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NCI Alliance for Nanotechnology in Cancer

Phase II Program Summary 2010–2015

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OFFICE OF CANCER NANOTECHNOLOGY RESEARCH

CENTER FOR STRATEGIC SCIENTIFIC INITIATIVES

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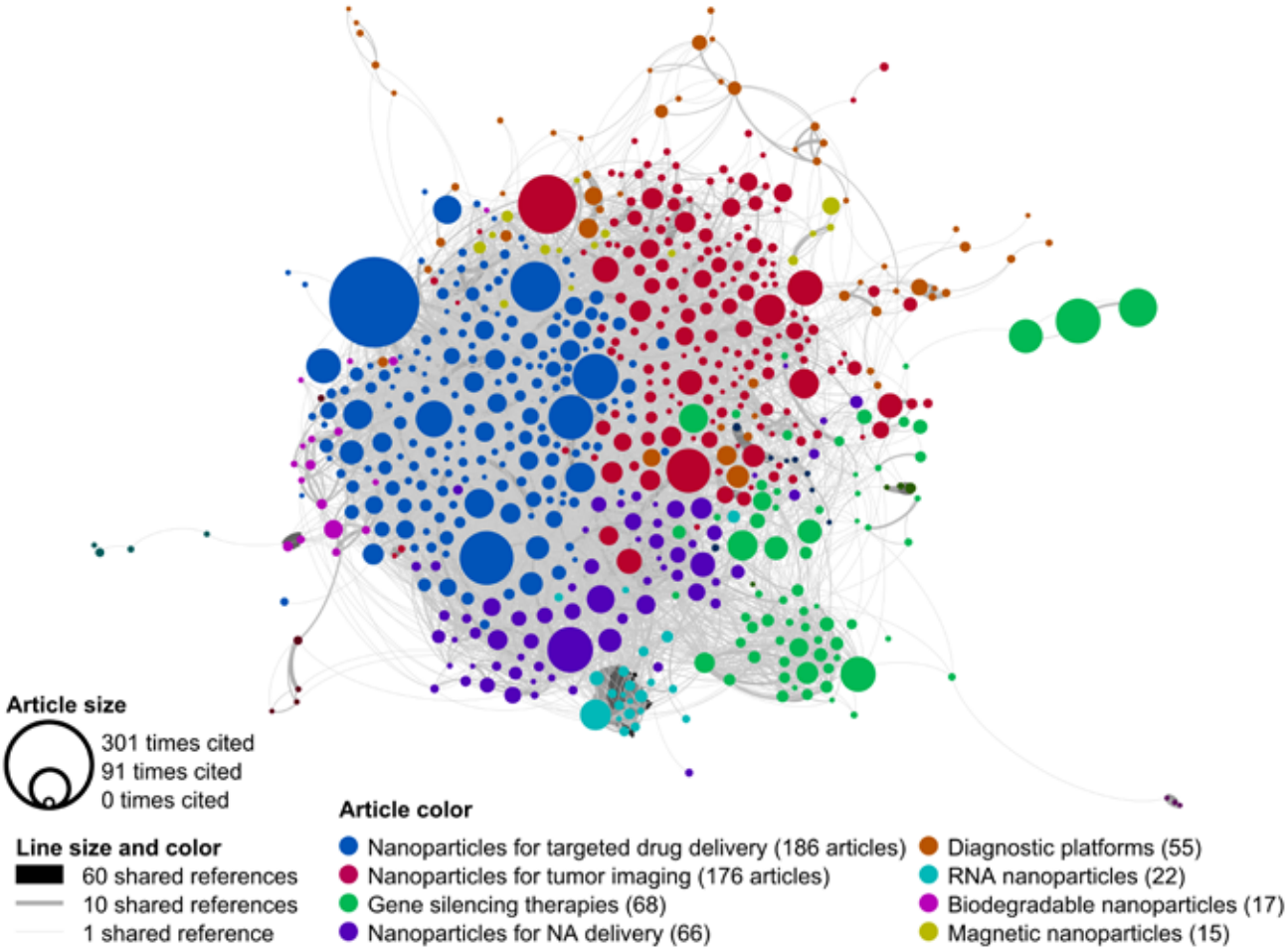
INTRODUCTION

Phase II of the National Cancer Institute (NCI) Alliance for Nanotechnology in Cancer program (Alliance; nano.cancer.gov) was characterized by many research and translational achievements During Phase II (2010-2015), awardees have reported more than 25 distinct nanomaterial delivery vehicles and numerous innovative technologies in over 1,000 publications. Adding this number to publications generated during Phase I of the program, over 2,800 reports from Alliance investigator laboratories have been published since the beginning of the Alliance program in 2005. In addition to publications, Alliance investigators have received more than 40 patents, and started over 75 companies focused on diagnostics, therapeutics, and research materials and services. Many of these companies have received NCI Small Business Innovation Research awards, some are engaged in discussions with the U.S. Food and Drug Administration (FDA) in preparation for Investigational New Drug applications, and two are engaged in clinical trials. In addition, Alliance-affiliated devices and therapeutics are being tested in more than 15 cancer related clinical trials and Institutional Review Board approved studies in humans. We have also seen the successful training of next-generation cancer nanotechnologists through the Alliance Cancer Nanotechnology Training Centers and Pathway to Independence Awards (K99/R00). During the program, the K99/R00 awardees transitioned from their postdoctoral positions into assistant professorships at universities across the country. More details about Alliance research and training successes achieved during Phase II can be found in **Scientific and Translational Advances**.

The Nanotechnology Characterization Laboratory (NCL), a formal scientific partnership of the Alliance, FDA, and the National Institute of Standards and Technology, continued to grow and serve the community during Phase II. Since it began accepting applications in 2005, NCL has characterized more than 300 different nanoparticles. NCL has also worked extensively with the FDA to address challenges in nanomedicine. More on the NCL and its accomplishments can be found in "Nanotechnology Characterization Laboratory—Accomplishments" in the **Scientific and Translational Advances** section.

Phase II also saw the development of several collaborations amongst Alliance awardees and opportunities for external partnerships. Through collaborative Challenge Projects, Alliance investigators worked with one another and the outside community to combine research strategies in order to create more effective diagnostics and therapeutics. Many of these projects have resulted in long-term collaborations and joint publications. Information about these efforts can be found in "Research Collaborations across the Alliance" in **Collaborations**. An update on the Translation of Nanotechnology in Cancer consortium ("Translation Of Nanotechnology In Cancer (TONIC): Accelerating Translation through Public-Private Partnerships") is also included in this section. Initiated in 2011, TONIC aims to accelerate the translation of nanomedicines to the clinic by bringing Alliance investigators and industry together to discuss nanotechnology opportunities. The TONIC consortium's achievements to date are highlighted in this story.

As a continuation of an initiative started during Phase I, efforts to support and promote nanomaterial data deposition and sharing were expanded by the growth and further development of two centralized databases for nanomaterial characterizations—the cancer Nanotechnology Data Portal (caNanoLab) and the Nanomaterial Registry. During Phase II, each has enhanced their capabilities to better serve nanotechnology researchers and promote data exchange. Details about these databases and the nanoinformatics efforts supported by the program office are detailed in "Nanomaterial Data Sharing: Support for Nanoinformatics and Databases" in the **Collaborations** section.



Bibliographic coupling network of a subset of original research papers supported by the NCI Alliance for Nanotechnology in Cancer, and published between 2010 and 2014 (665 publications). Each dot (node) represents an original research paper, dot color indicates algorithmically-derived topic, and dot size indicates total citation count as of September 2015. Gray lines indicate shared references between two papers; larger and darker lines indicate larger numbers of shared references between the connected papers. Papers were clustered into groups using a community detection algorithm that identifies sets of papers that are more densely connected to each other than to other papers in the network. Since papers that are topically related to each other tend to refer to the same previous literature, groups of papers that share references tend to have common topics. Network analysis performed using the Science of Science Tool (Sci2); visualization performed using Gephi. Image courtesy of Christopher Belter, NIH Library.

As the Alliance prepares to kick-off Phase III, we in the program office take the opportunity to review Alliance successes in this Program Summary. Although we primarily highlight Phase II, Phase III of the program would not be possible without the successes of the first phase of the Alliance as well. We end this Summary with a description of the numerous resources developed by Phase II Alliance investigators that are available to be shared with the wider cancer nanotechnology community (see **Resources**). Our goal for this next phase is to continue to add to this list of sharable resources and program successes. We hope you enjoy reading about the many accomplishments of the Phase II Alliance in the following pages.

SCIENTIFIC and TRANSLATIONAL ADVANCES

4 CENTERS OF CANCER NANOTECHNOLOGY EXCELLENCE (CCNEs): PROGRAM SUMMARIES

The CCNEs served as the core of the NCI Alliance for Nanotechnology in Cancer. NCI funded nine multidisciplinary Centers that were focused on using nanotechnology for cancer research discovery purposes, and the development of clinical tools and technologies to improve cancer detection, diagnosis, and treatment.

23 CANCER NANOTECHNOLOGY PLATFORM PARTNERSHIPS (CNPPs): PROJECT SUMMARIES

The CNPPs were multidisciplinary partnerships designed to support defined research projects that addressed major barriers and fundamental questions in cancer using solutions from nanotechnology. NCI funded 12 partnerships that used a team research approach to make innovative discoveries in basic and pre-clinical cancer research.

46 CANCER NANOTECHNOLOGY TRAINING CENTERS (CNTCs): TRAINING SUMMARIES

The CNTCs educated and trained graduate students and postdoctoral scientists from diverse fields in the use of nanotechnology-based approaches to advance understanding of cancer biology and applications of cancer nanotechnology to the clinic. NCI funded six training centers that brought in trainees with various research backgrounds and mentors with backgrounds in nanotechnology, cancer biology, and clinical oncology.

52 PATHWAY TO INDEPENDENCE AWARDS IN CANCER NANOTECHNOLOGY RESEARCH(K99/R00s): TRAINING SUMMARIES

K99/R00 awards were made to postdoctoral scientists working in the area of cancer nanotechnology to enable the transition from mentored postdoctoral training positions to independent research positions. NCI funded seven K99/R00s, with the ultimate goal of maintaining a well-trained pool of new investigators focused on cancer nanotechnology research.

59 NANOTECHNOLOGY CHARACTERIZATION LABORATORY (NCL)

NanoSystems Biology Cancer Center 2 (NSBCC)

CALIFORNIA INSTITUTE OF TECHNOLOGY
PRINCIPAL INVESTIGATORS: JIM HEATH, PhD, LEROY HOOD, MD, PhD, AND MICHAEL PHELPS, PhD

OVERVIEW

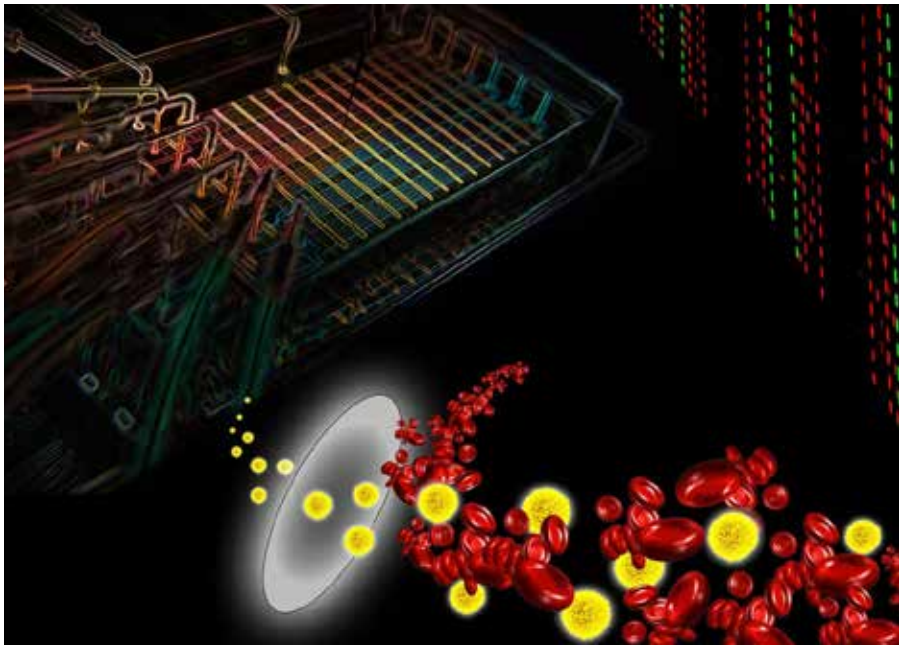
The NanoSystems Biology Cancer Center (NSBCC) began with a vision to utilize the measurement and analytical needs of systems approaches to cancer biology and clinical oncology to drive the science and engineering of new technologies. The goal was to then move these technologies to clinical care and research settings through the UCLA Jonsson Comprehensive Cancer Center and other partners. From the beginning, discovery science, technology development, and clinical application were tightly integrated within the NSBCC's Projects and Cores to develop *in vitro* and *in vivo* techniques and tools for early detection, diagnosis, and targeted therapies for melanoma and glioblastoma. NSBCC's work on polymer nanotherapeutics has helped to push siRNA cancer therapies closer to clinical reality and enabled development of low-toxicity chemotherapies, while single cell studies of immunotherapies and phosphoprotein signaling pathways are helping to broaden the patient populations that can benefit from immuno- and targeted therapies. Work on enabling technologies for multiplex proteomics and PET molecular imaging probe synthesis are expected to increase accessibility of these approaches to precision medicine. Given these goals, NSBCC researchers anticipate their technologies will have a significant impact on future cancer research and care. Additional information about the Projects and Cores can be found at http://nano.cancer.gov/action/programs/caltech.

SCIENTIFIC AND TECHNOLOGICAL ACHIEVEMENTS

Microchip platform for multiplex single-cell functional proteomics— Researchers at this Center have applied single cell proteomics studies towards understanding patient responses to immunotherapy in clinical trials. These technologies were used to provide a minimally invasive (via blood) method for tracking patient response to immunotherapies such as adoptive cell transfer of engineered anti-tumor T cells. This method defined a new metric for the quality of the anti-tumor immune response (the polyfunctionality strength index, or pSI), that is predicated upon the fact that anti-tumor T cells that secrete the largest numbers of different proteins also secrete, by far, the highest copy numbers of individual proteins, and so dominate the anti-tumor immune response[1]. The technologies also quantified the effect of epitope spreading, as well as how the regulatory influence of the immune system can detrimentally affect an adoptive cell transfer immunotherapy regimen. This technology has been translated into the commercial sector for cancer immunotherapy applications by Isoplexis (www.isoplexis.com), a company co-founded by former NSBCC postdoc Dr. Rong Fan (now on the faculty at Yale), and with scientific support from NSBCC Project Lead Dr. Toni Ribas.

Multiplex and quantitative single phosphoproteomic assays— A related suite of technologies was used at the NSBCC to study signaling pathways from tumor tissue via multiplex and quantitative single cell phosphoproteomic and metabolite assays[2]. New software algorithms developed by the NSBCC analyzed how these signaling pathways respond to therapeutic perturbations in a manner that allows for the anticipation of resistance, and the identification of therapeutic strategies that can stave off resistance[3]. It was also of value for identifying rapid resistance (adaptation) mechanisms[4,5]. This approach was applied to glioblastoma multiforme tumor models, patient tumor tissues, and melanoma tumor models, and is currently undergoing clinical translation into a CLIA (Clinical Laboratory Improvement Amendments) laboratory.

Nanoparticle drug delivery systems— Research at the NSBCC continued to support development of the polycyclodextrin/camptothecin nanodrug, CRLX101. NSBCC researchers and colleagues in industry correlated animal data to clinical data and developed methods to measure these nanoparticles in human tissue, important steps in translating nanoparticle therapeutics into clinical use[6]. NSBCC researchers also obtained biopsies in



Schematic of an SCBC microchip platform for analyzing the functional properties of tumor-antigen specific T cells collected from the blood or tumor tissues of cancer patients participating in cancer immunotherapy clinical trials.

one clinical trial (https://clinicaltrials.gov/ct2/show/NCT01612546) and showed that systemically administered nanoparticles reside in the tumor area, but not in adjacent, healthy tissue.

TRANSLATIONAL ACHIEVEMENTS

The NSBCC has numerous translational successes, including nine clinical trials on CLRX101, developed with Phase I Alliance support and under continuous study at the Phase II NSBCC (https://clinicaltrials.gov; NCT01612546, NCT01803269, NCT02187302, NCT02010567, NCT01652079, NCT02187302, NCT01652079, NCT01803269, NCT02010567). A molecular diagnostic developed at the Institute of Systems Biology, partially with NSBCC support, is now available from Integrated Diagnostics, Inc. (www.indidx.com). This diagnostic, the Xpresys test, is a multiplex blood proteomic assay to determine if a lesion identified by CT scan is likely to be benign or malignant. Other technologies, including the single cell proteomics suite, are being tested in clinical settings and are planned for entry into a CLIA laboratory setting.

1. Ma, C., et al. Multifunctional T-cell analyses to study response and progression in adoptive cell transfer immunotherapy. Cancer discovery 3, 418-429 (2013).
2. Xue, M., et al. Chemical methods for the simultaneous quantitation of metabolites and proteins from single cells. Journal of the American Chemical Society 137, 4066-4069 (2015).
3. Wei, W., et al. Hypoxia induces a phase transition within a kinase signaling network in cancer cells. Proceedings of the National Academy of Sciences of the United States of America 110, E1352-1360 (2013).
4. Nathanson, D.A., et al. Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. Science 343, 72-76 (2014).
5. Gini, B., et al. The mTOR kinase inhibitors, CC214-1 and CC214-2, preferentially block the growth of EGFRvIII-activated glioblastomas. Clinical cancer research : an official journal of the American Association for Cancer Research 19, 5722-5732 (2013).
6. Eliasof, S., et al. Correlating preclinical animal studies and human clinical trials of a multifunctional, polymeric nanoparticle. Proceedings of the National Academy of Sciences of the United States of America 110, 15127-15132 (2013).

Dartmouth Center for Cancer Nanotechnology Excellence

DARTMOUTH COLLEGE
PRINCIPAL INVESTIGATORS: IAN BAKER, PhD, AND KEITH PAULSEN, PhD

OVERVIEW

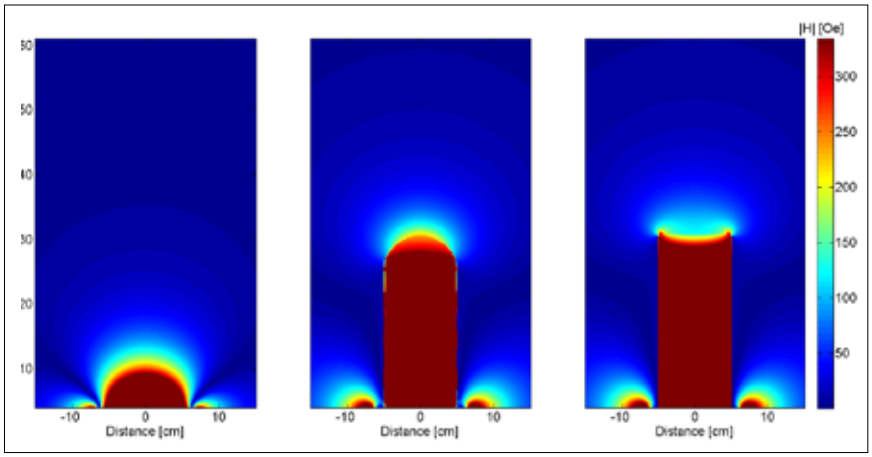
The objective of this Center was to develop and use novel, biocompatible antibody-targeted magnetic nanoparticles (mNPs) for the treatment of tumors using magnetic hyperthermia. In combination with an alternating magnetic field (AMF), mNPs were designed to be the heat source to severely damage or destroy targeted tumors. While the effort initially focused on breast cancer and ovarian tumors, the approach is applicable to most cancers. Four Projects and three Cores were dedicated to optimizing targeted delivery of mNPs to tumors, developing methods to quantify ligand binding *in vivo*, developing instrumentation to generate and apply AMF, and investigating the potential of mNP hyperthermia as an immunotherapy. Taken together, these efforts are leading to the design of protocols for effective hyperthermia treatments. Work on immunostimulation via hyperthermia, utilizing low risk iron oxide nanoparticles and AMF technology to stimulate systemic anti-tumor immune response, has the potential to be used in treatments for patients with potentially metastatic tumors. Demonstrating the safety and clinical value of neoadjuvant immunotherapy using mild hyperthermia could open a new conceptual area for further development and eventually lead to inclusion of such approaches as part of treatments for primary high-risk disease. Additional information on the Projects and Cores can be found at http://nano.cancer.gov/action/programs/dartmouth and http://engineering.dartmouth.edu/dccne.

