intelligencer, NanoBioNews, ScienceNews, and various commercial news media.

Based on the insights and technology developed from this project, we have substantially expanded our research in nanotechnology-based brain tumor therapy with funding secured from various sources, including grants from NIBIB, NCI, GMS, and funding support from the Seattle Children's Hospital and Regional Medical Center, HQ foundation, and other private donors. In addition, with the knowledge and skills gained from this project, we were able to secure a T32 training grant aimed at training next generation of scientists and physicians with expertise in interdisciplinary areas of nanotechnology, physical sciences, and oncology.

Zhang's Nanoplatform team at University of Washington has developed iron oxide nanoparticle systems that can cross the blood brain barrier, illuminate brain tumors through both MR and fluorescence imaging, and deliver therapeutics specifically to tumors. The nanoparticles are biocompatible, biodegradable, have excellent colloidal stability, and long blood circulation time.



NANOTHERAPEUTIC STRATEGY FOR MULTIDRUG RESISTANT TUMORS

Northeastern University in collaboration with Mass General Hospital
Principal Investigator:
Mansoor Amiji, Ph.D.

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.

We have a longstanding interest in targeting *de novo* ceramide production and metabolism. Studies in the laboratory have focused on the generation of different nanoparticles and combination with ceramide to overcome MDR in cancer. In one of our studies, ceramide was combined with the chemotherapeutic drug paclitaxel and delivered to ovarian cancer MDR cells. Poly(ethylene oxide)-modified poly(epsilon-caprolactone) (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 (**Figure 1**). 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 IC50 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 due to 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. We evaluated whether co-administration with

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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. A polymer-blend nanoparticle system was engineered to incorporate temporally controlled sequential release of the combination drug payload. The paclitaxel was encapsulated in the pH-responsive rapid releasing polymer, poly(beta-amino ester) (PbAE), while ceramide was present in the 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 due to a longer retention time and an enhanced tumor accumulation relative to the administration of free drugs. 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. This combination therapy circumvents another cellular mechanism whereby MDR develops by lowering the threshold for apoptotic signaling. *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 over a period of treatment with the 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. In conclusion, these results suggest nanotechnology based targeting ceramide metabolic and cell death signaling pathways is an attractive approach to overcoming MDR.

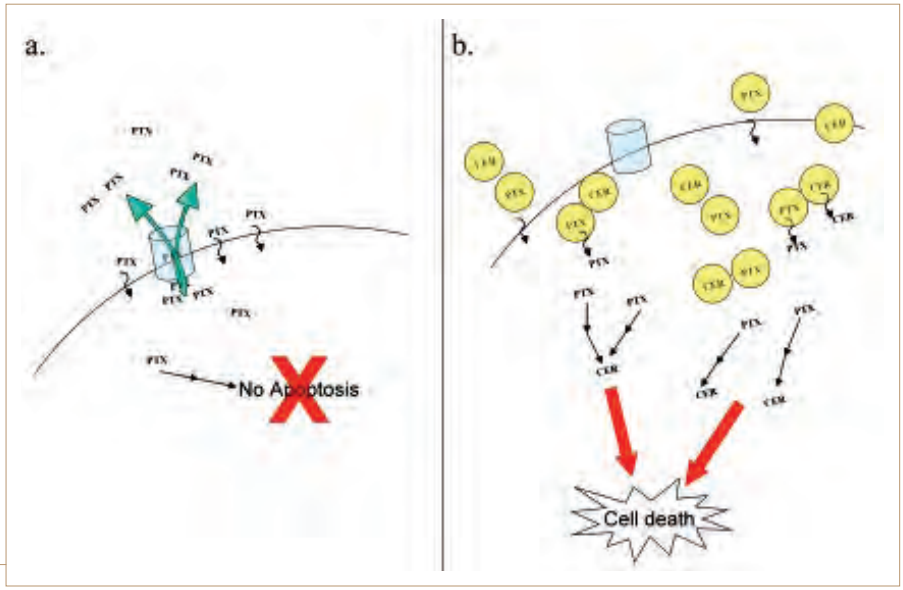

FIGURE 1: *Schematic illustration for the mechanism whereby multifunctional nanoparticle therapy functions in multidrug resistant 9MDR) tumors.* ***a)*** *In the MDR cancer cell, chemotherapeutic drugs (e.g. paclitax el, PTX) that diffuse into the cells are readily effluxed out by the drug efflux transporters (e.g. P-glycoprotein), resulting in lower intracellular concentrations of the drugs. The fraction of drug does that remains in the cell initiates cell death by activating the apoptotic cascade. However, the absence of ceramide (CER) causes the apoptotic cascade reaction to never reach completion and the tumor or cells continue to remain alive.* ***b)*** *Nanoparticle carrying PTX and CER are internalized by endocytosis into the cells, thus bypassing the membrane-bound efflux transporters, and release the content inside the cells. Once released, PTX again initiates cell death by activating apoptotic cascade reaction and, in the presence of CER, this process does go to completion resulting in cell death.*

NEAR-INFRARED FLUORESCENCE NANOPARTICLES FOR TARGETED OPTICAL IMAGING

The University of Texas M. D. Anderson Cancer Center
Principal Investigator: Chun Li, Ph.D.

During the entire granting period, we have been focused on two major areas. The first focus is to address the fundamental question as to how we can achieve efficient delivery of nanoparticles to solid tumors after systemic administration. The second focus is to develop 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 of less than 100 nm, including polymeric, semiconductor, and gold nanoparticles, we have arrived at the following conclusions. First, most nanoparticles coated with polyethylene glycol (PEG) displayed prolonged blood half-life. However, blood half-life is also a function of the particle size: smaller particles having thicker PEG coating display longer blood circulation time. Second, in general, nanoparticles with longer blood resident times have higher uptake in solid tumors. Moreover, other factors such as compressibility of nanoparticles can also influence extravasation of nanoparticles from tumor blood vessels into the extravascular space. Third, active targeting with significantly improved tumor deposition can be achieved using nanoparticles coated with homing ligands as compare to non-targeted nanoparticles. We have found that the nature of the homing ligand (i.e. size, binding affinity, ability to internalize by cancer cells) can have profound effect on the efficiency of targeted nanoparticle delivery. In the second area of our research, we have developed and evaluated nanoparticle platforms for dual near-infrared fluorescence optical/magnetic resonance imaging, and near-infrared fluorescence optical/nuclear imaging. Most significantly, we have demonstrated the feasibility and advantage of using nanoparticles integrating both diagnostic capability (nuclear imaging, photoacoustic imaging, and magnetic resonance imaging) and therapeutic capability (drug delivery, photothermal therapy). For example, we have used photoacoustic tomography to guide photothermal ablation therapy using targeted hollow gold nanospheres. We have also developed chelator-free positron emitter ^{64}Culabeled semiconductor CuS nanoparticles that can be simultaneous used for positron emission tomography, radiotherapy, and photothermal therapy. These novel nanoparticles will certainly be the focus of the next phase of our studies as we advance our laboratory findings into the clinical trial studies.

Example of Success Stories:

1. Previously, we have found that while using antibody as the homing ligand resulted in only moderate increase in tumor uptake of antibody-conjugated hollow gold nanospheres (HAuNS), using much small peptide as the homing ligand led to remarkably increased tumor uptake of peptide-bound HAuNS. This is exemplified in our studies of

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melanoma-targeted HAuNS for potential selective photothermal ablation in melanoma (*Lu et al, Clin Cancer Res., 15: 876-886, 2009*). Currently, we have successfully scaled-up the production of HAuNS and arranged with Nanotechnology Characterization Laboratory for toxicity studies of these targeted HAuNS. We are collaborating with Nanospectra Biosciences, Inc. as we advancing HAuNS as a novel class of gold nanostructure for targeted, near-infrared light-mediated theranostic applications.

2. We validated "photothermal transfection" strategy that enables selective cytoplasmic delivery of small siRNA through photothermal effect mediated by HAuNS upon NIR light irradiation (**Figure 1**). Folate-conjugated HAuNS carrying siRNA recognizing NF-κB p65 subunit exhibited significantly higher tumor uptake than non-targeted nanoconstructs in HeLa tumors expressing folate receptors (**Fig. 1A**). Efficient down-regulation of NF-κB p65 was achieved only in tumors irradiated with NIR light, but not in non-irradiated tumors grown in the same mice (**Fig. 1B**). Combined treatments with NF-κB p65 siRNA and irinotecan caused substantially enhanced apoptosis *in vivo* only upon NIR irradiation (data not shown). Moreover, efficient RNAi was achieved at a relatively low laser dose that did not cause ablation of tumor cells (**Fig. 1C**). This can have significant implication for clinical application of PTA therapy in that tumor cells with sub-optimal laser exposure may be killed by therapeutic RNAi during PTA therapy (*Lu et al., Cancer Res., 70: 3177-3188, 2010*).

3. We have developed a novel fabrication method for encapsulation of doxorubicin (DOX) in the interior of HAuNS (DOX@HAuNS, **Figure 2**). Exceptionally high payload of DOX (>60% by weight) could be loaded into HAuNS. Importantly, we found that the release of DOX from DOX@HAuNS could be triggered by NIR laser light (**Fig. 2B**) (You et al., accepted).