SCIENTIFIC AND TECHNOLOGICAL ACHIEVEMENTS

Magnetic hyperthermia as an adjuvant therapy—Researchers at this Center showed that mild hyperthermia generated by mNPs with an applied AMF alone or combined with radiation or chemotherapy is capable of improving treatment efficacy by approximately 25-30%, without any increase in normal tissue toxicity or complications[1]. Studies were performed in two different spontaneous canine oral tumor models in which hyperthermia treatment reduced the rate of cancer recurrence, and hence reduced post-treatment morbidity. Post treatment survival time and the quality of life was significantly extended and/or improved in all tested canines.

Immune response elicited by magnetic hyperthermia—Researchers at this Center also showed that mild hyperthermia generated by direct intratumoral injection of mNPs and applied AMF not only damaged or destroyed the tumor target, but also stimulated a systemic immune response[2]. The immune response was similar to the "abscopal" effect recognized for radiation therapy, which is dependent on the immune system and in particular requires CD8+ T cells. The most interesting aspect of these studies was that there is a narrow range of thermal dose that has the optimal effect—necrosis in combination with systemic immune stimulation. These studies provided a fundamental new approach to treat tumors prior to surgical removal in order to generate anti-tumor immune responses that could recognize and eliminate metastatic disease.

***In vivo* diagnostic methods with improved sensitivity—**Researchers at this Center developed methods that allow more sensitive detection of mNPs for imaging, along with spectroscopy to measure the concentration of selected molecules at the nano-molar range[3]. These capabilities can potentially be used to monitor dynamic changes in the tumor microenvironment, including changes in levels of soluble molecules or stiffness of the microenvironment matrix[4]. The improved detection sensitivity was achieved by placing the targeted magnetic nanoparticles in an AMF and observing the harmonics of the resulting magnetization, which are affected by a variety of parameters of the local environment in which the particles reside, as well as their interactions with target molecules. For example, particles outside cells, bound to the membrane, or inside cells will give rise to different harmonic signals and provide information on the microscopic location of the particles.



Calculated AMF distribution above a pancake coil without and with differently-shaped magnetic field concentrators.

TRANSLATIONAL ACHIEVEMENTS

Based on preclinical efforts made with mNP targeting, magnetic hyperthermia in combination with conventional cancer treatment therapies, and radiation sensitization arising from magnetic hyperthermia, a Phase I clinical trial has been designed to show the safety of clinically-relevant levels of mNP and AMFs in patients. The proposed magnetic hyperthermia/ AMF technology has been submitted to the U.S. Food and Drug Administration for Investigational Device Exemption approval.

1. Petryk, A.A., Giustini, A.J., Gottesman, R.E., Kaufman, P.A. & Hoopes, P.J. Magnetic nanoparticle hyperthermia enhancement of cisplatin chemotherapy cancer treatment. International journal of hyperthermia : the official journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group 29, 845-851 (2013).
2. Toraya-Brown, S., et al. Local hyperthermia treatment of tumors induces CD8(+) T cell-mediated resistance against distal and secondary tumors. Nanomedicine : nanotechnology, biology, and medicine 10, 1273-1285 (2014).
3. Zhang, X., et al. Molecular sensing with magnetic nanoparticles using magnetic spectroscopy of nanoparticle Brownian motion. Biosensors & bioelectronics 50, 441-446 (2013).
4. Weaver, J.B., Rauwerdink, K.M., Rauwerdink, A.M. & Perreard, I.M. Magnetic spectroscopy of nanoparticle Brownian motion measurement of microenvironment matrix rigidity. Biomedizinische Technik. Biomedical engineering 58, 547-550 (2013).

Center for Cancer Nanotechnology Excellence at Johns Hopkins

JOHNS HOPKINS UNIVERSITY
PRINCIPAL INVESTIGATORS: PETER SEARSON, PhD, AND MARTIN POMPER, MD, PhD

OVERVIEW

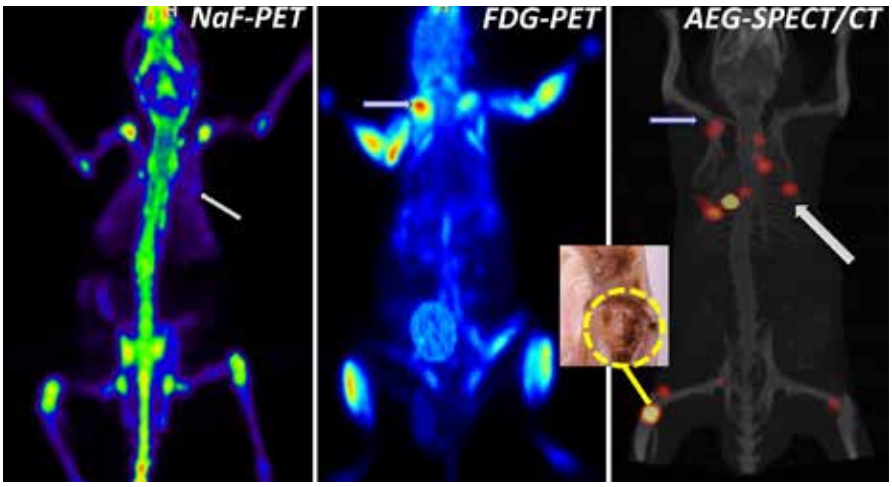
The objective of this Center was to integrate nanotechnology-based diagnostic and therapeutic tools and post-therapy monitoring as a comprehensive solution to lung and pancreatic cancer care. Supporting a community of researchers in the physical sciences, engineering, cancer biology, and oncology, this Center tackled several scientific and translational fronts, including: (1) screening DNA methylation in bodily fluids for early cancer diagnostics and post-therapy monitoring, (2) developing magnetic resonance imaging methods to non-invasively quantify vaccine-mediated antigen delivery to lymph nodes, and (3) designing nanoparticles that solubilize poorly water-soluble drugs or penetrate mucus barriers for improved drug delivery. The Center was located in the Institute for NanoBioTechnology at Johns Hopkins (http://inbt.jhu.edu), which brings physicians, scientists, and students together to explore new findings in science and technology at the boundaries between nanoscience and medicine. Additional information about this Center's Projects and Cores can be found at http://nano.cancer.gov/action/programs/johnshopkins and http://ccne.inbt.jhu.edu.

SCIENTIFIC AND TECHNOLOGICAL ACHIEVEMENTS

High throughput DNA methylation screening system— Researchers at the Johns Hopkins Center used silica superparamagnetic nanoparticles (SSPs) to develop a single tube technique for extracting DNA. In conjunction with an automated sample processing platform based on magnetofluidic manipulations, also developed by this Center, the SSP-based technique enables the integration of sample preparation and genetic analysis within discrete droplets, including cell lysis, DNA binding, washing, elution, amplification, and detection steps. As such, this technology can perform integrated and automatic analysis, which could minimize manual labor and time, while providing more reproducible results for high throughput DNA methylation screening of bodily fluids for early cancer diagnostics and post therapy monitoring.

Cancer vaccination optimization using dual-mode imaging— For the first time, researchers at the Center have successfully combined two non-invasive imaging techniques, magnetic resonance imaging (MRI) and bioluminescence imaging, to visualize both the afferent and efferent arms of the cellular immune response to a whole cell tumor vaccine, GVAX. Using this dual-mode imaging approach, the group observed adjuvant effects of a toll-like receptor agonist on the immune response to GVAX on both arms, illustrating the utility of quantitative non-invasive imaging as a platform to screen and evaluate vaccine strategies[1]. The researchers used magnetovaccination to enable MRI monitoring of antigen capture and subsequent migration of antigen presenting cells to the draining lymph node. The advantage of this approach is that superparamagnetic iron oxide nanoparticles have been used clinically, and it is therefore conceivable that magnetovaccination could be directly applied to evaluate cancer vaccine responses in human subjects with little or no modification.

Mucus-penetrating nanoparticles—Mucus forms a highly viscous gel layer that lines the airway epithelium and presents a major delivery issue for therapeutic agents intended for pulmonary diseases, particularly cancer. Researchers in this Center have developed a strategy to overcome the viscosity of airway mucus using a biodegradable mucus-penetrating particle (MPP) platform from which a wide array of drugs can be released in a controlled manner (Cx-MPP). The newly engineered Cx-MPP provides improved lung pharmacokinetics, leading to an enhanced anti-cancer effect in an orthotopic model of lung cancer. By conjugating Cx-MPP with laminin receptor targeting short peptides, the group demonstrated effective targeting of Cx-MPP to small cell lung cancer cells (which overexpress laminin receptors on their cell surface) while retaining the mucus-penetrating property.



Systemic, nanoparticle-based, molecular-genetic imaging of prostate cancer in vivo (right image) identifies lesions not seen with conventional molecular imaging [18F-sodium fluoride (18F-NaF, left) and 18F-fluorodeoxyglucose (18F-FDG, center) positron emission tomography][3].

TRANSLATIONAL ACHIEVEMENTS

Cancer Targeting Systems, a company derived from the Center, recently secured $10 million in investment funds for gene promoter-based cancer imaging and therapy[2]. The promoter can be delivered systemically within an FDA-approved linear polyethyleneimine nanoparticle for the purpose of detecting and treating cancer metastases. Center scientists have reduced this platform to practice in experimental models of metastatic melanoma, breast cancer, and prostate cancer (see image). The funds will be used to initiate a 12-patient Phase I clinical trial dose escalation trial.

Center scientists have been integral to the planning and oversight of the Center for Translational Molecular Imaging (CTMI), which opened in January 2015 on the Johns Hopkins Bayview Campus. A major goal of the CTMI is to produce nanomedicines according to current Good Manufacturing Practice for first-in-human studies.

1. Kadayakkara, D.K., Korrer, M.J., Bulte, J.W. & Levitsky, H.I. Paradoxical decrease in the capture and lymph node delivery of cancer vaccine antigen induced by a TLR4 agonist as visualized by dual-mode imaging. Cancer research 75, 51-61 (2015).
2. Bhang, H.E., Gabrielson, K.L., Laterra, J., Fisher, P.B. & Pomper, M.G. Tumor-specific imaging through progression elevated gene-3 promoter-driven gene expression. Nature medicine 17, 123-129 (2011).
3. Bhatnagar, A., et al. AEG-1 promoter-mediated imaging of prostate cancer. Cancer research 74, 5772-5781 (2014).

MIT-Harvard Center of Cancer Nanotechnology Excellence

MASSACHUSETTS INSTITUTE OF TECHNOLOGY
PRINCIPAL INVESTIGATORS: ROBERT LANGER, ScD, AND RALPH WEISSLEDER, MD, PhD

OVERVIEW

The overall goal of this Center was to develop new cancer therapeutics based on nanoparticle delivery of chemotherapeutics and small interfering RNAs (siRNAs), as well as new diagnostic tools using *in vitro* and implantable devices. The team included members from MIT, Harvard, and the major Partners Healthcare teaching hospitals. Five Projects and three supporting Cores were dedicated to the investigation of novel nanoparticle-combination therapies for improved targeting of prostate cancer, new siRNA delivery and targeting strategies for use in treatment of ovarian cancer, next generation magnetic nanoparticles and diagnostic magnetic resonance systems for circulating cancer cell detection and molecular analysis, and nanotechnology-based systems for molecular and biomedical sensing. Center members have been very active in translating and commercializing their academic discoveries and established at least 14 start-up companies (BIND, BLEND, T2 Biosystems, Taris, Lumicell, Microchips, Selecta, Layerbio and others). Two of these companies, BIND Therapeutics and T2 Biosystems, have gone public while five are conducting clinical trials. A total of over 600 million dollars was raised by these nanotechnology companies. In addition, several nanotechnology patents from Center investigators were licensed to established companies. Additional information on the Center's Projects, Cores, and related technologies can be found at nano.cancer.gov/action/programs/mit, ki.mit.edu/approach/partnerships/ccne, www.bindtherapeutics.com, and www.t2biosystems.com.

SCIENTIFIC AND TECHNOLOGICAL ACHIEVEMENTS

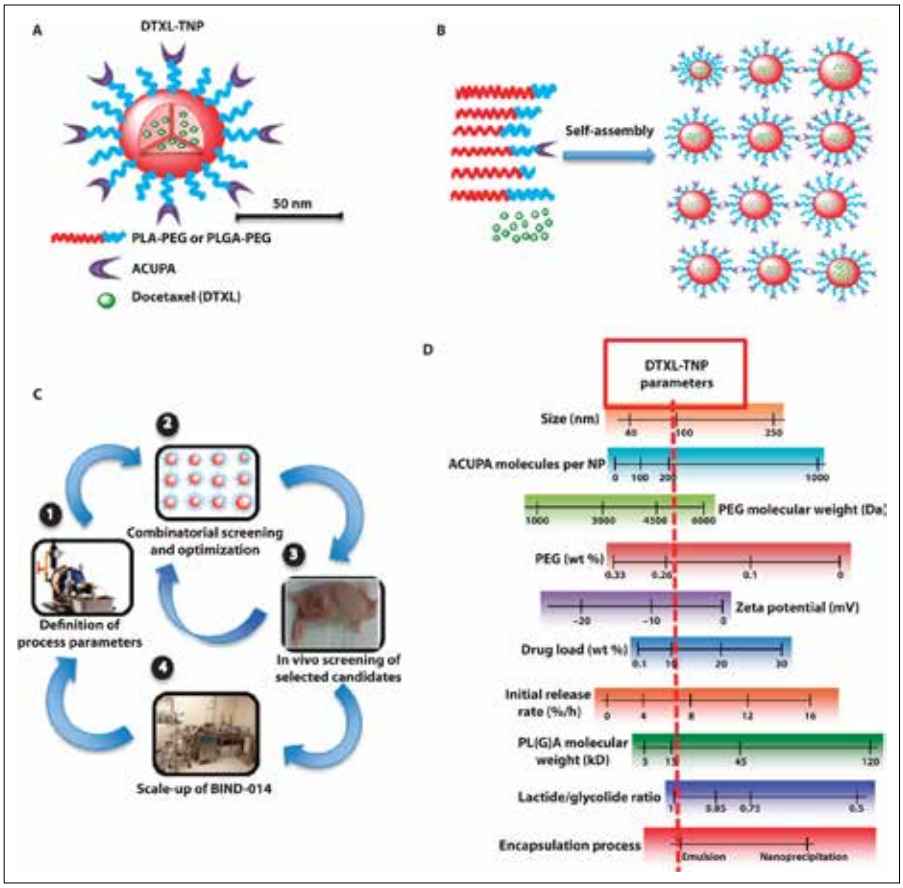
Development of targeted docetaxel nanoparticle therapy— Targeted polymeric nanoparticles (TNPs) have the potential to overcome the toxicity and efficacy limitations of traditional cytotoxic agents by delivering a greater fraction of the administered drug directly to cancer cells. However, TNPs have not advanced beyond early-phase testing in humans due to key challenges to define the optimal physiochemical parameters for multi-functional capabilities *in vivo*. To address these challenges, researchers in this group investigated a new approach to the development, optimization, and clinical manufacturing of TNPs. By varying the particle size, targeting ligand density, surface hydrophilicity (to protect from immune surveillance), drug loading, and drug release properties, Center researchers created a combinatorial library of more than 100 TNP formulations of prostate membrane surface antigen (PMSA)-targeted NPs containing the chemotherapeutic docetaxel (DTXL) for screening. Based on physicochemical properties, promising DTXL-TNPs were selected and tested in tumor-bearing mouse models. *In vivo* information was used for further optimization in DTXL-TNP composition and process. The optimized DTXL-TNP was shown to release DTXL in a controlled manner without associated toxicity to these animals. Further, this DTXL-TNP was shown to be efficacious in a mouse model of prostate cancer, slowing tumor growth to 26%, whereas free DTXL could not stop tumor growth over the same period of time[1]. Finally, a clinical study in patients with advanced solid tumors was carried out to determine tolerability in humans and to obtain an initial assessment of patient efficacy (data collected through clinical trials conducted on the lead candidate BIND-014 by Bind Therapeutics). Study results indicated that DTXL-TNP displays characteristics similar to those found in animal studies, an important finding when moving drugs to the clinical setting. Additional Phase I and II clinical trials were conducted on patients with or without castration resistant prostate cancer, prostate cancer, non-small cell lung cancer, and KRAS positive non-small cell lung cancer.

Diagnostic μ-NMR devices for sensitive and direct detection of proteins, circulating tumor cells, and microvesicles—There is a growing need for portable devices that offer fast, highly sensitive, and quantitative technologies to detect and profile cancer signatures in biological samples. New, miniaturized diagnostic magnetic resonance (DMR) devices were first developed by Center researchers to profile proteins and cells in unprocessed biological samples[2]. Most recently, a third generation version of this device (DMR-3) packaged a μ-NMR sensor with smartphone data readout and microfluidic sample handling into a device suitable for bedside use in the clinic. The sensor works by exploiting the difference in changes in the relaxation time of water molecules surrounding magnetic nanoparticles (MNPs) bound to the cell of interest from unbound nanoparticles. To improve DMR sensitivity for cell detection, the group synthesized and optimized MNPs exhibiting higher transverse relaxivity while maintaining small enough size (< 50 nm in hydrodynamic diameter) for optimal binding to the cell surface. Studies showed that by using these MNPs and targeting three established tumor markers (EGFR, HER2/neu or EpCAM receptors), DMR detected as few as two cancer cells in 1- μL sample volumes of unprocessed fine-needle aspirates of tumors and profiled the expression of several cellular markers in less than 15 min[3,4]. The panel was later expanded to four markers consisting of MUC-1, EGFR, HER2 and EpCAM; it had 96% accuracy, and results were returned within 60 minutes. Further studies involving this marker panel demonstrated detection of circulating tumor cells (CTCs) from whole and unprocessed blood in clinical samples, and showed this sensor outperformed CellSearch (a clinically approved method for CTC detection)[5]. Thus, this μ-NMR platform has the potential to benefit a broad range of diagnostic applications in clinical oncology.

Targeted tumor-penetrating siRNA nanocomplexes for credentialing the ovarian cancer oncogene ID4— RNA interference (RNAi) is a potential means to silence expression of candidate genes *in vivo*, particularly for "undruggable" gene products. However, systemic delivery of small interfering RNA (siRNA) to tumor has been challenging due to rapid clearance, limited tumor penetration and susceptibility to serum nucleases and endosomal entrapment. To overcome the challenges in systemic delivery of siRNA, Center researchers developed a platform for the discovery and initial validation of cancer targets. The technology combines a systematic effort to identify amplified and essential genes in human cancer cell lines and tumors with a novel modular delivery technology. Tumor-penetrating nanocomplexes (TPNs) that comprise siRNAs combined with tandem tumor-penetrating and membrane-translocating peptides enable the specific delivery of siRNAs deep into the tumor parenchyma. TPNs were used *in vivo* to evaluate inhibitor of DNA binding 4 (ID4) as a novel oncogene, following its discovery from a large scale screening effort called Project Achilles. Treatment of ovarian tumor-bearing mice with ID4-specific TPNs suppressed growth of established tumors and significantly improved survival. These observations not only credentialed ID4 as an oncogene in 32% of high-grade ovarian cancers, but also provided a framework for the identification, validation, and understanding of potential therapeutic cancer targets[6].

TRANSLATIONAL ACHIEVEMENTS

The consortium has conducted a number of clinical trials. All therapeutic clinical trials are listed at ClinicalTrials.gov (https://clinicaltrials.gov; NCT01792479, NCT01300533, NCT01812746, NCT01824303, NCT01051336, NCT01478893, NCT01158079, NCT00882180) and primarily involve the companies BIND, Taris, Selecta, and Alnylam. Approximately 250 patients have been enrolled in these clinical trials. Very active testing of blood from healthy human volunteers for the optimization of magnetic nanosensor was also carried out based on IRB protocols approved at Harvard and Massachusetts General Hospital.



Combinatorial screening and optimization of DTXL-TNPs. (A) Schematic of DTXL-TNP, a PSMA-targeted polymeric nanoparticle (NP) composed of a hydrophobic poly-lactic acid (PLA) polymeric core encapsulating docetaxel (DTXL) and a hydrophilic PEG corona decorated with small molecule (ACUPA) targeting ligands. (B) Generation of a library of DTXL-TNPs prepared by particle self-assembly. (C) Development and clinical translation of PSMA-targeted DTXL-TNPs. (D) Range of formulation parameters and physicochemical properties evaluated during evaluation of DTXL-TNPs, with optimized DTXL-TNP parameters and target parameters indicated by the red dotted line. Reprinted with permission from[1].

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Center for Translational Cancer Nanomedicine

NORTHEASTERN UNIVERSITY
PRINCIPLE INVESTIGATORS: VLADIMIR TORCHILIN PhD, DSc, AND NAHUM GOLDBERG, MD

OVERVIEW

The objective of this Center was to develop, characterize, and scale-up novel engineered multifunctional nanocarriers for targeted delivery of various drugs to treat pancreatic, ovarian, lung, prostate, and brain cancers. Comprised of four integrated Projects and three supporting Cores with well-connected goals, the Center aimed to develop combination therapies targeting refractory and multidrug resistant (MDR) tumors. The nanoparticle constructs developed were based upon liposomal and polymeric nanoparticles (NPs) and self-assembling nanosystems that carry nucleic acids, drugs, and/or imaging agents. These systems allow for co-delivery of therapeutic agents at high local concentrations directly to cancer cells. To date, a set of novel nanopreparations targeting MDR tumors and joint treatments involving chemotherapeutic nanopreparations and radiofrequency ablation were generated and characterized by the Center. Additional information about the Projects and Cores of this Center can be found at http://nano.cancer.gov/action/programs/northeastern.

SCIENTIFIC AND TECHNOLOGICAL ACHIEVEMENTS

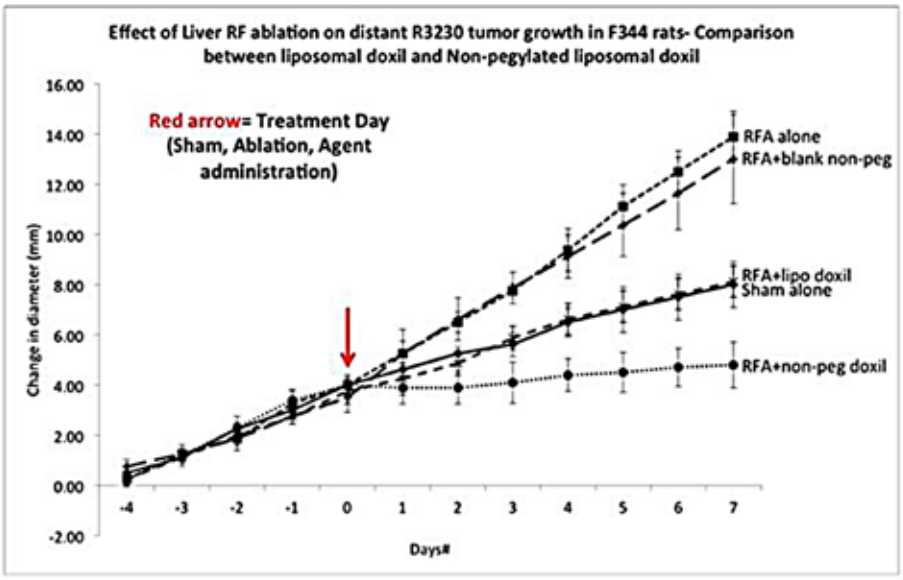
Stimuli-sensitive combination micelles targeting MDR tumors— Researchers at this Center generated a variety of nanoformulations that can respond to cues from the tumor microenvironment such as low pH, elevated redox status, high enzymatic activities, and low oxygen levels to promote controlled, targeted release of therapeutic agents. With the co-delivery of chemotherapeutic drugs and siRNAs to suppress drug resistance mechanisms, these stimuli-sensitive combination nanopreparations allow for the simultaneous targeting of multiple pathways and action through different mechanisms. When tested against MDR cancers, the nanopreparations demonstrated significantly enhanced therapeutic activity of these combination nanoformulations *in vitro* and *in vivo*[1-4].

Image-guided radiofrequency tumor ablation—In a joint application of chemotherapeutic nanopreparations and radiofrequency ablation (RFA), Center researchers also showed the alteration of nanocarrier properties can improve targeting of specific tissue reactions such as local growth factor production and suppression of unwanted post-RFA pro-oncogenic effects on distant tumors. Specific alterations were associated with more than doubled survival times in two mouse models using pro-apoptotic and/or heat-shock suppressive nanoagents. Further experiments demonstrated that targeting specific cellular populations such as macrophages might be an effective way to block unwanted off-target effects of RFA[5-7].

Redox-responsive type B gelatin nanovector for combination treatment—Another successful combinatorial approach developed by Center researchers involved an epidermal growth factor receptor (EGFR)-targeted redox-responsive gelatin nanoparticle system. This system modulates the release of plasmid DNA that expresses a tumor-suppressor protein (p53) and an anti-tumor chemotherapy drug (gemicitabine) for effective combination treatment of pancreatic ductal adenocarcinoma. This combined treatment could circumvent the limitations of previously developed single agent nanoparticle systems as evidenced by its ability to induce cell apoptosis[8].

TRANSLATIONAL ACHIEVEMENTS

This Center has closely collaborated with Nemucore Medical Innovations (NMI; http://www.nemucore.com, a clinical development company) to push their nanoformulations through the translational pipeline. Specifically, NMI's lead program, NMI-900, will be entering Phase II clinical trials in solid and hematological cancers during 2016. Two additional candidates based on multidrug resistant ovarian cancer research derived from the Center (NMI-300 and NMI-500) will be ready at some point in 2016 for entrance into Phase I clinical trials



Distant tumor growth stimulation after hepatic radiofrequency (RF) ablation is suppressed with adjuvant liposomal doxorubicin. RF ablation (RFA) of normal liver (as is performed in every clinical case) can stimulate distant subcutaneous R3230 breast tumor growth compared to sham treatment (no RFA). The addition of a single dose of adjuvant PEGylated liposomal doxorubicin (RFA + lipo Doxil) at the time of ablation can suppress this unwanted effect by increasing local periablational injury in partly injured liver and infiltrating cells. Use of adjuvant non-PEGylated liposomal doxorubicin formulation suppresses distant tumor growth even further (compared to all arms) such that development of the optimal nanoformulation can be used to maximize local injury and minimize systemic unwanted effects.

at Fox Chase Cancer Center. NMI is also collaborating with the Translational Genomics Research Institute to develop NMI-800, a targeted nanomedicine loaded with therapeutic molecules for patients suffering from glioblastoma.

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Nanomaterials for Cancer Diagnostics and Therapeutics

NORTHWESTERN UNIVERSITY
PRINCIPAL INVESTIGATORS: CHAD A. MIRKIN, PhD, AND STEVEN T. ROSEN, MD

OVERVIEW

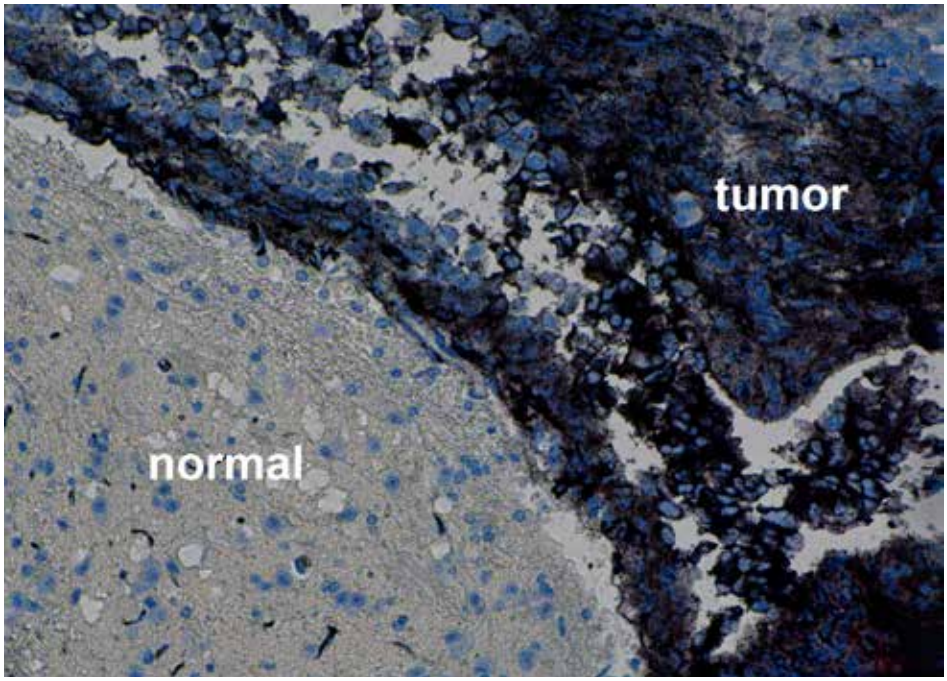
The Northwestern Center catalyzed discovery and development of transformative nanotechnology innovations for translation into cancer-relevant clinical applications. Operating primarily within a single university and organized around four interdisciplinary team Projects, the Center focused on developing: (1) NanoFlare diagnostic tests for circulating tumor cells in breast cancer, (2) magnetic contrast agents based on nanostructures or nanodiamonds for molecular imaging of brain and pancreatic cancer, (3) spherical nucleic acid (SNA) nanoparticle conjugates targeting brain and pancreatic tumors, and (4) fundamental research into three-dimensional cell culture matrices and high-resolution nanolithography. Researchers from this Center have contributed to the establishment of LS-CAT BioNanoprobe, a Life Science Collaborative Access Team at Argonne National Laboratory's Advanced Photon Source, which is devoted to sub-100 nm high-resolution X-ray imaging and spectroscopy for life sciences. The Center was also able to realize over $33 million in new grant funding from other sources. The combination of nanotechnology-based diagnostics, imaging agents, and therapeutics developed by the Northwestern Center has significant potential for clinical utility, and demonstrated use in many other forms of cancer research and treatment. The success of this effort is evidenced by a few of the achievements noted below, as well the licensing of 49 technologies to industry, and the launching of four new companies based on the Northwestern Center research. Additional information on this Center's Projects and Cores can be found at http://nano.cancer.gov/action/programs/northwestern and http://www.nu-ccne.org.

SCIENTIFIC AND TECHNOLOGICAL ACHIEVEMENTS

Spherical nucleic acids nanoplatform for gene silencing— Researchers at the Northwestern Center have completed the first preclinical studies on SNA nanoparticle conjugates as a treatment for glioblastoma. SNAs are gold nanoparticles surrounded by densely packed, highly organized nucleic acids tailored to recognize any sequence. SNAs were designed to deliver glioma-suppressive miRNA-182 and siRNAs targeted to silence Bcl2L12, an overexpressed protein that plays an important role in driving the pathogenesis of glioblastoma and mediating therapeutic resistance[1,2]. Gene silencing using siRNA and miRNAs conjugated to these SNAs represents a promising new approach for systemic RNA interference-based therapy of this aggressive malignant brain tumor in humans. This project is one of the first to report stable and robust RNAi delivery to intracranial tumors, as SNAs have the capacity to cross both an intact and tumor-compromised blood brain barrier, and has helped develop SNAs as a platform for biotherapeutic gene silencing in the central nervous system.

NanoFlares for detection of live tumor cells in blood— Researchers from this Center have also used DNA-functionalized SNAs to develop and validate a fluorescent detection method, which they termed NanoFlare technology. NanoFlares can be used to recognize unique populations of circulating tumor cells in the peripheral blood, including from breast cancer patients, that can be isolated live and used for further downstream analysis[3]. This capability may provide a powerful new way to assess the risk of individual patients for cancer recurrence and response to treatment. NanoFlares are currently commercially available for use as intracellular probes to detect and quantify RNA in living cells. There are now over 1700 commercial NanoFlare detection probes (See EMD Millipore SmartFlares ™).

Carbon nanomaterials in treatment of chemotherapy-resistant cancers—Through studies of liver and breast cancer models *in vivo*, Center researchers found that nanodiamonds linked to the chemotherapeutic doxorubicin significantly reduced the size of tumors in mice, and increased survival with minimum off-target toxicity. This was the first work to demonstrate the translational potential of nanodiamonds in the treatment of chemotherapy-resistant cancers. Rodent and



High magnification image of a brain section showing the transition between tumor and normal brain. Silver staining (dark spots) indicates the presence of large amounts of spherical nucleic acid (SNA) gold nanoparticles in the tumor.

non-human primate studies confirmed nanodiamond safety—and a maximum tolerated dose (MTD) study was completed. Long-term toxicity (blood, urinalysis, pathology) evaluations indicated nanodiamonds are well tolerated. These studies represent a significant milestone towards clinical validation[4-6].

TRANSLATIONAL ACHIEVEMENTS

One of the most relevant translational achievements of the Northwestern Center was the preclinical evaluation of SNAs designed to target Bc12L12 and steps taken towards clinical translation. Non-human primate and non-primate toxicology studies were completed, with no significant clinical observations. These results will enable the filing of an Investigational New Drug application with the U.S. Food and Drug Administration, a major step towards human clinical trials. For the NanoFlare project, Center investigators have also begun patient recruitment to study the diagnostic and prognostic potential of this platform.

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Stanford University Center for Cancer Nanotechnology Excellence

STANFORD UNIVERSITY
PRINCIPAL INVESTIGATORS: SANJIV SAM GAMBHIR, MD, PhD, AND SHAN WANG, PhD

OVERVIEW

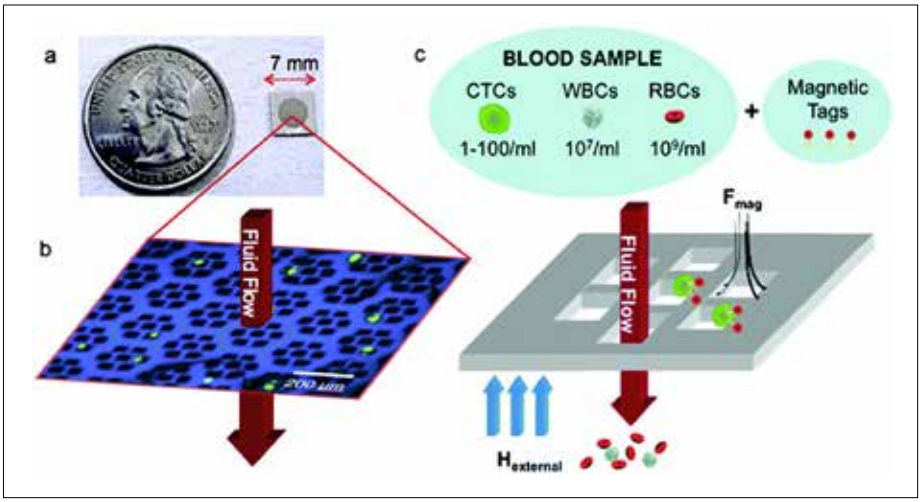
The Stanford Center's research program was centered on the vision that *in vitro* diagnostics used in conjunction with *in vivo* diagnostics can markedly impact future cancer patient management. The merger of nano-based *in vitro* and *in vivo* technologies was directed toward enabling earlier detection of ovarian cancer and prediction and monitoring of response to lung cancer therapy. The Center had two major arms: (1) *in vitro* genomic/proteomic/cellomic nanosensors and (2) *in vivo* molecular imaging with primarily gold-based nanoparticles and magnetic resonance imaging (MRI) with novel self-assembling nanoparticles. Technologies were developed to isolate, capture, and enrich circulating tumor cells from patients prior to initiation of therapy as well as during treatment to interrogate these cells in a highly detailed manner. Changes at the molecular level were measured within individual cells, on the cell membrane, and in cell secretions. Measuring these changes is critical to enable earlier detection and therapy monitoring using either *in vitro* sensors or molecular imaging. The Center's interactive and cohesive program imagined, invented, and innovated for the benefit of cancer patients, aiming to dramatically improve cancer rates while reassuring patients that their treatment is effective. Additional information about the Projects and Cores can be found at http://nano.cancer.gov/action/programs/stanford and http://mips.stanford.edu/grants/ccne-t/projects.html.

SCIENTIFIC AND TECHNOLOGICAL ACHIEVEMENTS

Nano-sensing platform—The Center supported development of a nano-sensing platform that relies on giant magnetoresistive (GMR) elements to detect biomolecules labeled with magnetic nanoparticles, analogous to optical readout of a fluorescent label in an ELISA immunoassay. Through simultaneous use of multiple GMR elements, this multiplexed platform offers a wide dynamic detection range that is not confounded by background signal from biological matrices, and provides high sensitivity for dependable measurement of multiple cancer biomarkers in blood or serum. This platform was further validated for use in the early detection of ovarian cancer by using patient biomarker panels (HE4, AGR2, C1orf, VTCN and PEBP) identified in collaborations through the Canary Foundation and the Fred Hutchinson Cancer Center. The device is compatible with point-of-care applications and has been commercialized by MagArray, Inc. (http://magarray.com).

Photoacoutic nano-imaging platform—Center researchers have developed a nanoimaging platform based on enhanced ultrasound imaging capabilities and targeted nanoparticles for non-invasive assessment of prostate cancer. The platform consists of an imaging probe that combines a fiber optic light guide with a two-dimensional capacitive micromachined ultrasound transducer array to enable transrectal photoacoustic imaging of the prostate. This imaging probe is currently being tested in clinical trials at Stanford University Cancer Institute (https://clinicaltrials.gov/ct2/show/NCT02365883). Commercialization of a fully three-dimensional photoacoustic computed tomography instrument for preclinical studies is being pursued by industrial partner Endra, Inc (http://www.endrainc.com), a company spun out from the Center.

Self-assembling nanoparticles—Center researchers developed and demonstrated efficacy of controlled self-assembly of small molecules into fluorescent nanoparticles in human xenograft mouse models receiving chemotherapy. Activated caspase-3/7, a marker of cell death, triggers a condensation reaction in molecular precursors developed by Center researchers, generating fluorescent nanoparticles in apoptotic cells and tumor tissue responding to chemotherapy. The fluorescent signal acts as an indicator of caspase activity, enabling non-invasive monitoring of therapeutic effectiveness[1]. The approach could be generalizable to other enzymatic targets and even other diseases.



Overview of the magnetic sifter, nano-sensing platform device. (a) Single magnetic sifter die and (b) optical micrograph showing a section of the patterned pore array (artificially colored blue) and cultured H-1650 lung tumor cells (green) captured by the magnetic sifter. Pores are 40 × 40 μm squares. (c) Capture principle. A whole blood sample is labeled with magnetic tags and pumped through the pores during the application of an external magnetic field. Magnetically labeled target cells are captured at the pore edges where high magnetic field gradients exist. Unlabeled cells pass through the pores.

TRANSLATIONAL ACHIEVEMENTS

The underlying ideas driving the Center's clinical translational efforts were rooted in fundamental principles of cancer biology and the belief that therapies can be monitored by changes in cancer cell gene expression that lead to changes in proteins expressed on the surface of cancer cells, and secretion of proteins and micro/nanovesicles from cancer cells. Furthermore, pre-clinical *in vivo* cancer models and cancer patients that respond to therapy, compared to those that do not, should show different protein expression profiles on the surface of their cancer cells and in the blood. Silica-based Raman nanoparticles and endoscopic imaging technology developed using these principles are at an advanced stage of review by the US Food and Drug Administration for colorectal cancer imaging. Researchers continue to work with the Nanotechnology Characterization Laboratory and the Food and Drug Administration to bring these to the clinically translated stage. The associated Raman endoscope has been tested in human patients at Stanford and the photoacoustic instrument has been used in prostate and breast cancer patients at the Stanford Hospital and Clinics.

The Center has spun out seven companies (MagArray Inc., ImaginAB, Zymera, Endra Inc., Nine Point Medical, CellSight and Nvigen) to commercialize nanotechnologies developed by its researchers. Five of these companies have both clinical and research grade products on the market.

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Carolina Center for Cancer Nanotechnology Excellence

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
PRINCIPAL INVESTIGATORS: JOSEPH DESIMONE, PhD, AND JOEL TEPPER, MD

OVERVIEW

The objective of this Center was to address two key aspects of a successful cancer control strategy: targeted delivery of multimodal therapies and early detection. Within this objective, five Projects and three supporting Cores were dedicated to the development of targeted methods for the delivery of biological, chemo- and radiotherapies against lung and brain cancers, including the delivery of multiple payloads, and the augmentation of a carbon nanotube-based imaging device for early detection and characterization of breast cancer. Collectively, these efforts drove the preclinical validation of several nanoparticle-based therapies and diagnostic devices towards clinical and commercial applications. To facilitate the clinical translation and commercialization of these technologies, the Center developed industrial partnerships with the following companies: Liquidia, Inc. (engineered vaccines and inhaled therapeutics, http://www.liquidia.com), Qualiber, Inc. (gene-based and small molecule drug delivery technologies, http://www.qualiberinc.com), and Particle Sciences (drug delivery formulations and support, http://www.particlesciences.com). Additional information about the Projects and Cores can be found at http://nano.cancer.gov/action/programs/unc and http://nano.unc.edu.