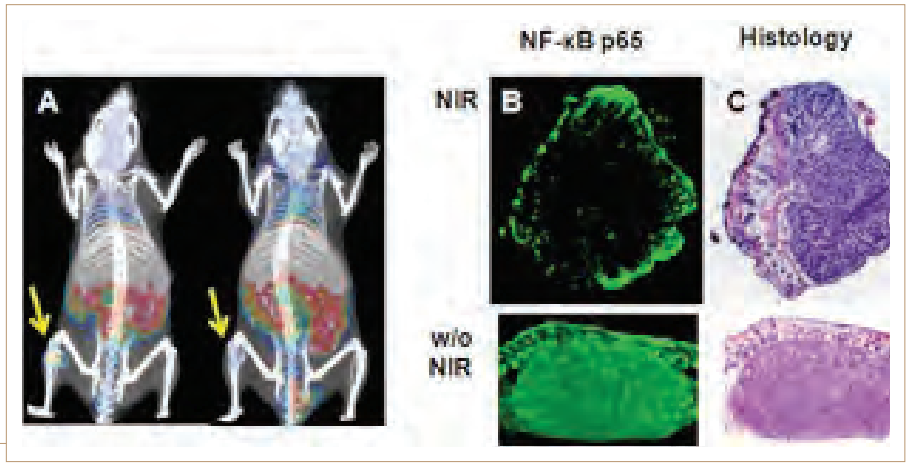

FIGURE 1: ***(A)*** *μPET/CT images of mice bearing s.c. HeLa cells expressing folate receptors after injection of ^{64}Cu-labeled folate-HAuNS-siRNA nanoconstructs (left) and HAuNS-siRNA as a control (right). Arrows: tumors.* ***(B)*** *NF-κB p65 expression in tumors exposed to NIR laser (top) and without laser irradiation (bottom).* ***(C)*** *H&E stained tumors from (B).*

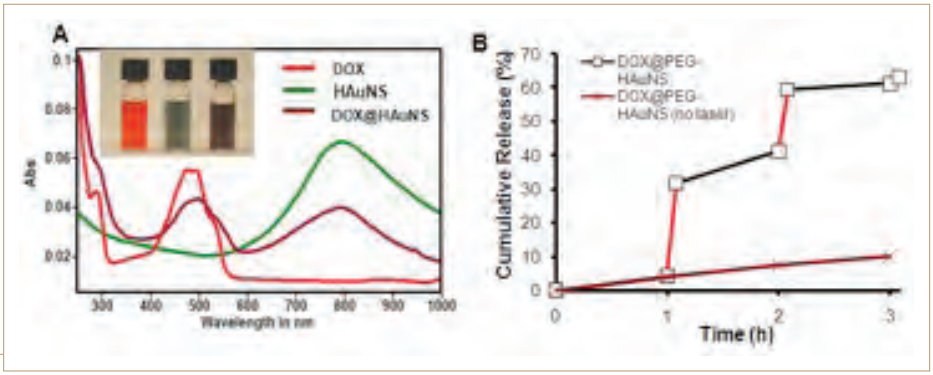

FIGURE 2: ***(A)*** *Photographs and absorption spectra of aqueous solutions of DOX, HAuNS, and DOX@HAuNS. The green color of HAuNS was turned to dark red owing to exceptionally high DOX payload.* ***(B)*** *NIR light triggered release of DOX from DOX@HAuNS in a pulsatile fashion.*

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4. We evaluated the potential of HAuNS as a new contrast agent for photoacoustic tomography (PAT). PAT also referred to as optoacoustic tomography (OAT) is a novel hybrid imaging modality that employs nonionizing optical radiation and ultrasonic detection. HAuNS displayed strong resonance absorption tuned to the near infrared (NIR) range, with an absorption peak at 800 nm, whose photoacoustic efficiency is significantly greater than that of blood. Following surface conjugation with thiolated poly(ethylene glycol), the pegylated HAuNS (PEG-HAuNS) had distribution and elimination half-lives of 1.38±0.38 and 71.82±30.46 h, respectively. Compared with PAT images based on the intrinsic optical contrast in nude mice, the PAT images acquired within 2 h after intravenous administration of PEG-HAuNS showed the brain vasculature with greater clarity and detail. The image depicted brain blood vessels as small as ~100 μm in diameter using PEG-HAuNS as contrast agents (**Figure 3**). Preliminary results showed no acute toxicity to the liver, spleen, or kidneys in mice following a single imaging dose of PEG-HAuNS. Our results indicate that PEG-HAuNS are promising contrast agents for PAT, with high spatial resolution and enhanced sensitivity (*Lu et al., Biomaterials, 31: 2617-2626, 2010*).