SCIENTIFIC AND TECHNOLOGICAL ACHIEVEMENTS

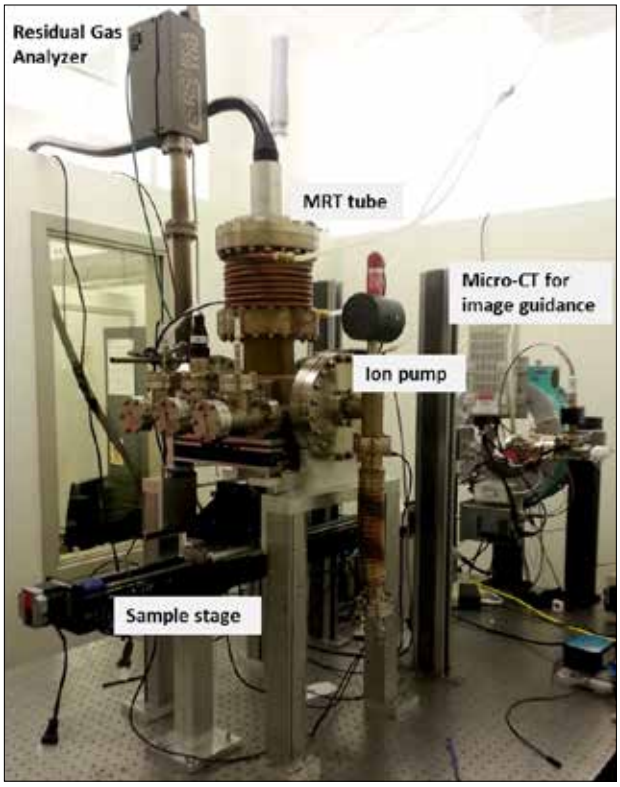
Carbon nanotube (CNT)-based systems for radiation therapy and diagnostic medical imaging—Synchrotron microbeam radiation therapy (MRT) is an experimental treatment with a high therapeutic ratio between cancerous tumors and normal tissue. Until recently, the outsized dimensions of the technology for generating the beams have limited its clinical use. Using carbon nanotube-based field emission X-ray technology, researchers at this Center developed the first tabletop microbeam irradiator, which is currently being tested as a brain cancer treatment in preclinical trials. Building on this success, a second-generation MRT system was developed and shown to irradiate multiple lines simultaneously at a higher dose rate per line, resulting in an almost 20 times higher dose rate[1]. Subsequent developments included an image guidance technique for targeted delivery of narrow microbeams to small tumors, and a nanoparticle-terminated fiber-optic detector for real-time microbeam dosimetry to measure the continuous dose rate at the microbeam peak and the lateral beam shape[2,3].

Using the same technology, Center researchers also developed a CNT x-ray based stationary digital breast tomo-synthesis (s-DBT) system for early detection of breast tumors. Current DBT scanners use a single rotating x-ray source that requires long scanning times, and can lead to patient discomfort from breast compression, motion blurring, system instability, and limited spatial resolution. The CNT x-ray based s-DBT system can overcome these limitations by utilizing a stationary x-ray source array that generates beams from different viewing angles without mechanically moving the x-ray tube, eliminating motion blurring[4].

Cisplatin-containing hydrogel nanoparticle—PEGylation (coating with polyethylene glycol) is a common surface modification approach to improving the stability and *in vivo* performance of nanoparticles for systemic drug delivery. Researchers at this Center systematically investigated the effect of surface PEG density on the loading and release of cisplatin from PRINT hydrogel nanoparticles (PRINT-Platin)[5]. The Center researchers demonstrated the PEGylation density-dependent loading of cisplatin for PRINT hydrogel nanoparticles and analyzed its effect on circulation persistence and sustained drug release *in vivo* to find the optimal formulation. Presently, PRINT particles are in the last stages of preclinical testing in orthotopic lung carcinoma.

TRANSLATIONAL ACHIEVEMENTS

The most relevant translational achievements included the construction of the first prototype s-DBT system. It has been successfully calibrated and has passed all electrical and radiation safety tests. The prototype is currently installed in the



Picture of the desktop microbeam radiation therapy (MRT) system with integrated micro-CT mounted on the optical table.

mammography clinic at the UNC Cancer Hospitals, where it was shown to produce better image quality at the same entrance dose as that of 2D mammography (standard detection device). This system could greatly enhance prognosis by providing a wider angular range, better sensitivity, and higher spatial resolution that can improve the detection of microcalcifications, potential precursors to invasive cancer. Currently, there are several ongoing clinical trials of this device (https://www.clinicaltrials.gov; NCT01773850, NCT02008032).

Additionally, two drug delivery systems developed by the Center have cleared testing at the NCI Nanotechnology Characterization Laboratory (NCL). The optimal formulation based on a novel lipid/calcium/phosphate nanoparticle (LCP) platform was shown to be effective in triggering apoptosis in tumor cells and in dramatic inhibition of tumor growth, while demonstrating limited off-target toxicity[6,7]. SBIR Phase II funding has been secured for the potential commercialization of this formulation. The other was based on a highly scalable oil-core ("BTM") nanocapsule. When compared to Taxol, the best BTM formulation demonstrated prolonged circulation and superior antitumor efficacy in an orthotopic non-small cell lung cancer mouse model[8,9]. The UNC Cancer Research Fund has also provided $800,000 to the most advanced particle system for further Investigational New Drug enabling studies.

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Texas Center for Cancer Nanomedicine

UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER
PRINCIPAL INVESTIGATORS: DAVID G. GORENSTEIN, PhD, MAURO FERRARI, PhD, ANIL SOOD, MD, GABRIEL LOPEZ-BERESTEIN, MD, AND JENNIFER L. WEST, PhD

OVERVIEW

The objective of the Texas Center was to develop and translate nanotechnology-enabled innovations for improving the outcome of patients with ovarian or pancreatic cancer. The main research focus areas were targeted multifunctional nanotherapeutics and post-therapy monitoring tools for these cancer types, early pancreatic cancer diagnosis using *in vitro* assays and devices, and *in vivo* imaging techniques. The combination of four Projects and three Cores supported this Center. Importantly, the work derived from the Center included capabilities to scale-up nano- and micro-particles in-house via the development of a current Good Manufacturing Practice (cGMP) facility, a requirement for successful bench to bedside translation, and partnerships with industry—AAVP Biosystems (https://gust.com/companies/aavp_biosystems_inc). Additional information on the Center's Projects and Cores can be found at http://nano.cancer.gov/action/programs/uthsc.

SCIENTIFIC AND TECHNOLOGICAL ACHIEVEMENTS

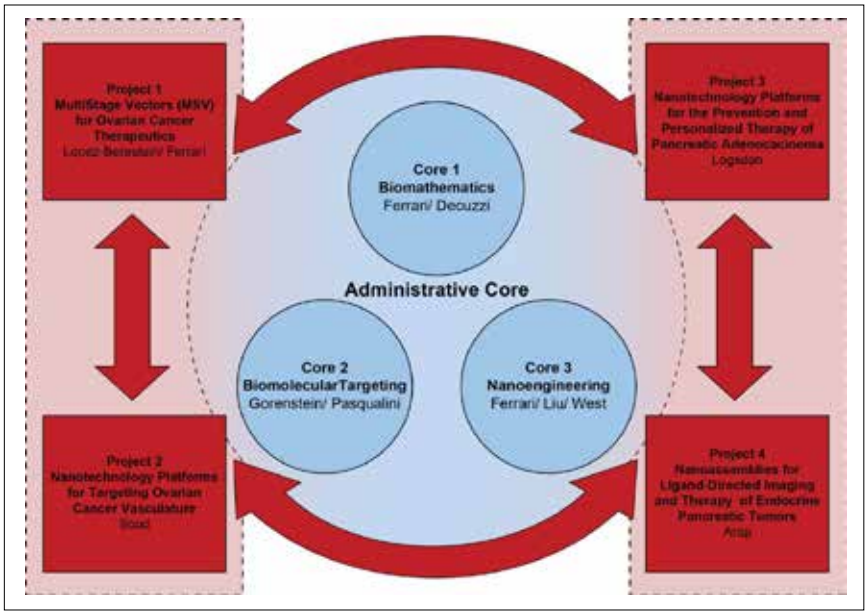
Multistage delivery system for ovarian cancer therapeutics— This delivery system consists of a biodegradable porous silicon-based particle that can transport siRNAs or small molecule inhibitors incorporated into nanoliposomes. Center researchers have demonstrated the effectiveness of this multistage vector (MSV) system loaded with nanoliposomes containing siRNAs that target EphA2, which is overexpressed in many cancers including ovarian cancer. In this work, they demonstrated that the multistage approach was successful for tumor tissue-targeted delivery and sustained release of siRNA in murine cancer models. Silencing was sustained for up to two weeks following a single administration, and therapeutic efficacy could be attained with less frequent administration, as compared to the liposomal formulation alone[1].

Tumor vasculature-targeting delivery system—To selectively target the tumor vasculature, researchers from this Center have been working on a bead-based library selection approach using human tumor-derived endothelial cells to identify highly selective thioaptamer ligands for targeted delivery of nanoparticles[2]. They have demonstrated that a chitosan nanoparticle attached to a targeting thioaptamer was specific *in vivo* for the tumor vasculature, and was more effective than chitosan nanoparticles alone for delivery of siRNA therapeutics into the tumor microenvironment. They also demonstrated that targeting the tumor vasculature is an effective strategy for thioaptamer-conjugated MSV to deliver siRNA to tissues that are normally hard to reach such as the bone marrow[3].

Combination treatment for pancreatic cancer—After discovering that pancreatic stellate cells (involved in pancreatic cancer pathogenesis) shared characteristics with monocyte-macrophage lineage (MML) cells, researchers from this Center tested whether these stellate cells would be affected by MML cell inhibitors. *In vivo*, these inhibitors inactivated pancreatic stellate cells, reduced fibrosis, inhibited tumor growth, and increased tumor cell death in a mouse model of pancreatic cancer. These anti-tumor effects were enhanced when the inhibitors were combined with albumin-bound paclitaxel (FDA approved nab-paclitaxel, Abraxane). These results will support further studies with the MSV system, and suggest that targeting pancreatic stellate cells and tumor cells with MML cell inhibitors, in combination with Abraxane, may be a novel therapeutic approach[4].

TRANSLATIONAL ACHIEVEMENTS

One of the most important translational achievements of the Texas Center is the submission of an Investigational New Drug application to the U.S. Food and Drug Administration for the nanoliposomal formulation of EphA2 siRNA. This formulation will soon enter a Phase I, first in human clinical trial and has the potential to yield important data relevant to other genes that are traditionally considered undruggable.



Interaction of Texas Center for Cancer Nanomedicine Projects and Goals.

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Nanobioconjugate Based on Polymalic Acid for Brain Tumor Treatment

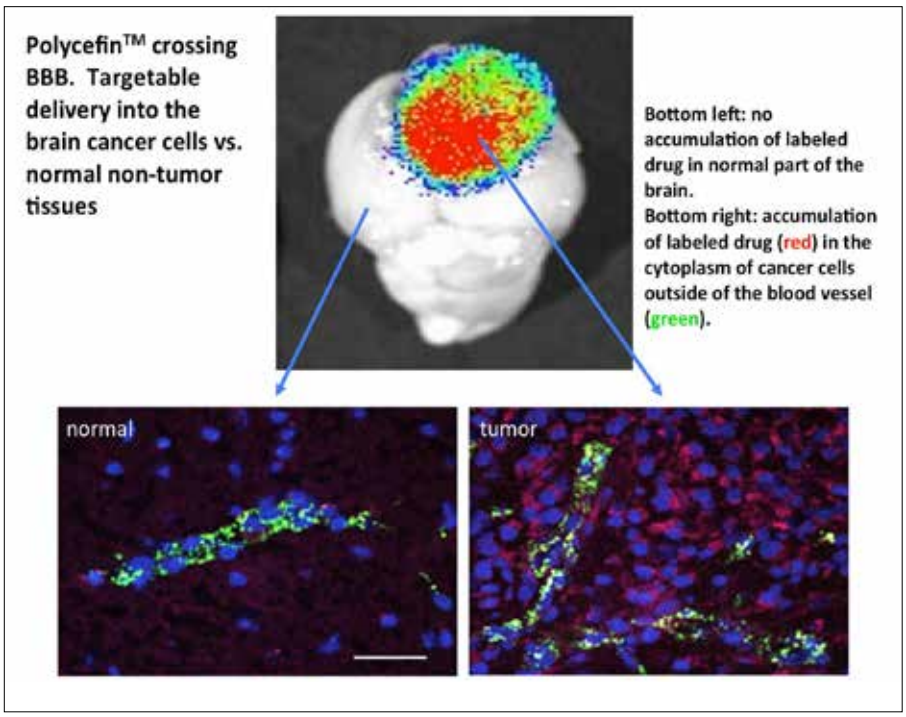
CEDAR-SINAI MEDICAL CENTER
PRINCIPLE INVESTIGATOR: JULIA Y. LJUBIMOVA, MD, PhD

OVERVIEW

The goal of this platform project was to develop a polymalic acid (PMLA)-based nanodelivery system to improve treatment outcomes in patients with gliomas that are largely incurable by current therapy. Obtained from a slime mold, PMLA forms the backbone of an antitumor nanobioconjugate drug, Polycefin™ family, developed by the platform investigators and shown to be non-toxic, non-immunogenic, modifiable, and most importantly, able to cross the blood brain and blood tumor barriers. Anti-brain cancer nanobioconjugates can be modified with various functional components for targeting, controlled release and tracking *in vivo*. The investigators aimed to optimize antitumor nanobioconjugates for clinical use by performing detailed physicochemical characterizations and preclinical evaluations. In the longer term, the development of effective Polycefin nanodrugs and an understanding of the underlying functional mechanisms could result in potent treatments for lethal gliomas that can be coupled to other emerging treatment strategies. Information about this platform award can be found at http://nano.cancer.gov/action/programs/platforms/cedarsmc.asp and Dr. Ljubimova's website at http://www.cedars-sinai.edu/Research/Faculty-Directory/Bios/Julia-Ljubimova-MD-PhD.aspx.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

The Ljubimova group has designed a prototype of an anti-tumor nanobioconjugate for targeted delivery of morpholino antisense oligonucleotides (AON) that block the synthesis of a protein required for glioma growth, laminin-411. By attaching two AONs against laminin-411 and antibodies that target brain endothelial and tumor cells through the transferrin receptor, the group demonstrated the ability of targeted Polycefin to cross the blood brain barrier, release AONs into the cytoplasm of cells, and suppress primary and metastatic brain cancers after systemic injection[1-5]. Studies further aimed to elucidate the molecular mechanisms of drug release in the cytoplasm via an attached pH-activated endosomal escape moiety, which was found to be due to induced membrane leakage[2,6,7]. These results were used to guide the selection of a lead nanodrug candidate in preparation for advanced preclinical studies of the polymalic acid nano platform, and are relevant to the development of other nanodrugs intended for delivery of oligonucleotides and drugs that act in the cytoplasm[8-10]. Efforts within this platform were also focused on moving the PLMA platform and Polycefin™ closer to clinical application[4,10]. The most significant achievement in this area was the completion of comprehensive toxicity studies of the PMLA platform and Polycefin nanodrug, necessary for an Investigational New Drug submission to the U.S. Food and Drug Administration.



Data related to this image can be found in Ding et al.[1]

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5. Patil, R., et al. MRI virtual biopsy and treatment of brain metastatic tumors with targeted nanobioconjugates: nanoclinic in the brain. ACS nano 9, 5594-5608 (2015).
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Targeting SYK Kinase in B-lineage ALL with CD19 Specific C-61 Nanoparticles

CHILDREN'S HOSPITAL LOS ANGELES
PRINCIPLE INVESTIGATOR: FATIH UCKUN, MD, PhD

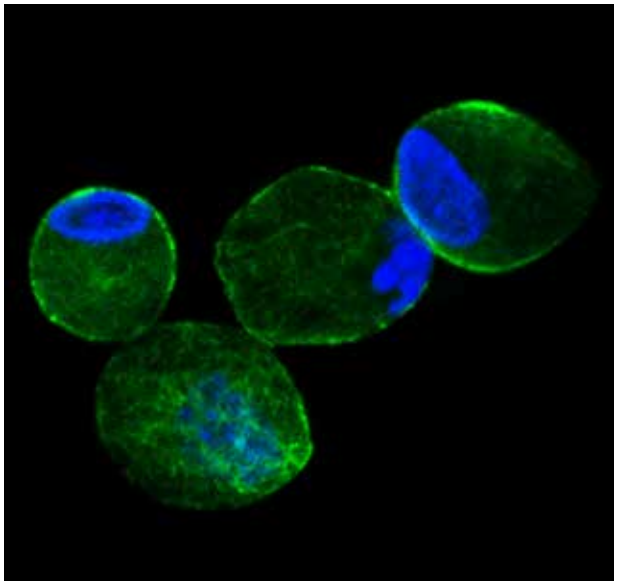
OVERVIEW

The main objective of this platform project was to design effective nanoparticle-based treatment strategies for B-lineage acute lymphoblastic leukemia (ALL), the most common form of childhood cancer. Despite major improvements in the treatment of B-lineage ALL, achieving long-term survival in patients who fail frontline chemotherapy regimens remains an unmet medical need. Dr. Uckun's laboratory showed that C-61 (also known as C61 and SYKINH-61) could act as an inhibitor of spleen tyrosine kinase (SYK), a promising therapeutic target in chemotherapy-resistant B-lineage ALL and other diseases. Platform investigators hypothesized that encapsulating C-61 in liposomal nanoparticle therapeutics may further improve its potency and broaden its therapeutic window especially if the treatment is combined with standard chemotherapy or radiation. Within this objective, specific goals were set to develop iterative generations of nanoparticle constructs of C-61 to improve the formulation. This approach could provide a foundation for therapeutic innovation against therapy-refractory leukemias and, when combined with standard anti-leukemic chemo- or radiotherapy, could lead to more effective and less toxic anti-leukemic treatment strategies. Information related to this platform award can be found at http://nano.cancer.gov/action/programs/platforms/chla.asp and Dr. Uckun's website at https://www.linkedin.com/in/fatihuckun.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

SYK is a master regulator of apoptosis, and its anti-apoptotic function is implicated in several hematological malignancies including B-lineage ALL. Researchers of this CNPP award targeted SYK for personalized nanotherapy of childhood leukemia. Through their work they found that C-61 is a potent and selective SYK inhibitor that can induce apoptosis in radiation-resistant human B-lineage leukemia/lymphoma cells[1]. Subsequently, their work focused on generating targeted liposomal nanoparticles to enable more specific delivery of C-61 to ALL cells[2]. Because the CD-19 receptor is expressed on radiation-resistant, aggressive B-precursor ALL cells, antibodies specific for this receptor were used to decorate C-61-loaded nanoparticles to generate targeted C-61 nanoparticles, which was more effective than their untargeted counterpart in inducing apoptosis in ALL cells[3].

Identification and pre-clinical evaluation of stable and potent lead CD-19-specific C-61 nanoparticle formulations in mouse models were the most relevant translational achievements of this award. The development of cell-type specific nanoparticles targeting the SYK-dependent anti-apoptotic survival mechanism in leukemic cells was a significant step towards achieving effective systemic delivery of this formulation, and increased intratumoral/intracellular delivery. Dr. Uckun's and his colleagues' ongoing work, which is focused on enhancing the efficacy of this nanoformulation by altering the tumor microenvironment, may also provide generally applicable insights into improved drug delivery.



Confocal microscopy images of ovarian cancer cells treated with the liposomal C-61 nanoparticle for 24 hours. Cancer cells show changes consistent with nanoparticle-induced apoptosis (programmed cell death), including shrinkage and fragmentation of nuclei and destruction of tubulin cytoskeleton. Blue: DAPI staining of nucleus, Green: tubulin.

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Toxicity and Efficacy of Gold Nanoparticle Photothermal Therapy in Cancer

EMORY UNIVERSITY
PRINCIPLE INVESTIGATORS: DONG M SHIN, MD, AND MOSTAFA EL-SAYED, PhD

OVERVIEW

The objective of this platform project was to optimize plasmonic photothermal therapy (PPTT) for maximum effectiveness and minimal toxicity in the treatment of squamous cell carcinoma of head and neck cancer (SCCHN). Presently, these patients have little or no treatment options after failure of standard chemotherapy, surgery, and/or radiation. Elongated gold nanoparticle (gold nanorod) PPTT can overcome challenges facing conventional treatment such as drug resistance and surgical infections while minimizing toxicity to healthy cells. Researchers of this platform award investigated gold nanoparticle surface properties and tumor targeting ligands along with different laser treatment strategies to identify the most favorable parameters for low dose near-infrared (NIR) light tumor ablation. Findings were validated in xenograft mouse models, and extensive pharmacokinetic and biodistribution studies were performed to establish a safe and effective treatment methodology for *in vivo* application of these gold nanorods. Information about this platform award can be found at http://nano.cancer.gov/action/programs/platforms/emory.asp, https://winshipcancer.emory.edu/bios/faculty/shin-dong-moon.html, and http://www.chemistry.gatech.edu/faculty/el-sayed.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

Platform researchers have synthesized high quality gold nanorods with high yield. Because the choice of nanorod size (in length x width) is a compromise between the total amount of light absorbed and the fraction that is converted into heat, platform researchers coupled extensive modeling of gold nanorod field and heat generation under laser irradiation with *in vitro* PPTT studies to determine a lead gold nanorod candidate for *in vivo* studies[1]. PPTT methodology was also optimized during this project; parameters considered included nanorod dosage, laser power, route of administration (intratumoral vs. intravenous), and frequency of administration (single vs. multiple treatments)[2]. *In vivo* efficacy studies found significant tumor growth inhibition in xenograft models of SCCHN, with no serious off-target toxicities noted, and showed no long term toxicity for up to fifteen months in mice. These studies were also used to modify a tumor pharmacodynamics model to describe the kinetics of SCCHN human xenograft tumor growth in mice under treatment with PPTT and derive a maximal dose of gold nanorods for treatment.

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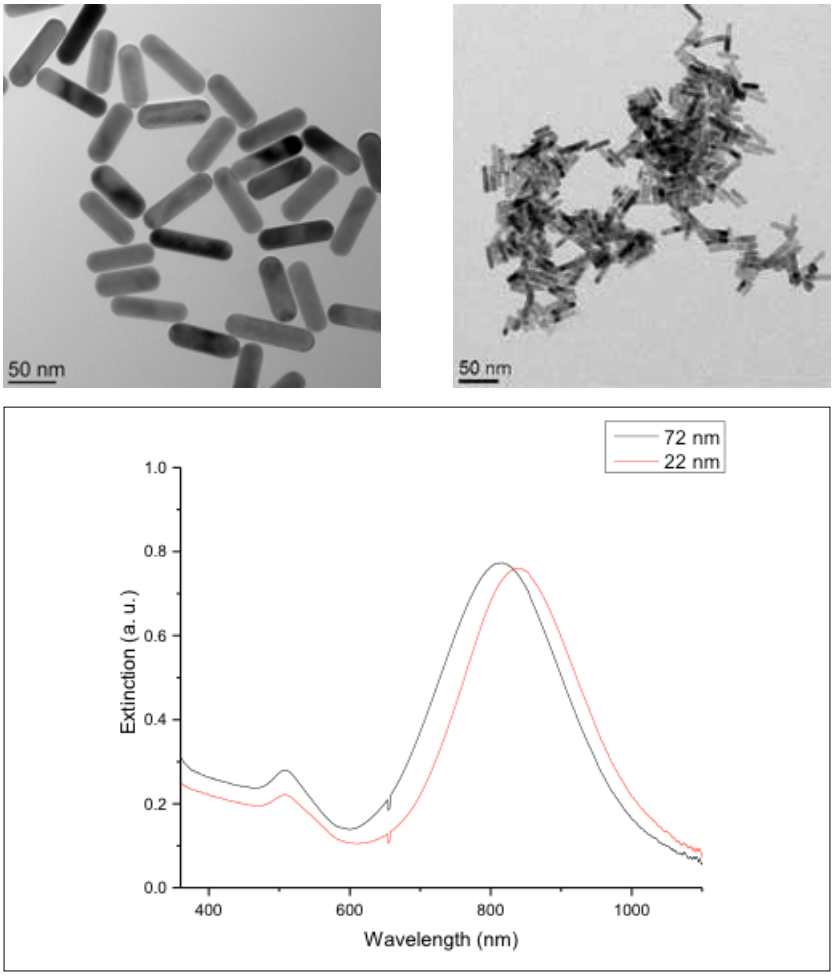
2. Ali, M.R., Panikkanvalappil, S.R. & El-Sayed, M.A. Enhancing the efficiency of gold nanoparticles treatment of cancer by increasing their rate of endocytosis and cell accumulation using rifampicin. Journal of the American Chemical Society 136, 4464-4467 (2014).



(a) Transmission electron microscopy of large rods (length 72±7 x width 19±4 nm), (b) small rods (length 22±3 x width 6±1 nm) of cetyltrimethylammonium bromide-stabilized gold nanorods. (c) Ultraviolet-visible absorbance spectra of the large (length 72 nm, black) and small (length 22 nm, red) gold nanorods. The surface plasmon resonance peaks of the large and small gold nanorods were 767 nm and 797nm, respectively.

Theranostic Nanoparticles for Targeted Treatment of Pancreatic Cancer

EMORY UNIVERSITY
PRINCIPLE INVESTIGATORS: LILY YANG, MD, PhD, AND HUI MAO, PhD

OVERVIEW

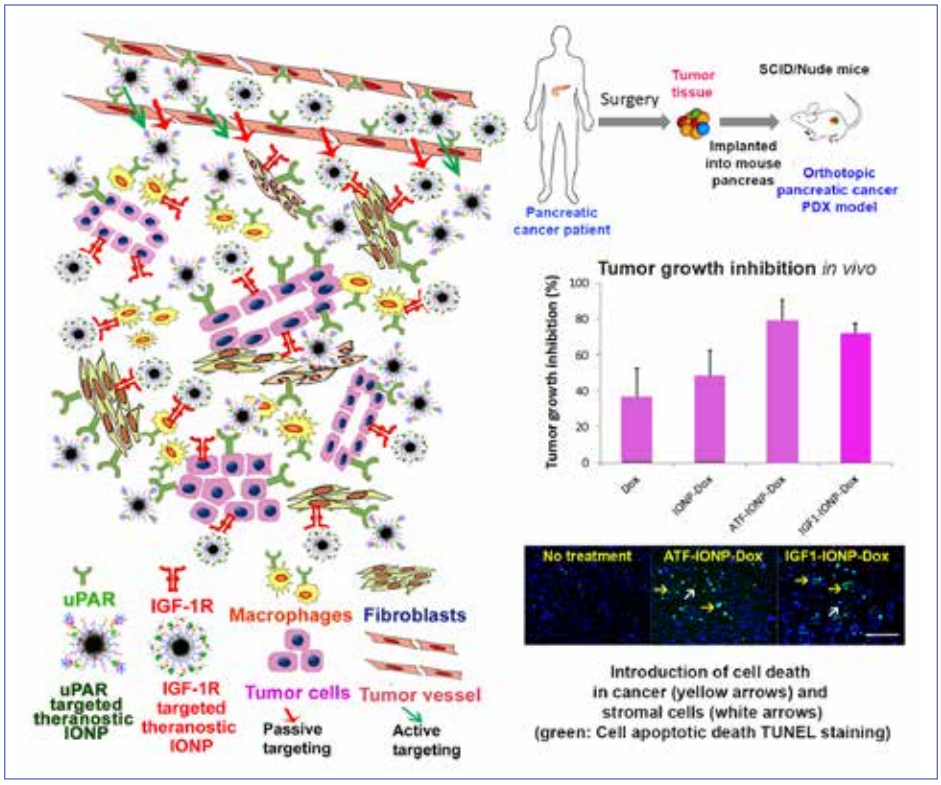
The objective of this platform project was to develop a multi-functional theranostic iron oxide nanoparticle (IONP) platform that combines tumor imaging and tumor targeted drug delivery to overcome physical and intrinsic barriers that confer drug resistance in pancreatic cancer. Engineered and surface functionalized theranostic IONPs targeted cell surface receptors that are highly expressed in both pancreatic cancer and tumor stromal cells, to break the tumor stroma barrier and improve intratumoral delivery and distribution of the therapeutic agents. The targeted delivery of theranostic IONPs not only could be monitored by magnetic resonance imaging, but also facilitated the direct interaction and internalization of drugs into pancreatic cancer cells, resulting in enhanced therapeutic responsiveness. Ultimately, the results of this project may further advance the field of nanomedicine and clinical translation of versatile nanotheranostic platforms. Information related to this platform award can be found at http://nano.cancer.gov/action/programs/platforms/emorysm.asp, and Drs. Yang's and Mao's websites at https://winshipcancer.emory.edu/bios/faculty/yang-lily.html and www.corelabs.emory.edu/csi/about_us/bios/mao_hui.html.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

In this project, platform researchers confronted the pancreatic tumor stroma by identifying and validating cell surface receptor targets expressed on both pancreatic ductal carcinoma and tumor stromal cells—urokinase plasminogen activator receptor (uPAR) and insulin-like growth factor 1 receptor (IGF-1R). IONPs were functionalized with the receptor-binding domain of uPA or human IGF-1 to enable efficient delivery of IONP-bound drugs into pancreatic tumors with cells expressing these receptors. The researchers then demonstrated the target specificity of intravenously or intraperitoneally delivered nanoparticles using near infrared optical and magnetic resonance imaging, as well as histological and chemical analyses. Studies were done using various types of animal models developed by the platform project team, including tumor cell line, patient tissue derived xenograft (PDX), and transgenic mouse models[1,2]. Multimodal *in vivo* imaging strategies and techniques were also developed to improve monitoring of nanoparticle-drug delivery and assessment of tumor response to therapy[1-3].

Significant translational progress was made on the development of targeted theranostic IONPs carrying single or multiple therapeutic agents for chemo- and RNAi therapy[1,2,4]. The platform researchers demonstrated targeted delivery of nanoparticle-drug carriers into pancreatic cancer and tumor stromal cells and efficient induction of cell death in those cell populations in pancreatic cancer PDX models. They also discovered the differential effects of conventional chemotherapy and theranostic IONP treatment on the amount, localization and types of tumor associated macrophages. To further clinical translation, they investigated systemic toxicity, dose, biodistribution, and pharmacokinetics of theranostic nanoparticles in animal models.

1. Lee, G.Y., et al. Theranostic nanoparticles with controlled release of gemcitabine for targeted therapy and MRI of pancreatic cancer. ACS nano 7, 2078-2089 (2013).
2. Zhou, H., et al. IGF1 Receptor Targeted Theranostic Nanoparticles for Targeted and Image-Guided Therapy of Pancreatic Cancer. ACS nano 9, 7976-7991 (2015).
3. Wang, L., et al. Ultrashort echo time (UTE) imaging of receptor targeted magnetic iron oxide nanoparticles in mouse tumor models. Journal of magnetic resonance imaging : JMRI 40, 1071-1081 (2014).
4. Cho, Y.S., et al. Targeted delivery of siRNA-generating DNA nanocassettes using multifunctional nanoparticles. Small 9, 1964-1973 (2013).



Theranostic nanoparticles targeting pancreatic cancer and stromal cells.

Combinatorial-designed Nano-platforms to Overcome Tumor Drug Resistance

NORTHEASTERN UNIVERSITY
PRINCIPLE INVESTIGATORS: MANSOOR AMIJI, PhD, AND ZHENFENG DUAN, MD, PhD

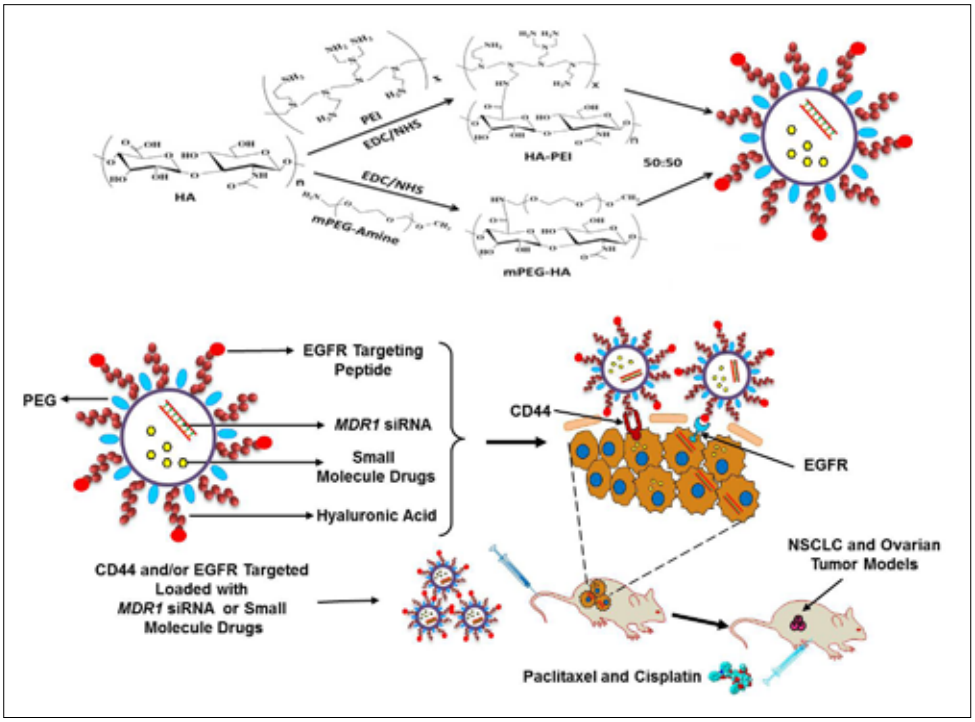
OVERVIEW

Bringing together teams from Northeastern University and Massachusetts General Hospital, the goal of this platform partnership was to use combinatorial design principles to effectively tailor self-assembled nano-formulations that target multidrug resistant (MDR) forms of ovarian cancer and lung cancer. The strategy used was centered on the development of biocompatible dextran- and hyaluronic acid based polymeric nano-assembled structures encapsulating small interfering RNAs (siRNAs) and microRNAs (miRs) that can silence MDR-associated genes in cancer cells. When co-delivered with anticancer drugs, these formulations can enhance the cell kill effect in resistant cancer cells, providing a step toward the advancement of nanotechnology-based gene therapy and drug delivery strategies for overcoming chemoresistance in ovarian cancer and lung cancer. Information related to this platform award can be found at http://nano.cancer.gov/action/programs/platforms/northeastern.asp, and Drs. Amiji's and Duan's websites at www.northeastern.edu/amijilab/people/prof-amiji and www.dfhcc.harvard.edu/insider/member-detail/member/zhenfeng-duan-md-phd.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

MDR in ovarian cancer and lung cancer remains a major obstacle for successful treatment. One of the most promising approaches to overcoming MDR is by inhibiting the expression of the MDR1 or MRP1 genes, both of which are involved in pumping out foreign substances from cells and the development of drug resistance. By knocking down the expression of these genes, sensitivity to chemotherapeutics can be restored in resistant cancer cells. In this project, platform award researchers designed a series of polymeric self-assembling nanoparticle platforms for targeted delivery of silencing siRNAs and miRs to MDR1 genes, along with small molecule drugs for synergistic therapeutic effects in refractory diseases. These platforms were evaluated *in vitro* and *in vivo*, leading to the development of self-assembling nanoparticles composed of hyaluronic acid (HA), which has high specificity and affinity for CD44 receptors overexpressed in metastatic and recurrent ovarian and lung cancer models.

Subsequently, the investigators used these nanosystems to target resistance-related anti-apoptotic genes (bcl-2 and survivin) in cisplatin-resistant lung tumors. In an MDR lung cancer model, the delivery of encapsulated siRNAs and cisplatin reversed resistance to cisplatin and delayed tumor growth significantly[1]. They went on to demonstrate that systemic administration and delivery of anti-MDR1 siRNA nanoparticles followed by chemotherapeutic drug treatment (paclitaxel) suppressed MDR1 gene expression and tumor growth in an MDR ovarian cancer mouse model[2]. The investigators have also shown that these siRNAs along with small molecule drugs can be delivered using EGFR-targeted nanoassemblies to a specific tumor site. These results provided evidence that HA-based self-assemblies may represent a clinically relevant system for systemic delivery of siRNA-based anticancer therapeutics for the treatment of resistant ovarian and lung tumors.



Schematic of in vitro and in vivo evaluation of multifunctional combinatorial-designed nanoparticles composed of biodegradable hyaluronic acid (HA) for selectively targeting tumors and cell penetration, high siRNA binding affinity, and simultaneous delivery of small molecule drugs along with siRNA. This system offers additive and synergistic therapeutic effects.

Tumor Targeted Nanobins for the Treatment of Metastatic Breast and Ovarian Cancer

NORTHWESTERN UNIVERSITY
PRINCIPAL INVESTIGATORS: THOMAS O'HALLORAN PhD, AND VINCENT CRYNS, MD

OVERVIEW

The overall objective of this platform project was to develop a translational pipeline of nanoparticle-based anticancer drugs for the treatment of ovarian and metastatic breast cancers. Specifically, new polymer-caged nanobins (PCNs) were developed based on liposome templates in which potent anticancer drugs were combined with surface-tethered antibodies that target tumor specific epitopes. These PCNs possess pH-responsive characteristics that can be used to trigger the release of encapsulated payload (such as arsenous acids and/or aqua-cisPt) inside the liposomal core under mild acidic conditions. Newly developed agents were screened in an experimental matrix and their antibody-tethered constructs were evaluated in several orthotopic animal models of breast and ovarian cancers. Additional information about this platform award can be found at http://nano.cancer.gov/action/programs/platforms/northwestern.asp and Dr. O'Halloran's website at http://sites.northwestern.edu/ohalloran.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

Arsenic compounds (ATO, As_2O_3) have emerged as front line agents for the treatment of acute promyelocytic leukemia; however, expansion of clinical utility to other cancers has been limited by toxicity at higher doses. Similarly, cisplatin is commonly used in the treatment of solid tumors, but therapeutic applications are limited by serious systemic toxicities and rapid drug de-activation. PCNs were shown to overcome these limitations and diminish systemic toxicity of chemotherapeutic payloads while retaining or increasing the therapeutic activity in ovarian and breast tumors, as well as metastatic tumors[1,2]. The targeting agents combined with PCNs were antibodies to the urokinase plasminogen activator (uPA) and its receptor (uPAR). uPA and uPAR are a ligand-cell surface receptor system that is frequently overexpressed in tumor and tumor-associated cells but rarely in quiescent, normal tissues. Both are critical players in cancer metastasis and promote receptor-mediated endocytosis of drugs targeted to uPA, which, in turn leads to higher levels of drug retention and accumulation within tumors. Using *in vitro* uptake and cell viability experiments with metastatic breast cancer cell lines, the group observed that targeting liposomal drugs to uPA and uPAR with monoclonal antibodies led to increased cellular drug accumulation and cell death. The group also developed protocols for producing multigram scale batches of sterile nanobins with the ability to scale production using established and validated drug manufacturing processes, and extensive analytical methods to accurately characterize the targeted liposomal drugs emerging from this platform.

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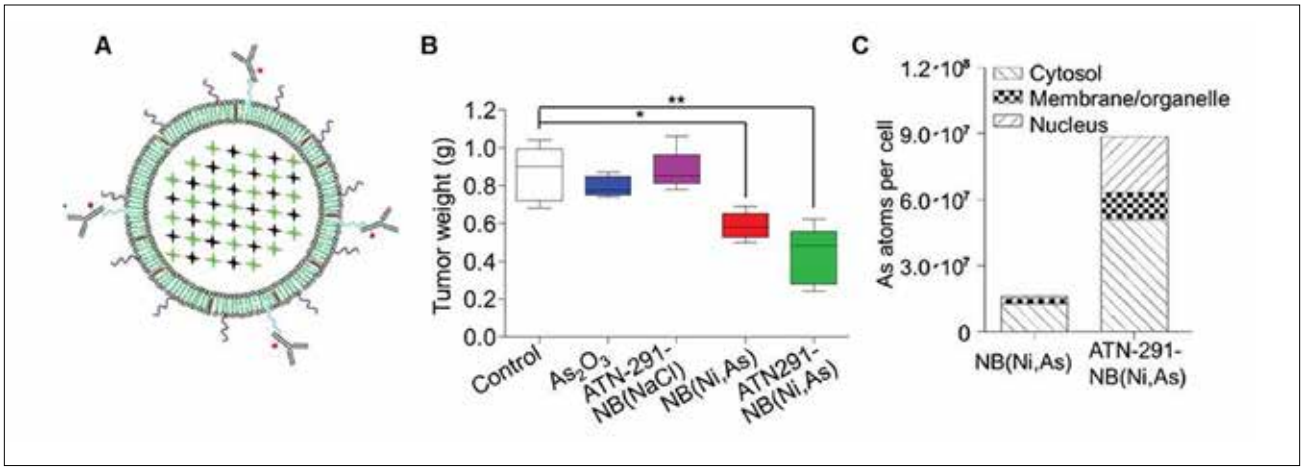

Targeted arsenic-loaded nanobins accumulate in cells and are efficacious. (A) Nanobin formulation with crystalline nickel-arsenic (Ni, As) payload targeted to urokinase plasminogen activator (uPA) with antibody ATN291. (B) uPA-targeted nanobin (ATN291-NB (Ni, As)) shows increased efficacy in HeyA8 ovarian cancer model compared to untargeted nanobin (NB (Ni, As)) and free drug (As203). (C) uPA-targeting results in increased accumulation of arsenic atoms per cell and in the nucleus in vitro. Reprinted with modifications from Zhang et al. with permission from the American Association for Cancer Research[2].