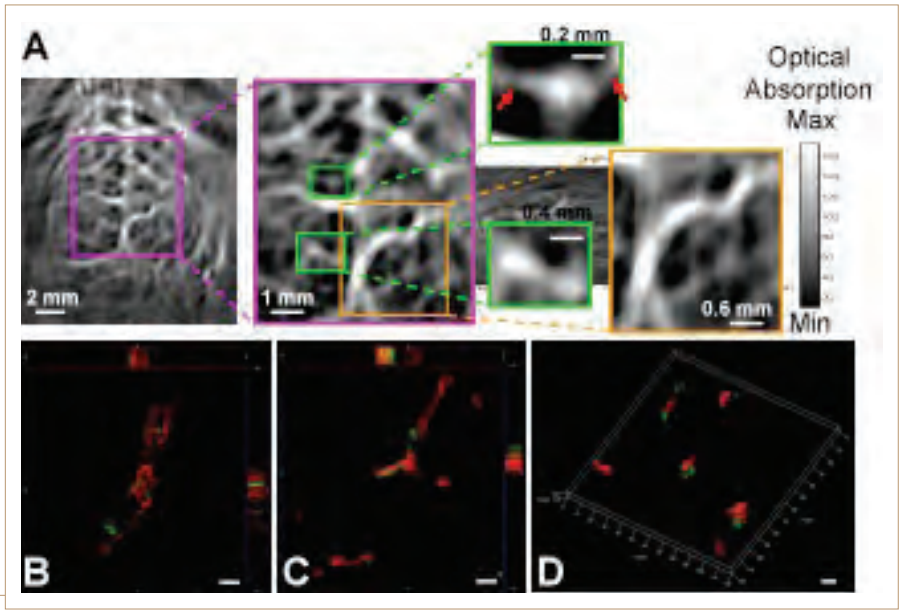


FIGURE 3: ***(A)*** *Enhanced photoacoustic signals revealed clear and detailed structure of large (yellow-framed picture) and small (green-framed picture) blood vessels in the mouse brain at higher magnification 2 h after intravenous injection of PEG-HAuNS. Arrows represent the small blood vessels with a diameter of about 100 μm, which can be seen in the contrast-enhanced images. (B-D) Distribution of PEG-HAuNS in brain vessels 2 h after injection (Bar=10 μm). Brain vessels were stained with anti-CD31 antibody (red fluorescence), while the scattering signals of gold particles were detected under a dark field (pseudo-green). Z-stack images showed the particles located on the luminal side of brain blood vessels* ***(B and C)****. In brain capillaries, three-dimensional reconstruction images show that the particles colocalized or stayed adjacent to the brain capillary endothelial cells* ***(D)****.*

NOVEL CANCER NANOTECHNOLOGY PLATFORMS FOR PHOTODYNAMIC THERAPY AND IMAGING

Roswell Park Cancer Institute
Principal Investigator:
Ravindra K. Pandey, Ph.D.

Objective: The objective of this project was to compare the encapsulation post-loading and covalent conjugation of the photosensitizers and the imaging agents (PET & Optical) to ORMOSIL and PAA Nanoparticles and select the best multifunctional platform for tumor imaging and therapy.

1. NPs formulations of photosensitizer did not inhibit their $^{1}O_{2}$ production efficiency, a key cytotoxic agent in PDT.

2. Compared to encapsulation, the post-loading approach was more efficient.

3. HPPH-cyanine dye (multifunctional agent for fluorescence imaging and PDT) required an 8-fold higher therapeutic dose than the imaging dose). Interestingly, PAA NPs loaded with the individual compounds had imaging and therapeutic doses similar to the parent compounds, but with enhanced tumor-specificity.

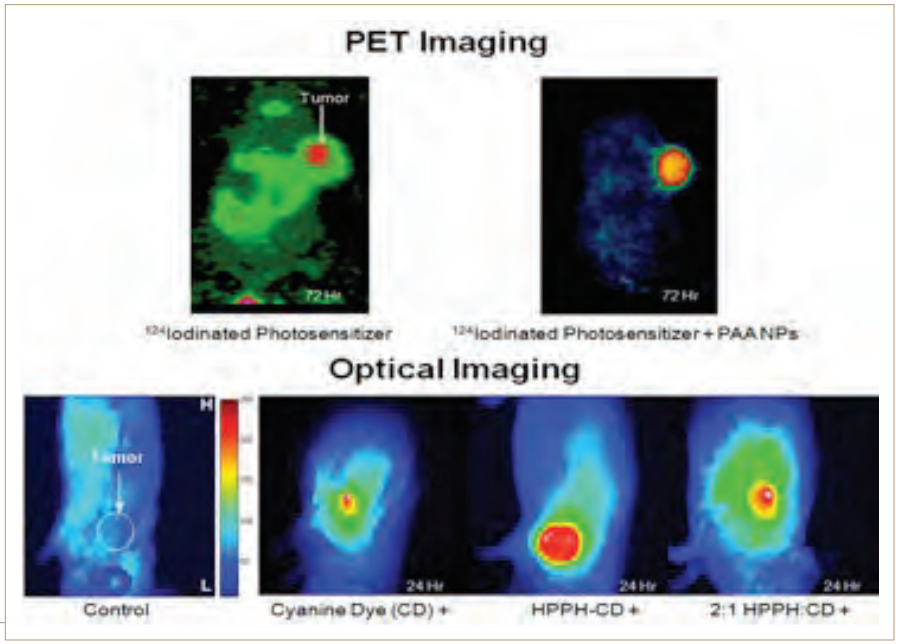
4. The ^{124}I-PS shows a remarkable capability of imaging RIF, Colon, Gl261, U87, pancreatic and 4T1 (breast cancer) tumors with improved contrast of breast cancer metastasis to lung over that obtained by ^{18}F-FDG, a clinical standard. Optimally designed targeted PAA NPs further improved the PS imaging and therapeutic potential.

5. The PET imaging capability (tumor contrast) of ^{124}I-PS was significantly increased whereas the liver and spleen uptake show a remarkable decrease in the presence of (blank) PAA and ORMOSILNPs. The addition of cRGD, F3-Cys or F3-PEG may further enhance its target specificity.

6. The presence of F3-Cys peptide at the periphery of PAA NPs significantly enhances the NPs targeting capability in 9L glioma tumors (*in vitro*).

7. At the imaging or therapeutic doses, the PAA NPs (targeted or non-targeted) did not produce any morbidity or mortality and the mice appear normal at 60 days after the treatment.

8. Finally, compared to ORMOSIL, the PAA nanoparticles were more effective for tumor-imaging/therapy and did not produce any toxicity in mice.



PAA Naoplatform for PET and Fluorescence Imaging. *Polyacrylamide (PAA) NPs enhance the tumor-imaging contrast of ^{124}I-photosensitizer and the cyanine dye based fluorophore for PET and fluorescence imaging respectively (Investigators: Pandey, R. K., Roswell Park Cancer Institute, Buffalo and Kopelman, R., University of Michigan, Ann Arbor).*

PHOTODESTRUCTION OF OVARIAN CANCER: ERBB3 TARGETED APTAMER-NANOPARTICLE CONJUGATE

Massachusetts General Hospital
Principal Investigator:
Tayyaba Hasan, Ph.D.

The broad goal of this cancer nanotechnology platform project (CNPP) has been to develop new approaches using targeted nanotechnology and optical imaging for detection, treatment, and therapy monitoring of cancer. The primary molecular target selected for this study was the epidermal growth factor receptor (EGFR) and the targeting vector used was either a commercially available antibody (cetuximab) or an aptamer which recognizes this trans-membrane receptor. The aptamer has been developed in collaboration with the University of Texas which was initiated as a result of the annual nano-alliance meeting held in Chicago in September, 2008. The diseases we have focused on are ovarian, pancreatic and brain (glioblastoma multiforme) cancers with testing being performed both *in vitro* and *in vivo* orthotopic models. We have developed 3D *in vitro* models of these three cancers that recapitulate the disease more effectively than the monolayer cultures that have been traditionally used. The technology used for triggering cancer cell death is photodynamic therapy (PDT), a photochemistry based approach where a light activatable molecule (photosensitizer, PS) is excited with light to generate cytotoxic species. PDT has inherent dual selectivity built into it: first from the preferential localization of the PS and second from the spatial confinement of light. The PDT platform is approved for the treatment of a number of cancer and non-cancer pathologies. The broad strategy of the project has been to co-deliver multiple therapeutic and imaging agents using a single nanoscale delivery vehicle.

Some of the major accomplishments of this MGH-CNPP include the following.

1. To combine nanotechnology with PDT, we have designed, synthesized and characterized a construct called "nanocell" in which the PS is non-covalently trapped inside a polymer nanoparticle (NP) and these NPs, along with additional therapeutic and imaging agents, are encapsulated inside liposomes. These nanocells have been used to deliver several therapeutic biomolecules like antibodies intracellularly to cancer cells. Intracellular localization of these agents was confirmed both *in vitro* and *in vivo* cancer models by confocal microscopy.

2. Our studies have demonstrated a dramatic increase in cytotoxicity when a PS and Avastin (anti-VEGF antibody) are delivered intracellularly via a nanocell. Further, these nanocells significantly enhanced overall treatment efficacy by dramatically reducing the local and metastatic tumor burden in mice that were implanted with orthotopic pancreatic tumors. This work was selected for a press conference followed by a press

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release at the recent "Molecular Targets and Experimental Therapeutics" meeting held in November 2009 in Boston, MA. This meeting was sponsored by AACR, NCI and EORTC. Further this work was also highlighted by the MIT-based online journal Technology Review.

3. Using the same nanocell construct design we have also co-encapsulated other agents like cetuximab and a small molecule receptor (MET) tyrosine kinase inhibitor PHA665792 along with the PDT agent to affect a triple combination therapy that targets the EGFR-MET crosstalk in the orthotopic pancreatic cancer mouse model. Again, dramatically reduced tumor weight and significantly lower occurrence and extent of metastases was seen with these nanocell-based treatments.