Preclinical Platform for Nanoparticle-based Theranostics in Pancreatic Cancer

RICE UNIVERSITY
PRINCIPAL INVESTIGATOR: NAOMI HALAS, PhD, DSc

OVERVIEW

The goal of this platform partnership was to develop a panel of hybrid magneto-fluorescent nanoparticles as multimodal imaging and therapeutic agents for the diagnosis and treatment of pancreatic cancer. Bringing together nanotechnology researchers at Rice University and researchers with expertise in imaging, radiation therapy, and translational research at MD Anderson Cancer Center and Baylor College of Medicine, this project planned to combine photothermal therapy and contrast agent-mediated imaging in the same gold-iron oxide nanoparticle system. As a result, this approach would enable whole body small animal imaging studies and testing of pancreatic cancer treatments, generating particle platforms that may serve as the foundation for other therapeutic developments. Information related to this platform award can be found at http://nano.cancer.gov/action/programs/platforms/rice.asp and Dr. Halas' website at http://halas.rice.edu/home.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

The plasmon resonance properties of gold nanoparticles enable dual functionality as imaging agents and photothermal therapeutics by alterations in the wavelength of illumination in the near infrared (NIR) region of the spectrum. Researchers of this platform award used these properties to create two distinct types of nanoparticles. The first was a gold nanoshell encapsulated in silica epilayers containing iron-oxide and the NIR dye indocyanine green, resulting in theranostic gold nanoshells. These nanoshells were conjugated with an antibody against neutrophil gelatinase-associated lipocalin (NGAL), which is overexpressed in multiple cancer cell types, and used in these studies to specifically target pancreatic cancer cells. Anti-NGAL-theranostic gold nanoshells were shown to target pancreatic cancer cells *in vitro* and *in vivo*, and provided imaging in cells and mice using both NIR fluorescence and magnetic resonance imaging. These nanoparticles were also shown to mediate cancer cell death *in vitro* via NIR photothermal therapy[1].

The second nanoparticle fabricated by the Rice platform researchers was a sub-100 nm nanoparticle consisting of a gold nanoparticle core, a thin porous silica spacer layer, and a thin gold outer shell. Fluorescent dyes or paramagnetic agents were incorporated within the porous silica spacer layer to allow the tracking of nanoparticle uptake *in vivo*[2]. In addition, the group also found these smaller nanoparticles have better efficacy in photothermal therapy compared to their larger gold silica nanoshell counterparts, in part due to the decrease in overall diameter to below 100 nm, which allows greater tumor uptake[3]. Using gold for the final surface layer allows for tuning of the plasmon interaction between the gold core and gold shell such that absorption and scattering characteristics can be maximized in the NIR spectrum. In addition, by using gold for the final layer, biocompatibility and ease of surface functionalization were maintained in the nanoshell particles. The new particles have been named "nanomatryoshkas" (after matryoshka: the well-known nested Russian folk doll).

1. Chen, W., et al. Targeting pancreatic cancer with magneto-fluorescent theranostic gold nanoshells. Nanomedicine 9, 1209-1222 (2014).
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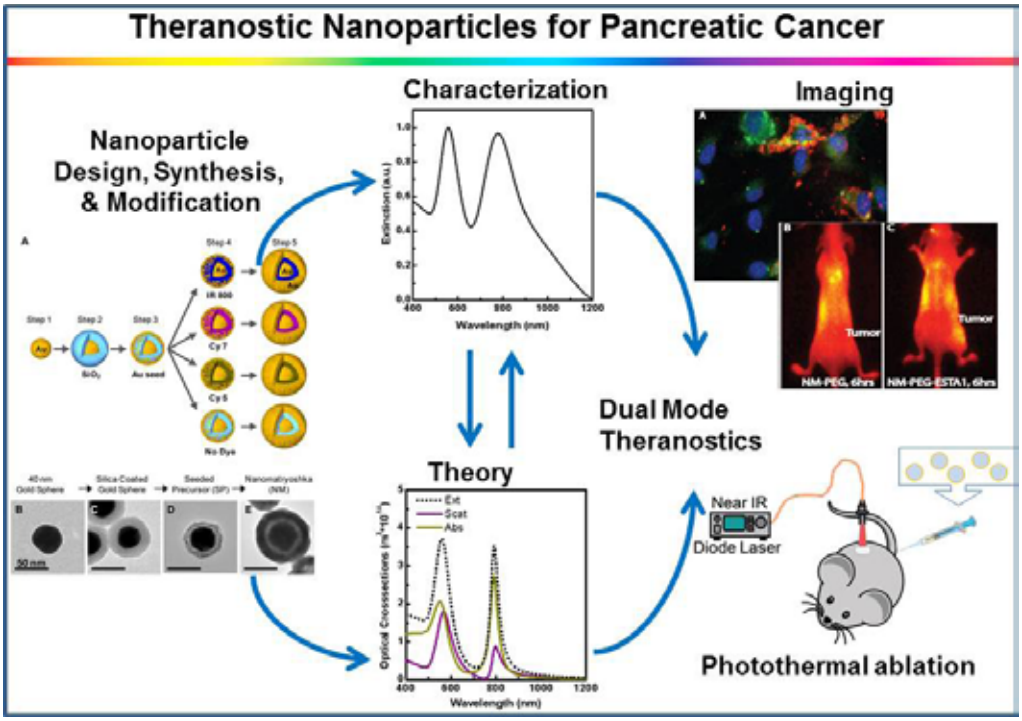

Overview of preclinical platform for theranostic nanoparticles in pancreatic cancer.

Nanoscale Metal-organic Frameworks for Imaging and Therapy of Pancreatic Cancer

UNIVERSITY OF CHICAGO/UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
PRINCIPLE INVESTIGATORS: WENBIN LIN, PhD, AND JEN JEN YEH, MD

OVERVIEW

This platform project focused on the development of new classes of hybrid nanomaterials, nanoscale metal-organic frameworks (NMOFs) and nanoscale coordination polymers (NCPs), for effective delivery of imaging and therapeutic agents to pancreatic, colon, and ovarian cancer. In particular, platform researchers designed NMOFs and NCPs containing magnetic resonance imaging contrast agents and chemotherapeutics for early diagnosis and more effective treatment of pancreatic cancer in mouse models. The project was grounded in Dr. Lin's expertise in NMOF and NCP chemistry, coupled to state-of-the-art mouse models developed by Dr. Yeh's laboratory. Platform research showed that NCPs/NMOFs enable efficient delivery of combination therapies for synergistic cancer treatment, including multiple chemotherapeutics, chemotherapeutics and biologics, and photodynamic therapy for the treatment of resistant cancers[1-4]. Information about this platform can be found at http://nano.cancer.gov/action/programs/platforms/uncc.asp, and Drs. Lin's and Yeh's websites at http://linlab.uchicago.edu and http://www.med.unc.edu/pharm/people/joint-faculty/jen-jen-yeh.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

The platform research team developed NCP particles that carry cisplatin (NCP-1) and oxaliplatin (NCP-2), with sizes and surface chemistry appropriate for *in vivo* applications[5]. PEGylated NCPs were shown to effectively avoid uptake by the mononuclear phagocyte system (MPS) *in vivo*, leading to long circulation times; the blood circulation half-lives were 16 hours and 12 hours for NCP-1 and NCP-2, respectively. These pharmacokinetic properties enabled *in vivo* applications of these particles in several different tumor mouse models. An NCP particle that carries both oxaliplatin and gemcitabine in high drug loadings was also synthesized for combination therapy, and this strategy is being extended to treat resistant lung and pancreatic cancers[1]. Furthermore, *in vitro* and *in vivo* characterizations of these NCPs were completed, and significant efforts are being devoted to clinical translation and commercialization of the NCP/NMOF technology. Currently, a $100,000 award from the University of Chicago Innovation Funds and a substantial amount of other investments are being used for Investigational New Drug-enabling preclinical studies by RiMO Therapeutics LLC with the goal of initiating the first-in-human Phase I study in summer 2016.

1. Poon, C., He, C., Liu, D., Lu, K. & Lin, W. Self-assembled nanoscale coordination polymers carrying oxaliplatin and gemcitabine for synergistic combination therapy of pancreatic cancer. Journal of controlled release : official journal of the Controlled Release Society 201, 90-99 (2015).
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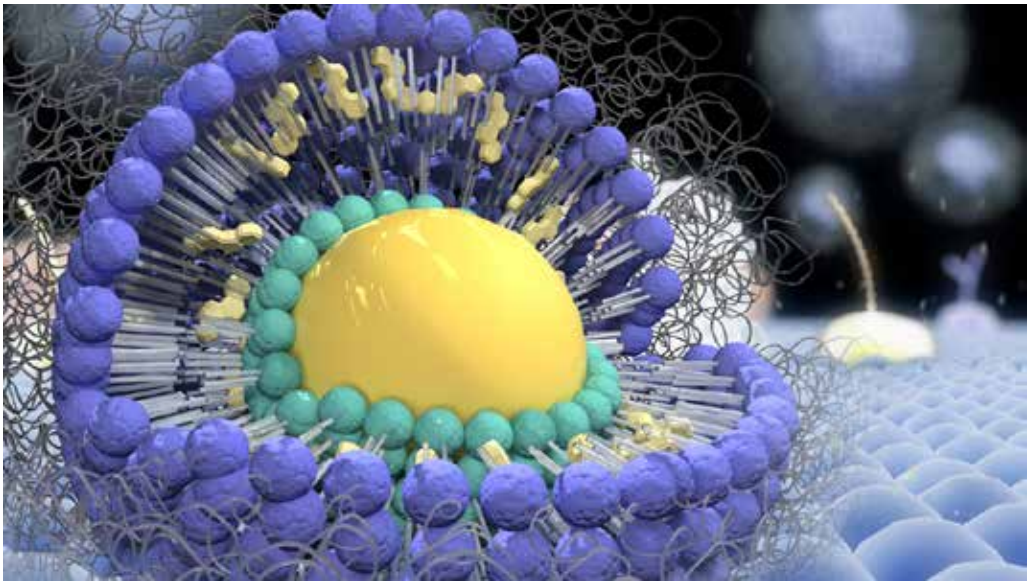


Schematic representation of the composition of nanoscale coordination polymers (NCPs).

RNA Nanotechnology in Cancer Therapy

UNIVERSITY OF KENTUCKY
PRINCIPAL INVESTIGATORS: PEIXUAN GUO, PhD, AND JOHN ROSSI, PhD

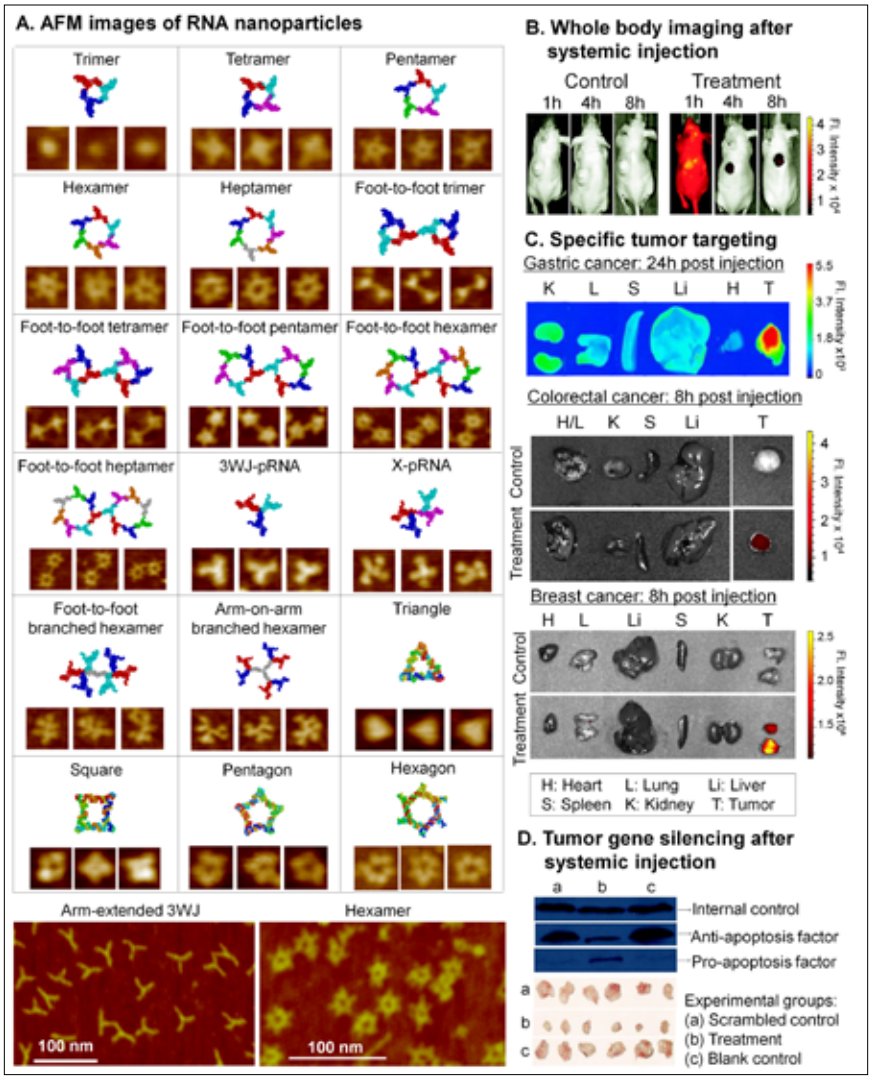
OVERVIEW

The objective of this platform project was to elucidate the principles underlying RNA nanoparticle assembly, and to develop methods to build RNA-based delivery vehicles capable of cancer cell recognition and gene silencing. Based on the motifs of dimers and trimers of bacterial DNA-packaging RNA (pRNA), these nanoparticles were developed with the capability of carrying targeting ligands, imaging agents for detection, and therapeutic siRNAs for treatment of various cancers including ovarian cancer and leukemia. The investigators also planned to develop methods for large scale production of stable RNA nanoparticle for clinical applications. Information related to this platform award can be found at http://nano.cancer.gov/action/programs/platforms/uc.asp.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

The Guo laboratory has discovered the pRNA-3WJ (Three-Way Junction) motif, which serves as the thermodynamically and chemically stable core of their fusion RNA complexes[1]. Since its discovery, they have constructed a variety of RNA nanoparticles using pRNA motifs as the scaffold with tunable angle, size, stoichiometry, and functionality[2]. Different functional modules incorporated into the RNA nanoparticles retain their folding, and independent functionalities such as specific cell binding and entry, gene silencing, catalytic function, and tumor targeting. The polyvalent nature of the pRNA motifs also allows for synergistic enhancement of gene silencing effects by carrying multiple siRNAs in one particle[3]. Pharmacokinetic studies indicated these particles have no detectable toxicity in mice and an *in vivo* terminal half-life of several hours, while the half-life of siRNA alone is only 0.25-0.75 hrs[4].

In collaboration with the laboratory of Dr. Mark Evers at the Markey Cancer Center at the University of Kentucky, researchers of this platform award showed targeting of RNA nanoparticles to colorectal cancer metastatic cells that had spread to the liver, lung, or lymph nodes. RNA nanoparticles did not accumulate in normal liver or lung parenchyma, demonstrating the therapeutic potential of these RNA nanoparticles as a specific delivery system for the treatment of colorectal cancer metastasis[5]. In collaboration with Dr. Carlo Croce's laboratory at Ohio State University, they further demonstrated the targeting of RNA nanoparticles to brain glioma in a patient-derived mouse model. In addition to targeting, they also showed that RNA nanoparticles carrying siRNAs can silence reporter gene expression in brain tumor cells both *in vitro* and *in vivo*. This demonstrates the potential of RNA nanoparticles in specific delivery of therapeutics to brain tumor cells without affecting healthy cells and tissues[6]. In further studies of the pRNA-3WJ motif scaffold, the Guo group constructed boiling-resistant triangular RNA nanoscaffolds, opening a new dimension of RNA functionality as a polymer feasible in industrial and nanotechnological applications[7].



Phi29 pRNA based RNA nanoparticles for cancer therapy. (A.) Atomic force microscopy images of polyvalent RNA nanoparticles using the pRNA scaffold. (B.) In vivo tumor targeting of Alexa-647 labeled RNA nanoparticles after systemic injection into xenograft mouse. PBS treated mouse was used as control. (C.) Specific targeting of RNA nanoparticles to colorectal cancer liver, lung, and lymph node metastases. Red: Alexa-647 labeled RNA; Green: metastatic cancer cells, K: kidney, L; liver, S: spleen, Li: Liver, H: heart, T: tumor. (D.) In vivo gene silencing after systemic inject of RNA nanoparticles containing siRNA, with increased expression of pro-apoptotic factor and decreased tumor size.

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Peptide-directed Protocells and Virus-like Particles: New Nanoparticle Platforms for Targeted Cellular Delivery of Multicomponent Cargo

UNIVERSITY OF NEW MEXICO
PRINCIPLE INVESTIGATORS: CHERYL WILLMAN, MD, AND C. JEFFREY BRINKER, PhD

OVERVIEW

The overall objective of this project was the development of therapeutic delivery platforms that address challenges associated with the use of nanoparticles as drug delivery vehicles in the context of treatment for acute lymphoblastic leukemia (ALL). Specifically, this included the development of protocells, mesoporous silica nanoparticles (MSNP) loaded with therapeutic and diagnostic agents (cargos) and encapsulated within lipid bilayers, and virus-like particles (VLPs) as drug-agnostic nanocarriers for targeted delivery. Although the primary focus of this project was ALL treatment, the platforms developed could be used against diverse cancer types. Protocells are robust, drug-agnostic, rationally designed nanodelivery systems that are "scale-up" ready and have the potential to offer a single delivery platform for the safe and effective delivery of therapeutics and/or *in vivo* diagnostics in multiple clinical indications. Information related to this platform award can be found at nano.cancer.gov/action/programs/platforms/unm.asp and Dr. Brinker's website at www.unm.edu/~solgel/index.html

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

At the beginning of this project, preliminary data regarding protocell nanoparticle development and optimization had been acquired using *in vitro* models. These studies revealed a number of highly advantageous properties of protocells including: target specificity, cargo capacity and diversity, enhanced bilayer stability, bilayer fluidity and resultant increased binding affinity at low peptide density, and no apparent immunogenicity or toxicity. These advantageous properties resulted in orders of magnitude improvements in chemotherapeutic potency relative to MSNP or liposomes individually, where it was shown that a single nanoparticle could kill an MDR cancer cell *in vitro*[1]. Since then the most significant scientific achievements have focused on engineering and optimizing the *in vivo* performance of MSNP and protocells through development of a colloidal/hydrothermal self-assembly approach that enables reproducible control of size, shape, pore size, and surface chemistry of MSNPs and protocells. In addition to this monodispersity and gram scale synthesis, particles were shown to display *in vivo* stability (based on size determined by dynamic light scattering, post-recovery from blood), targeted *in vivo* cancer cell binding (via peptides, affibodies and/or antibodies), cancer cell-specific cargo delivery (small molecule and nucleic acid therapeutics), and have demonstrated systemic distribution for enhanced tumor localization. Finally, an *ex ovo* avian embryo model was developed for direct observation and screening (i.e., microscopic and millisecond spatial and temporal resolution, respectively) of nanoparticle interactions with non-specific cells and tissues.

All protocell related intellectual property was licensed by Alpine Biotechnologies and then subsequently acquired by Oncothyreon in August 2014. Additionally, a sponsored research agreement is in place with members of the University of New Mexico platform award to allow for focused development of the protocell technology for specific clinical applications.

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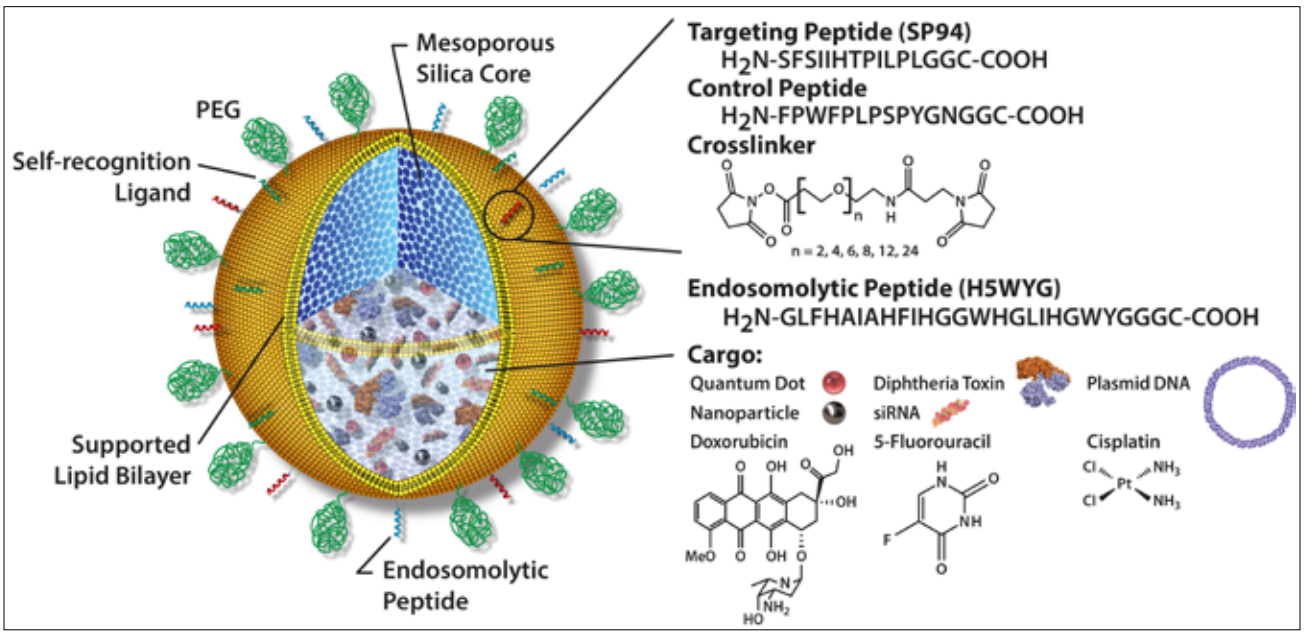

Schematic representation of the protocell (i.e., lipid bilayer encapsulated mesoporous silica nanoparticles) depicting composition, ligands that can be displayed on the nanoparticle surface, and different types of diagnostic and therapeutic agents that can be loaded as cargo.

High Capacity Nanocarriers for Cancer Therapeutics

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
PRINCIPAL INVESTIGATOR: ALEXANDER KABANOV, PhD, DSc

OVERVIEW

The goal of this platform project was to develop polymeric micelle carriers as effective drug delivery systems to overcome the limitations of low water solubility and bioavailability that plague many drugs used to treat breast cancer, such as taxanes. Joining pharmaceutical scientists at UNC Lineberger Comprehensive Cancer Center and polymer chemists at Technische Universitat Dresden, this platform award developed high capacity nanoformulations based on poly(2-oxazoline) (POx) amphiphiles. Drug delivery with POx micelles maximized delivery of active compounds while minimizing use of excipients that may cause adverse reactions. Information related to this platform award can be found at http://nano.cancer.gov/action/programs/platforms/unmc.asp and at Dr. Kabanov's website at https://pharmacy.unc.edu/Directory/kabanov.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

The nanoformulation developed in this work features well-controlled manufacturing and compliance to lyophilization procedures. It has the capacity for high drug loading (>45%), significantly decreasing the amount of excipient needed to solubilize chemotherapeutic drugs and avoiding excipient-related toxicity associated with paclitaxel (PTX). As a result, the PTX-POx micelle formulation has lower toxicity compared to both independently, and the *in vivo* treatment dose can be increased, resulting in increased drug accumulation in tumor[1]. Various mouse tumor models indicated an elevated drug exposure to tumor tissues, prominent antitumor activity, and superior antitumor activity compared to both Taxol® and Abraxane®[2]. This work was extended to formulating a number of other poorly soluble active pharmaceutical ingredient (API) classes, including other taxanes (microtubule disrupting agents), topoisomerase, and proteasome and heat shock protein inhibitors. Moreover, several of these compounds were co-formulated in the POx micelles and exhibited very high loading (loading capacity nearly 50%- i.e. drug: polymer wt. ratio 1:1) and synergy in anticancer activity. This promising preclinical data on POx/PTX nanoformulation provides a robust basis for translation into clinical trials.

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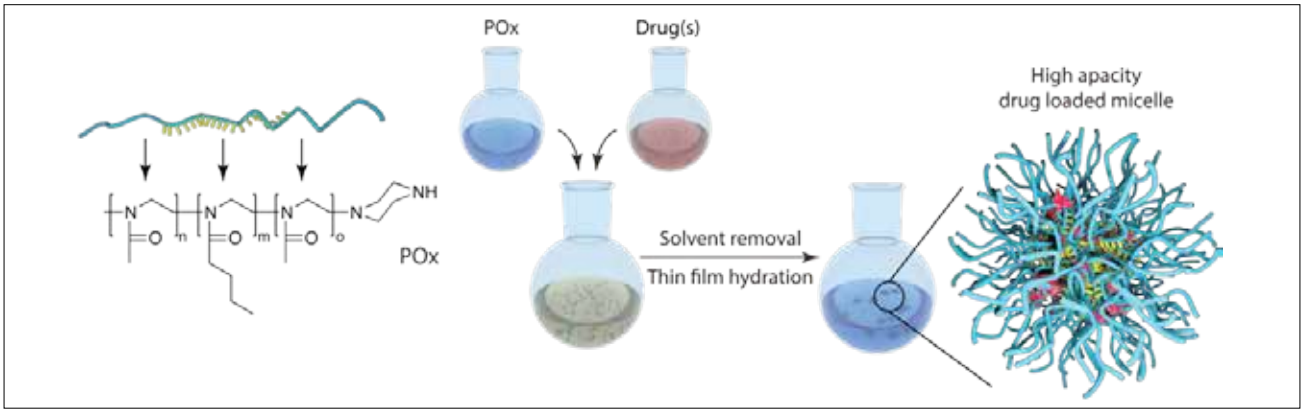

High capacity drug loaded micelles.

Magnetoresistive Sensor Platform for Parallel Cancer Marker Detection

UNIVERSITY OF UTAH
PRINCIPAL INVESTIGATORS: MARC PORTER, PhD, AND SEAN J. MULVIHILL, MD

OVERVIEW

The goal of this project was to develop a magnetoresistive microarray sensor platform for detecting multiple antigens at low concentrations, and adaption of this technology for effective detection of biomarker panels associated with pancreatic ductal adenocarcinoma (PDAC). Specifically, this platform aimed to develop methodology to simultaneously and efficiently capture distinct biomarkers from complex matrices and to develop a novel synthetic procedure to prepare ferromagnetic nanoparticle (NP) labels that do not cluster, enabling detection of individual labels. Information about this platform award can be found at http://nano.cancer.gov/action/programs/platforms/ut.asp and Dr. Porter's website at http://www.che.utah.edu/department/people/faculty/marc_d_porter.

SCIENTIFIC AND TRANSLATIONAL ACHIEVEMENTS

In the context of multiplexed immunoassays, cross reactivity between analytes and non-complementary capture antibodies can be problematic. To overcome problems with binding cross reactivity of antigens and non-complementary capture antibodies in multiplexed immunoassays, the investigators established two screening protocols to minimize the number of antibody-antigen screening permutations for selecting clinically sensitive and specific PDAC biomarkers. The first screening protocol used an in silico process—Basic Local Alignment Search Tool (BLAST) analysis—to identify potential cross reactors and to limit the number of ligands that undergo further solution-based evaluation. The second screening protocol used fluorescently labeled antigens and target antibodies in fluorescent anisotropy ligand receptor binding experiments. Affinity constants were then determined for a range of antibody-antigen pairs, and the most promising pairs were moved into immunometric screening experiments. These procedures selected four candidate PDAC markers (OPN, TIMP-1, MMP7, and CA 19-9), which were coupled with surface-enhanced Raman scattering (SERS) labels as readouts in a proof-of-concept nanoparticle-based biomarker immunoassay array[1].

Past works in designing nanoparticle (NP) labels for immunometric assays have shown that NPs larger than a few hundred nanometers can undergo ballistic deposition rather than pure diffusional mass transport in stagnant assays, and do not label surface-bound analytes as effectively as smaller NPs. For magnetic NPs (MNPs), however, the particle's magnetic moment (m) is responsible for the measured signal, and so a larger particle typically has a larger m compared to a smaller particle of the same material. Thus, a larger particle can be a more effective label, which is in opposition of the desired mass transfer characteristics. Therefore, small MNPs—typically diameters are kept below 250 nm—with large m are preferred. MNPs with large magnetic moment often aggregate in solution. To prevent aggregation, the group developed a novel approach to encapsulate ferromagnetic zinc ferrite nanocubes (ZFNCs) with silica after an intermediary layer-by-layer polyelectrolytes deposition step and was able to maintain stable suspensions of these MNPs. Silica-encapsulated MNPs not only reduce magnetic-induced aggregation, but also provide a surface which is biocompatible and can be functionalized to link molecular recognition moieties[2].

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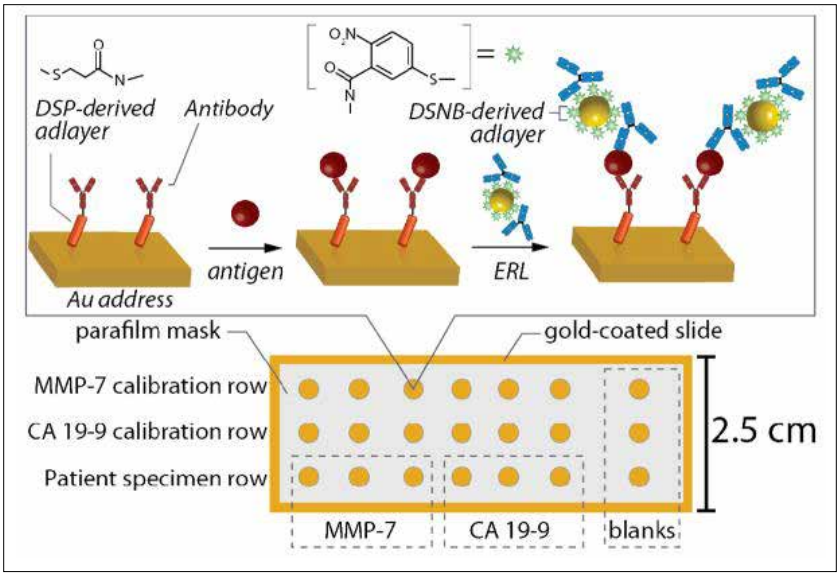


Schematic of SERS array for MMP-7 and CA 19-9 (reproduced from Granger et al.[1] with permission from the Royal Society of Chemistry).

Boston University Cross-Disciplinary Training in Nanotechnology for Cancer (XTNC)

BOSTON UNIVERSITY
PRINCIPLE INVESTIGATORS: BENNETT B. GOLDBERG, PhD, AND DOUGLAS FALLER, MD, PhD

OVERVIEW

The Boston University XTNC provided interdisciplinary training support to predoctoral and postdoctoral fellows whose research focused on nanotechnology applications for cancer diagnostics and treatment. Scientific areas included identification of rare circulating tumor cells, proteomics to detect nuclear matrix proteins and biomarkers for early stage tumor screening, and the development of nanotechnologies for early detection of precancerous and malignant lesions from biological fluids. Trainees participated in the university's annual Cancer Care for Engineers and Scientists conference and hands-on workshops to gain a greater understanding of clinical care. They also took Introduction to Nanomedicine, a cross-disciplinary course taught by faculty from science, engineering, and medical disciplines. In addition, trainees organized monthly nanomedicine journal club meetings in which they interacted with fellow trainees and researchers, and presented on topics of interest. Co-mentored research training, the primary focus of the XTNC, was mediated by coupling faculty and students from physical and biological sciences with medical researchers and clinicians, as well as the development of cross-fertilized research projects to better prepare trainees to overcome challenging scientific, cultural, and disciplinary barriers. Thirty-four predoctoral students and eight postdoctoral researchers from the natural sciences, engineering, and medicine were successfully trained during the Center's funding period. Additional information about this training center can be found at http://www.bu.edu/nano-bu/programs/xtnc.

PROGRAM ACCOMPLISHMENTS AND TRAINING IMPACT

To date, XNTC trainees have generated 136 publications and given 111+ conference presentations and posters. Additionally, trainees worked with CityLab, a biotechnology learning laboratory at Boston University School of Medicine, and Upward Bound, to develop cancer nanotechnology curricula for high school students and the general public (http://www.bu.edu/nano-bu/programs/xtnc/xtnc-outreach). This past academic year (2015-16), XTNC graduate student and postdoc trainees developed six evening programs for 24 high school students in CityLab's Urban Scholars program. Over the course of the year, trainees led the students through hands-on laboratory experiments and discussions about general principles and applications of nanotechnology, especially nanomedicine, and shared their experiences as graduate students and postdocs in nanotechnology cancer research. XTNC graduate students also developed lectures and laboratory activities for Boston University's Upward Bound Math-Science Program.

XTNC is committed to the creation and support of a diverse network of researchers linked by their work in cancer-related studies. As such, during the funding period, the program was evaluated using social network analysis (SNA) to examine the extent to which the training center had been effective in facilitating this cross-disciplinary network. SNA showed that the Center network over time showed significant growth and diffusion as well as very high levels of cohesion, especially given the steady growth in the number of participants and the turnover among the trainees. Currently, seven predoctoral trainees have graduated; three of them work in industry and four are postdoctoral researchers. Eight participants completed postdoctoral training in XTNC, four of whom work as scientists in industry, three have gone on to postdoctoral fellowships, and one is an assistant professor at Seattle University.

The Johns Hopkins Cancer Nanotechnology Training Center

JOHNS HOPKINS UNIVERSITY
PRINCIPAL INVESTIGATORS: DENIS WIRTZ, PhD, AND HAI-QUAN MAO, PhD

OVERVIEW

The objectives of the Johns Hopkins Cancer Nanotechnology Training Center (JHU CNTC) were to recruit scientifically diverse predoctoral students and train them to become scientists, engineers, and clinicians capable of developing new nanoscale materials for biological applications, or therapeutics and diagnostics for human cancers. To achieve this goal, all JHU CNTC fellows, were required to take two core courses, intensive nanobiotechnology laboratory "bootcamps", and laboratory courses in engineering and clinical oncology to develop and enhance their experimental and theoretical fundamentals. Trainees were also required to be co-mentored by at least one engineering faculty and one life science faculty or clinician. Over the course of the funding period, the JHU CNTC maintained a typical training cohort of five to six students, with a total of three two-year cohorts formed and 16 participants. The scientific backgrounds of predoctoral trainees included chemical engineering, biotechnology, materials science, biological engineering, bioengineering, biomedical engineering, biochemistry, biology, electrical and computer engineering, and pharmacology. Additional information about this training center can be found at the Johns Hopkins University Institute for NanoBioTechnology, which houses the JHU CNTC training program (http://inbt.jhu.edu).

PROGRAM ACCOMPLISHMENTS AND TRAINING IMPACT

Relevant educational training achievements included the success of the grand rounds journal tutorials. In these sessions, students were first involved in a traditional journal club that discussed various articles. These articles referenced the type of surgery they would be able to view the following month in grand rounds. It was a significant experience for the students to match scientific articles with real-life medical procedures and platforms. Students similarly were given the opportunity to participate in two "bootcamps" in cell biology and nanoparticle synthesis. These were three-week long, hands-on training in various molecular biology techniques, as well as nanoparticle synthesis techniques. The JHU CNTC also sought and received approval from Johns Hopkins University and the State of Maryland to offer a certificate program in Nanobiotechnology during the funding period. As a result, in addition to their PhD, each student that successfully completed the program received recognition in the form of a certificate of advanced study in nanobiotechnology. Currently, 13 students have completed the program and five have graduated.

UCSD Cancer Nanotechnology Training Center (CRIN)

UNIVERSITY OF CALIFORNIA SAN DIEGO
PRINCIPAL INVESTIGATORS: ANDREW KUMMEL, PhD, AND ROBERT G. MATTREY, MD

OVERVIEW

The UCSD training center provided training in cancer nanotechnology to predoctoral students, postdoctoral researchers and physician scientists. UCSD CRIN's goal was to recruit and cross-train young scientists from diverse research backgrounds to bring emerging tools and technologies from nanoscience, nanoengineering, mesoscale engineering, and imaging sciences to the care of cancer patients. The program supported two years of training in a translational medicine project under dual mentors, a basic research scientist and a clinician scientist. This laboratory based training was supplemented with didactic training in cancer biology, nanomedicine and technology commercialization. The program ensured cross-training with specialized curricula for trainees depending on their background, with separate tracks for trainees from medical and physical sciences. All the physical scientists completed courses in cancer biology and all biologists completed courses in nanotechnology. CRIN worked with three major National Cancer Institute funded efforts at the UCSD's Moores Cancer Center)—the Comprehensive Cancer Center, the Center of Cancer Nanotechnology Excellence (CCNE), and the *In vivo* Cancer and Cellular Molecular Imaging Center (ICMIC)—to provide resources and additional support for trainees. This enabled UCSD to maintain a four year cross-training program in cancer nanotechnology, with two years supported by CRIN and the additional two years supported through grants held by CRIN key faculty, or via fellowships and teaching assistantships at UCSD.

PROGRAM ACHIEVEMENTS AND TRAINING IMPACT

On average six PhD students and postdocs were trained per year, with a sizable number of under-represented minorities (URMs) and/or women trainees in physical sciences and engineering. All trainees who completed the program had first author publications, and a total of 29 publications resulted from CRIN supported projects. Four trainees have obtained F31 grants following completion of the program. CRIN leadership solicited feedback from trainees throughout the program's duration, to assess and improve program performance. Surveys of trainees show several clear trends: (1) dual mentors are the most valued non-financial program component; (2) research is considered most important in increasing interdisciplinary knowledge; (3) trainees were satisfied with interactions with clinical mentors and, finally, (4) the course in entrepreneurship was considered to be of extremely high value. This business training was an unusual and highly successful aspect of the program, evidenced not only by trainee enthusiasm, but by one trainee starting a company to further develop technology initially supported by his CRIN project.

Midwest Cancer Nanotechnology Training Center (M-CNTC)

UNIVERSITY OF ILLINOIS URBANA-CHAMPAIGN
PRINCIPAL INVESTIGATORS: RASHID BASHIR, PhD, AND ANN M. NARDULLI, PhD

OVERVIEW

The objective of the Midwest Cancer Nanotechnology Training Center (M-CNTC) was to create an effective interdisciplinary training program via four intellectual themes centered on cancer and nanotechnology: *ex vivo* diagnostics, *in vivo* imaging, therapeutic nanotechnology, and mechanobiology and nanotechnology. Educational activities were designed to create an environment based on an individual trainee's background, with guidance from co-advisors with diverse research and clinical expertise. A two-track curriculum provided both engineering and biology students a path to seamlessly integrate cancer nanotechnology courses and research in their PhD program beginning with an interdisciplinary course titled "BioNanotechnology and Nanomedicine: Applications in Cancer and Mechanobiology." Over the five year funding period, there were a total of 39 graduate and postdoctoral trainees in five cohorts, in which each cohort was supported for two years through the M-CNTC. The scientific backgrounds of trainees included biochemistry, bioengineering, biophysics, chemistry, electrical and computer engineering, food science and human nutrition, materials/mechanical science and engineering, nuclear, plasma, and radiological engineering, pathobiology, and veterinary medicine. Additional information about this training center can be found at http://www.istem.illinois.edu/resources/mcntc.html.

PROGRAM ACCOMPLISHMENTS AND TRAINING IMPACT

The Center established a Student Leadership Council (SLC, http://m-cntc.illinois.edu/slc.html) that played an integral role in the strategic directions of the M-CNTC through an infusion of critical thinking, leadership, communication, team building, and ethics training. The M-CNTC also successfully established the BioNanotechnology Summer Institute (http://nano.illinois.edu/summer-institute-2014), a two-week long summer school with hands-on laboratory modules in cancer nanotechnology taught by leading experts in the field. The Summer Institute was held prior to the start of each fall semester and was available to trainees from within and outside of the M-CNTC. In addition, a new course on cancer nanotechnology was developed that now has a permanent course number in the Department of Bioengineering, as well as a new transcriptable graduate concentration in Cancer Nanotechnology that allows students from across the college to include elements from the M-CNTC training grant into their respective PhD programs. Many clinical collaborations across the Carle Foundation Hospital, Mayo Clinic, and other clinical partners were established, resulting in the submission and funding of multiple grants and a continuation training grant in cancer nanotechnology. Assessment of the training center and courses were performed throughout the funding period. If interested in learning more, the results of these evaluations can be found at www.istem.illinois.edu/resources/mcntc.html.