4. Further we have tested the ability of our nanocells as contrast agents to detect ovarian cancer in an orthotopic *in vivo* model using a custom-built fluorescence 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. Using this non-targeted nanocell construct we have demonstrated their ability to detect micron size tumor nodules. Further we have used the microendoscope to establish nanocell-based drug delivery to the tumor tissue. The nanocells improve delivery of BPD as compared to verteporfin and can deliver higher payloads of Avastin *in situ* to the tumor.

5. We had anticipated that making these multifunctional constructs targeted will enhance the treatment outcome while providing improved disease imaging capabilities. To achieve this we have a conjugated targeting ligand (cetuximab or C225) that has a high selective affinity for tumor cell receptor, EGFR, directly on to the nanocell surface. Using both *in vitro* and *in vivo* models of glioblastoma multiforme we have studied drug delivery using this targeted nanocell (TNC) construct using hyperspectral imaging system. As shown in **Figure 1**, the targeted nanocells were selectively delivered only to the tumor in mice that were implanted subcutaneously with EGFR positive U87 human glioblastoma cells. Further, we have demonstrated that the EGFR-targeted nanocell effectively crosses the blood brain barrier and the contents of the nanocell: C225, Avastin, and BPD selectively accumulated in the orthotopic intracranial tumors 48 hours following intravenous injection. The tumor to normal fluorescence ratios for fluorescently labeled Avastin and C225 in the brain are both approximately 10 as determined by quantitative hyperspectral imaging (corrected for wavelength dependent light scattering in tissue).

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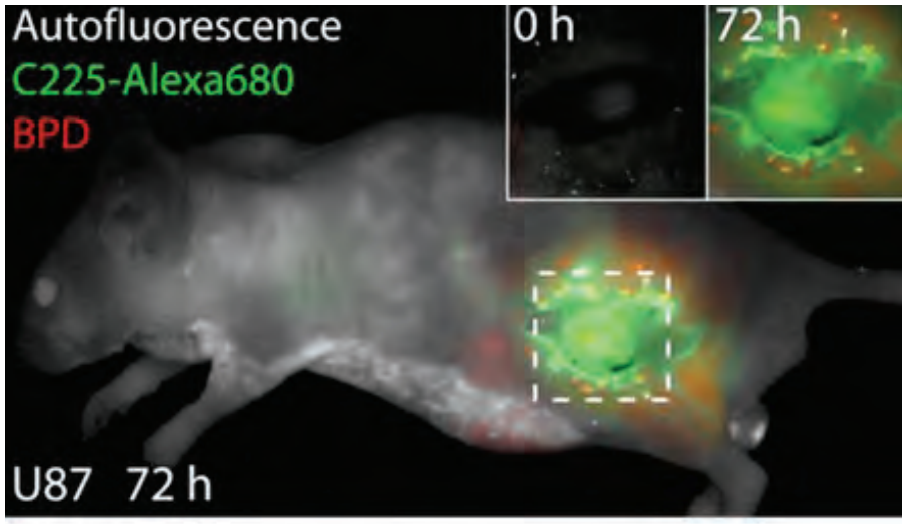
6. Using a liposomal nano-construct, we have also demonstrated for the first time, the specific delivery of a Ki-67 directed antibody and subsequent light-triggered death in a human ovarian cancer cell line OVCAR-5. Photoimmunoconjugate encapsulating liposomes (PICELS) were constructed from anti-pKi-67 antibodies conjugated to fluorescein isothiocyanate, as a photoactivatable agent followed by encapsulation in non-cationic liposomes. Nucleolar localization of the PICELS was confirmed by confocal imaging. Photodynamic activation with PICELS specifically killed pKi-67 positive cancer cells both in monolayer and in 3D cultures of OVCAR-5 cells.

A. TNC selectively accumulates in U87 tumors *in vivo*.



B. TNC is delivered intracellularly *in vivo*.

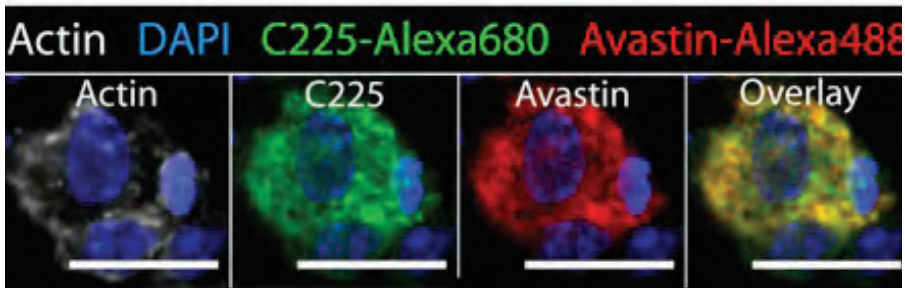


FIGURE 1: *EGFR targeted nanocells (TNC) is delivered specifically and intracellularly in vivo to subcutaneous U87 (EGFR+) tumors.* ***A.*** *TNC selectively accumulated in U87 (EGFR+) tumor cells (Top).* ***B.*** *Confocal microscopy of an ex vivo section from the tumor above shows that TNC delivers Avastin (red) and C225 (green) intracellularly. All colors were pseudo-colored in ImageJ; Scale bar= 20 µm.*

Nanotechnology Characterization Laboratory (NCL)

The NCL is a formal interagency collaboration among NCI, the National Institute of Standards and Technology (NIST), and the Food and Drug Administration (FDA). *The ultimate goal of the NCL's efforts is generation of data in support of regulatory review of nanotech constructs. NCL also serves as a bridge to take promising cancer nanotechnology research to the FDA. NCL provides both NIST and the FDA with collaborative research resources and NIST assists NCL with physicochemical characterization and standards development.*

NANOTECHNOLOGY CHARACTERIZATION LABORATORY CELEBRATES 5 YEARS OF OPERATION

The Alliance conceived the Nanotechnology Characterization Laboratory (NCL) in 2004 to help accelerate the clinical translation of promising cancer nanomedicines. The NCL, part of NCI's Alliance for Nanotechnology in Cancer, is a formal interagency collaboration among NCI, the U.S. Food and Drug Administration (FDA), and the National Institute of Standards and Technology (NIST). The NCL helps Alliance PIs take their research to the FDA and serves as an access point and resource for all researchers (academia, industry, and government) in the biomedical nanotechnology field. The NCL promotes knowledge sharing across the nanotech community, conducts structure activity relationship (SAR) studies to identify critical parameters related to nanomaterial safety and efficacy, facilitates the regulatory review of nanomaterials, and performs preclinical characterization of nanoparticles using standardized methods.

The NCL began operations in 2005. The NCL sought out, and hired its key scientists: Chemist Dr. Anil Patri, Toxicologist Dr. Stephan Stern, and Immunologist Dr. Marina Dobrovolskaia. Once hired, these NCL scientists immediately began developing and validating protocols for the preclinical characterization of biomedical nanomaterials — what would eventually become the "NCL Assay Cascade." To date, the NCL's Assay Cascade contains over 30 validated protocols, including assays such as size and stability determination, endotoxin quantification, analysis of complement activation, and *in vitro* cytotoxicity analysis. Many of these protocols have become voluntary consensus standards, adopted by standards developing organizations such as ASTM and ISO. In addition to physicochemical and *in vitro* immunology and toxicology assays, the typical NCL analysis pathway for a nanomaterial also includes *in vivo* animal studies including dose-range finding studies, single- and repeat-dose acute toxicity studies, and efficacy studies using transgenic and xenograft rodent tumor models. These experiments must be tailored to each individual nanomaterial under study.

The NCL Assay Cascade was developed in collaboration with NIST and the FDA. NCL scientists continue to work closely with NIST and the FDA to ensure the Assay Cascade remains applicable to a wide variety of nanomaterials and that it helps meet regulatory requirements. All of the NCL's protocols are available free-of-charge on the NCL website (http://ncl.cancer.gov).

The NCL began accepting nanomaterials for preclinical characterization in 2006. NCL selects nanomaterials based on an application process — applications are evaluated based on published criteria, with a primary focus on nano-sized formulations

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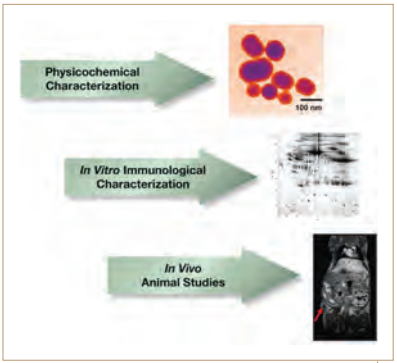

Characterizing Nanoparticles in the NCL Assay Cascade. *Nanotechnology strategies submitted to the NCL are characterized in a standardized assay cascade developed in collaboration with the National Institute of Standards and Technology (NIST) and the Food and Drug Administration (FDA). This three-tiered system for nanoparticle characterization consists of physicochemical, in vitro, and in vivo testing. Shown here (from top to bottom) is a transmission electron micrograph of a mesoporous silica nanoparticle (false color added), a two-dimensional gel electrophoresis separation of serum proteins, and a magnetic resonance image of a mouse bearing a flank xenograft tumor (indicated with the red arrow).*

and demonstrated efficacy in a biological system (more details about the application process and acceptance criteria can be found at http://ncl.cancer.gov). NCL has a nearly 50% acceptance rate for applications, and all NCL services are provided at no cost to the submitting investigator. Applications from Alliance-funded investigators are given special priority and NCL has an abbreviated application process for Alliance grantees, since NCI has already vetted their technologies. Once a nanomaterial comes into the NCL under a materials transfer agreement (MTA), NCL scientists collaborate with the submitting investigator to determine the best analysis pathway for their concept, i.e., which experiments will have the most benefit to progress towards an Investigational New Drug (IND) or Investigational Device Exemption (IDE) filing with the FDA. The characterization process can take more than a year to complete, depending on the developmental stage of the concept. Once complete, the NCL will provide the submitting investigator with the data from their characterization project in the form of a Client Report. This is a fully inclusive scientific document that details the results of all NCL experimentation. Generally these scientific reports are more than 100 pages in length and can be used to support IND or IDE applications or excerpted for scientific publications.

The NCL operated with a staff of just four scientists and three technicians in 2006, and were able to output four Client Reports the next year. Over the course of the last five years, the NCL staff and output have increased at a significant pace. NCL now employs more than 20 scientists and technicians in a broad range of disciplines including chemists, biologists, electron microscopy technicians, histology technicians, and a statistician. The NCL has now distributed more than 20 Client Reports.

NCL collaborations and resources have continued to grow for the past five years. The list of collaborators the NCL has worked with has grown to over 65 and includes laboratories from academia, small biotech companies, large pharmaceutical companies, and US government institutions. The NCL has characterized more than 180 nanomaterials in this time; this includes liposomes, dendrimers, quantum dots, polymers, emulsions, gold colloids, metal oxides, and fullerene derivatives. Continued acquisition of state-of-the-art instrumentation has been critical to the growth of the NCL. NCL instrumentation includes a three Tesla clinical MRI, five electron microscopes, an imaging mass spectrometer, and a wide range of other instruments for thorough chemical and biological characterization of nanomaterials.

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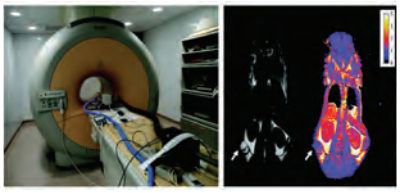

NCL has seen significant growth in its in vivo animal studies program over the last five years. *NCL has access to a clinical 3 Tesla MRI instrument. This instrument is equipped with a specially designed chamber that will house and image two rodents at a time. Shown on the right is an MR image of a mouse bearing a flank xenograft tumor. The mouse was injected with a nanomaterial imaging agent.*

One area in which the NCL has seen significant growth over the last five years is its *in vivo* animal studies. In 2007, the NCL performed seven animal studies. In 2009, this number had more than tripled to 22 animal studies. In 2010, the NCL announced a collaboration with the FDA's National Center for Toxicological Research (NCTR) — this collaboration will allow for GLP-compliant pharmacokinetic studies in non-human primates. NCL and NCTR anticipate conducting approximately three rhesus monkey studies per year.

Many Alliance investigators have been a part of the success and growth of the NCL over the past five years. Through 2007, the NCL had interactions with eight different Alliance Investigators: James Baker, Mansoor Amiji, Kattesh Katti, Robert Langer, Joseph DeSimone, Gregory Lanza, Shuming Nie, and Paras Prasad. Five new Alliance funded collaborations were begun in 2008, and by the close of 2009, the NCL had interacted with 20 different Alliance Investigators. Many of these interactions resulted in continued collaboration with the NCL, and now the NCL has successful collaborations with more than half of the eight CCNEs and twelve CNPPs.

Significant strides have been made towards all the NCL's objectives. SAR studies are an important part of what the NCL does; these help to shape the future development of biomedical applications for nanomaterials. Recently, the NCL has conducted SAR studies involving the penetration of titanium dioxide nanoparticles in rodents, pig, and human skin, macrophage uptake of gold nanoparticles, and studies concerning the role of nanomaterials in autophagy. The results of these studies are disseminated to the public to help fulfill NCL's objective of promoting knowledge sharing. NCL staff have contributed to approximately 30 publications since 2005, and they attend more than 20 scientific conferences and workshops each year. Additionally, the NCL provides support to caNanoLab, a web database designed to promote nanomaterial data sharing among Alliance members.

The last five years of NCL operation have been very successful. NCL data has been used to help several collaborators successfully apply for an IND or IDE with the FDA, advance their nanotech concepts to clinical trials, and to garner investment and interest. The NCL continues to strive to provide the nanotech community with the latest in nanotech research and help promote the clinical advancement of nanotechnology concepts. For more information about the NCL and on how the NCL may be able to help you advance a biomedical application of nanotechnology, please visit the NCL website, http://ncl.cancer.gov, or contact us at 301-846-6939.

The NCI Alliance Nanotechnology in Cancer Bulletin is a collaborative effort developed and facilitated by the Communications and Integration Working Group (CIWG) of the Alliance program. The group is currently led by Alliance co-chairs, Lynn Coulter (Washington U. S-CCNE) and Diane Clark Robinson (NSBCC-CCNE), with coordination from NCI co-chair, Krzysztof Ptak, Ph.D.

The CIWG's mission is to catalyze effective Alliance-wide and external communications, facilitate Alliance team science integration, create education outreach opportunities, and leverage best practices.

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