The University of Kentucky Cancer Nanotechnology Training Center

UNIVERSITY OF KENTUCKY
PRINCIPLE INVESTIGATORS: BRADLEY D. ANDERSON, PhD, AND B. MARK EVERS, MD

OVERVIEW

The University of Kentucky Cancer Nanotechnology Training Center (UK CNTC) provided advanced multidisciplinary training for predoctoral students and postdoctoral fellows in a collaborative environment intended to foster cancer nanotechnology research and the translation of laboratory results into cancer diagnostics and therapeutics. Led by a multidisciplinary team of mentors consisting of 20 nanotechnology researchers/biomedical scientists and 15 clinical oncologists, the research program included early detection and diagnosis, *in vivo* imaging, targeted delivery of therapeutics, and combination therapy. To ensure an individually tailored clinical experience, each trainee designed their own clinical exposure programs relevant to their interests and research projects, such as shadowing a clinical faculty member engaged in patient care. Over the course of the funding period, the UK CNTC maintained a steady-state of seven to eight predoctoral and postdoctoral trainees, with a total of 18 trainees by the end of the fourth year. The majority of the predoctoral trainees had scientific backgrounds in pharmaceutical science and allied fields or engineering disciplines, and worked toward PhDs in pharmaceutical sciences, chemical & materials engineering, or biomedical engineering. Additional information about this training center can be found at http://www.cntc.uky.edu.

PROGRAM ACCOMPLISHMENTS AND TRAINING IMPACT

Significant achievements of the UK CNTC included the production of more than 40 publications by trainees and the clinical exposure made available to trainees. The CNTC also brought together faculty focusing on nanotechnology with practicing oncologists and scientists engaged in drug discovery and basic biology, resulting in new collaborations and multidisciplinary grant applications. UK CNTC trainees were asked in a recent survey, “Has your CNTC traineeship influenced your career goals?” and “How so?” Greater than 90% responded positively, and indicated that interactions with clinicians and clinical research were important aspects. All PhD graduates are employed either in the pharmaceutical industry, as postdoctoral fellows in academia, or in one case, in the Chinese equivalent of the U.S. Food and Drug Administration. Two of the postdoctoral trainees accepted faculty positions.

Integrative Cancer Nanoscience and Microsystems Training Center

UNIVERSITY OF NEW MEXICO
PRINCIPAL INVESTIGATORS: JANET M. OLIVER, PhD, AND AHBAYA DATYE, PhD

OVERVIEW

The New Mexico Cancer Nanoscience and Microsystems Training Center (NM CNTC) was established in 2010 to integrate strengths in nanoscale engineering and physical sciences research, and training at the University of New Mexico (UNM) and at Sandia and Los Alamos National Laboratories (SNL, LANL). Programs of study focused on cancer discovery, prevention, diagnosis and treatment in the NCI-designated UNM Cancer Center. The program aimed to nurture new cancer-focused teams, while expanding student and postdoc involvement in established teams of cancer biologists/oncologists, physical scientists, and engineers. CNTC trainees also acted as translators between cancer biologists and nanoscientists and were drawn almost equally from the integrated Biomedical Sciences Graduate program in the UNM Health Sciences Center and the UNM School of Engineering and College of Arts and Sciences. Trainees were mentored by cross-disciplinary faculty teams and received an integrative educational program including professional development and coursework in cancer nanotechnology, such as courses in “Clinical Cancer Perspectives” and “Multi-Modality Imaging and Translational Medicine.” More information about the CNTC can be found at http://cntc.unm.edu.

PROGRAM ACCOMPLISHMENTS AND TRAINING IMPACT

One key training achievement of the CNTC programs, resulting directly from the requirement for co-mentors from complementary disciplines, was the emergence of new cancer research teams and collaborations; a new biotechnology company was formed based on their quantum dot camera design, which has also been brought into the clinic. Overall, the NM CNTC trainees have contributed to more than 60 publications in these and other areas since 2010. The program supported 19 graduate students and 11 postdocs over the funding period, and has been particularly successful in training women and minorities in cancer nanotechnology, with 18 women trainees (60% of total) and six Hispanic trainees (20% of total). Of the seven completed CNTC graduate students, two now hold staff positions in nanobiology teams at Sandia National Laboratories, one is an assistant professor in a UNM team working on therapeutic applications of magnetic nanoparticles, three have taken nanobiology-focused postdoctoral positions at other universities, and one is a resident in medical physics. Of the nine completed CNTC postdocs, four are now assistant professors (in Biomedical Engineering, Internal Medicine (2) and Pathology), one is an associate professor (in Pharmacy), one has a staff position at Sandia National Laboratories, and three were recruited to staff positions in cancer-focused biotechnology companies.

Enzyme-responsive Nanoemulsions as Tumor-specific Ultrasound Contrast Agents

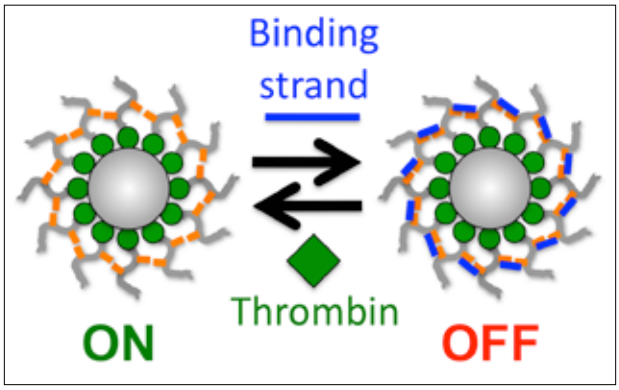
UNIVERSITY OF COLORADO, BOULDER
PRINCIPLE INVESTIGATOR: ANDREW P GOODWIN, PhD

OVERVIEW

The main objective of this project was to design soft colloids capable of changing their ultrasound contrast properties in response to elevated levels of tumor malignancy and angiogenesis biomarkers. The second objective was to design and validate superheated nanoemulsions capable of using increased ultrasound contrast to specifically sense elevated levels of the protein matrix metalloproteinase-2 (MMP-2), which is found in metastatic tumors. Additional information about Dr. Goodwin and his research can be found at http://nano.cancer.gov/about/meet/pathway_independence.asp#agoodwin and http://www.colorado.edu/goodwinlab.

SCIENTIFIC ACHIEVEMENTS

The most significant achievement of this award was the design of microbubbles encapsulated in a phospholipid-polymer shell coated with oligonucleotide-based cross-links containing DNA sequences with affinity for thrombin, a model biomarker. The presence of thrombin displaced the DNA crosslinks, reducing the stiffness of the shell and allowing the microbubble to emit selectively-detectable nonlinear echoes in response to ultrasound. After showing sufficient sensitivity *in vitro*, these bubbles were validated successfully in a rabbit thrombosis model, with full, activated response in the presence of thrombin only 40 seconds after injection[1]. Dr. Goodwin also designed a nanoemulsion system that functions as an in-solution sensor for biological targets. Hybridization of complimentary oligonucleotides in the shells of droplets in solution led to fusion and transfer of contents between droplets. Addition of target specific sequences to the oligonucleotides makes the fusion process sensitive to the presence of the target, and enables targets to be detected and quantified through monitoring of droplet fusion[2]. Thrombin could be detected by this system with sensitivity as low as 100 nM.



Schematic of thrombin-sensitive microbubbles in the absence (right) or presence of thrombin (left), which causes the displacement of crosslinking DNA sequences (blue strands, right image) and enables microbubble activation in response to ultrasound.

AWARD IMPACT ON CAREER PATH AND TRANSITION

Dr. Goodwin felt the K99/R00 award had a large and positive impact on his career, and that without the award he would most likely not be a professor today. Having independent funding on his curriculum vitae was invaluable for getting job interviews, and the freedom to develop ideas was absolutely essential for landing a tenure-track faculty position. Although his choices and plans for projects were still heavily influenced by the need to publish results relatively quickly, to establish follow-on funding and a secure, independent laboratory, this accelerated pace had positive benefits for training his own students, who are already engaged in productive research projects.

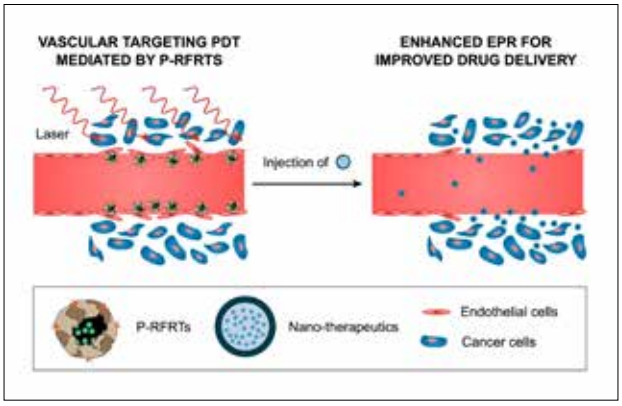
1. Nakatsuka, M.A., et al. *In vivo* ultrasound visualization of non-occlusive blood clots with thrombin-sensitive contrast agents. Biomaterials 34, 9559-9565 (2013).
2. Mohan, P., Noonan, P.S., Nakatsuka, M.A. & Goodwin, A.P. On-demand droplet fusion: a strategy for stimulus-responsive biosensing in solution. Langmuir : the ACS journal of surfaces and colloids 30, 12321-12327 (2014).

Nanoplatform Based, Combinational Therapy Against Breast Cancer Stem Cells

UNIVERSITY OF GEORGIA
PRINCIPLE INVESTIGATOR: JIN XIE, PhD

OVERVIEW

The main objective of this project was to develop powerful, nanoparticle-based platforms for combination cancer therapy. Dr. Xie developed numerous nanoparticles for drug delivery, including albumin coated iron oxide nanoparticles, ferritin nanoparticles, silica nanoparticles, Fe_5C_2 nanoparticles, and $SrAl_2O_4$:Eu nanoparticles, along with novel magnetic resonance imaging (MRI) contrast agents and nanoparticle-based dual MRI-optical imaging probes for tumor imaging and cell tracking. Additional information about his research can be found at http://nano.cancer.gov/about/meet/pathway_independence.asp#jxie and http://xie.uga.edu.



Nanoparticle mediated PDT permeabilizes tumor vascular, enhancing the EPR effect and subsequent nanotherapeutic delivery.

SCIENTIFIC ACHIEVEMENTS

Nanoparticle-based delivery is frequently used to improve drug accumulation in tumors and treatment efficacy. Dr. Xie's group demonstrated that RGD4C (targeting integrins) modified ferritin nanocages (RFRTs) can efficiently home to tumors after systematic injection. RFRTs could load large amounts of doxorubicin and exhibited a longer circulation half-life than untargeted RFRTs. As such, doxorubicin-loaded RFRTs showed higher tumor uptake (as much as 20 fold), better tumor growth inhibition, and less cardiotoxicity than free doxorubicin[1]. Most macromolecule or nanoparticle drugs/probes accumulate in tumors through the enhanced permeability and retention (EPR) effect. However, many tumors exhibit an inadequate EPR effect, causing inefficient drug delivery. Dr. Xie's group sought to improve the EPR effect by permeabilizing the tumor vasculature through photodynamic therapy (PDT). They found that RFRTs could encapsulate large amounts of a photosensitizer such as ZnF16Pc and deliver it efficiently to the tumor endothelium. With photo-irradiation at relatively low fluence rates (number of particles crossing per unit time), the treatment led to newly formed or enlarged gaps on tumor endothelial walls without collapsing the vessels. As a result, macromolecules or nano-particles administered after treatment were extravasated and accumulated more efficiently at tumors, without affecting their distribution in normal tissues. Using Doxil as a representative nanoparticle drug, they studied the impact of this procedure on cancer treatment and demonstrated improved efficacy and reduced systematic toxicity[2].

AWARD IMPACTS ON CAREER PATH AND TRANSITION

Dr. Xie felt that the K99/R00 award greatly facilitated his transition to a faculty position at the University of Georgia and the establishment of his independent research program. Within the three-year R00 phase, he published (including co-authored) 17 papers, filed three provisional patents, and successfully expanded his research into new exciting areas.

1. Zhen, Z., et al. RGD-modified apoferritin nanoparticles for efficient drug delivery to tumors. ACS nano 7, 4830-4837 (2013).
2. Zhen, Z., et al. Tumor vasculature targeted photodynamic therapy for enhanced delivery of nanoparticles. ACS nano 8, 6004-6013 (2014).

Next-Generation Quantum Dots for Molecular and Cellular Imaging of Cancer

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN
PRINCIPLE INVESTIGATOR: ANDREW M. SMITH, PhD

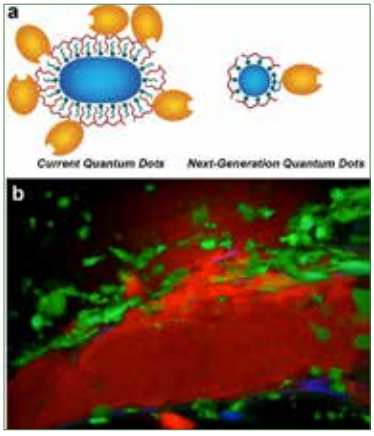
OVERVIEW

The goal of this project was to understand and improve nanoparticle drug delivery by developing a new series of quantum dots (QDs) for quantitative, multicolor *in vivo* imaging of the tumor microenvironment. This new class of quantum dots designed by Dr. Smith, called "Brightness Equalized Quantum Dots," or BE-QDs, provides equalized fluorescence brightness across a broad spectrum of colors and can be used to model nanoparticle drug formulations. Signals from these QDs may offer quantitative measurements associated with uptake and penetration in solid tumors under intravital microscopy. Additional information about Dr. Smith and his research can be found at http://nano.cancer.gov/about/meet/pathway_independence.asp#asmith and http://smithlab.bioen.illinois.edu.

SCIENTIFIC ACHIEVEMENTS

Dr. Smith focused his early efforts on establishing high yield, reproducible synthesis of BE-QDs and then investigated the underlying physics of the emission behavior of the BE-QDs[1,2]. His studies revealed that these particles were ideal for quantitative multiplexed imaging, as multiple fluorescent colors of BE-QDs have the same brightness in one-photon and two-photon excitation modes from the ensemble level to the single-molecule level. Dr. Smith's group further showed the accuracy of quantifying particle concentration in complex tissue (as detected through fluorescence intensity) was substantially improved when using BE-QDs in comparison with conventional fluorophores.

A major limitation in using QDs for microscopy is their intermittent light emission, known as "blinking," which leads to image frames without probe signals during the "off' period. To overcome this issue, Dr. Smith's group pursued synthesis of QDs with a graded alloy shell. The alloy shell suppressed blinking, with more than 25% of the alloy-shell QDs free of blinking behavior in both water and organic solvents, and the ability to provide continuous tracking of cell trajectories *in vitro*[3]. In collaboration with Dr. Peter Wang (University of California San Diego), these QDs have been investigated for quantitative assessment of invasion of living tumor cells[4].



(a) Current and next-generation quantum dots. (b) Imaging quantum dot diffusion in a mammary tumor using multiphoton intravital microscopy. Quantum dots, red; tumor cell expression of GFP, green; second harmonic generation from collagen, blue.

AWARD IMPACT ON CAREER PATH AND TRANSITION

The K99/R00 award had a very positive impact on Dr. Smith's capacity to transition into a faculty position, and attain a high level of achievement early in his independent career. The award helped him stand out in a very crowded field for academic positions. During the R00 phase, the benefits of the award were substantial, as it allowed him to have a lighter than normal teaching load and freed up startup package funds for use towards the support of additional personnel and projects beyond the scope of the R00 award.

1. Smith, A.M., Lane, L.A. & Nie, S. Mapping the spatial distribution of charge carriers in quantum-confined heterostructures. Nature communications 5, 4506 (2014).
2. Smith, A.M. & Nie, S. Bright and compact alloyed quantum dots with broadly tunable near-infrared absorption and fluorescence spectra through mercury cation exchange. Journal of the American Chemical Society 133, 24-26 (2011).
3. Lane, L.A., Smith, A.M., Lian, T. & Nie, S. Compact and blinking-suppressed quantum dots for single-particle tracking in live cells. The journal of physical chemistry. B 118, 14140-14147 (2014).
4. Chung, E.Y., et al. Activatable and Cell-Penetrable Multiplex FRET Nanosensor for Profiling MT1-MMP Activity in Single Cancer Cells. Nano letters 15, 5025-5032 (2015).

Theranostic Nanomedicine for Breast Cancer Prevention and Image-guided Therapy

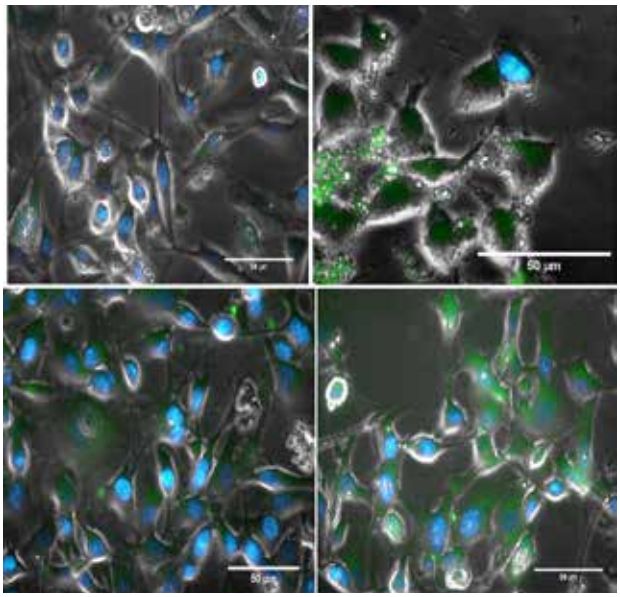
UNIVERSITY OF MASSACHUSETTS, LOWELL
PRINCIPLE INVESTIGATOR: PRAKASH R. RAI, PhD

OVERVIEW

The overall objective of this project was to develop new nano and optical imaging- based approaches for breast cancer prevention. To achieve this, Dr. Rai pursued a strategy of co-encapsulating multiple drugs in fluorescently labeled polymeric nanoparticles (NPs), which were either passively or actively targeted to tumor cells, and testing the efficacy of these NPs in three-dimensional *in vitro* models of cancer and *in vivo* orthotopic mouse tumor models. NP delivery and treatment response were evaluated under fluorescent confocal microscopy. Additional information about Dr. Rai and his research can be found at http://nano.cancer.gov/about/meet/pathway_independence.asp#prai and https://www.uml.edu/Engineering/Chemical/faculty/Rai-Prakash.aspx.

SCIENTIFIC ACHIEVEMENTS

The application of chemoprevention to humans has met with limited success, largely due to inefficient systemic delivery and the low bioavailability of promising agents. To achieve maximum effectiveness for chemopreventive agents, novel strategies are needed to enhance their bioavailability and reduce potential toxicity. For both treatment and prevention, Dr. Rai developed multifunctional targeted theranostic nanoconstructs (TNCs) to deliver multiple agents simultaneously, seeking synergistic activity to improve drug efficacy. For therapeutic TNCs, polymeric nanoparticles were formulated to co-encapsulate olaparib and sorafenib (poly ADP ribose polymerase, PARP, and kinase inhibitors, respectively) with cytotoxic paclitaxel and cetuximab, an inhibitor for epidermal growth factor receptors (EGFRs) that are highly expressed on the surface of triple negative breast cancer cells. Fluorescent imaging was used to establish pharmacokinetics and biodistribution, and to guide treatment and monitor response to TNC-based therapy. Preliminary data using these multi-agent constructs showed effectiveness in reducing tumor growth and burden in three-dimensional culture and orthotopic mouse models. To create chemopreventive nanoconstructs (CPNCs), Dr. Rai co-encapsulated anti-inflammatory, apoptosis-inducing, and glucogenesis-blocking drugs that are either in clinical use or have undergone clinical testing for chemopreventive use. The effectiveness of these agents in preventing tumor establishment and growth is being tested in orthotopic mouse models.



Confocal imaging of breast cancer cells (top left) with free drug (top right), passive nanoconstructs (bottom left) and TNCs (bottom right). Blue: DAPI staining of nucleus, Green: drug.

AWARD IMPACT ON CAREER PATH AND TRANSITION

Dr. Rai could not imagine being an independent investigator without the support provided by the K99/R00 award and feels he would have most likely looked towards an industry-based scientist position without it. He found the Alliance offered additional benefits to an early career researcher, especially the Alliance Principal Investigators' Meetings, which were an excellent opportunity to meet and discuss his work with other nanotechnology researchers. He hopes the network he established as an Alliance member will continue to help him build collaborations in the future.

Nanotechnology for Minimally Invasive Cancer Detection and Resection

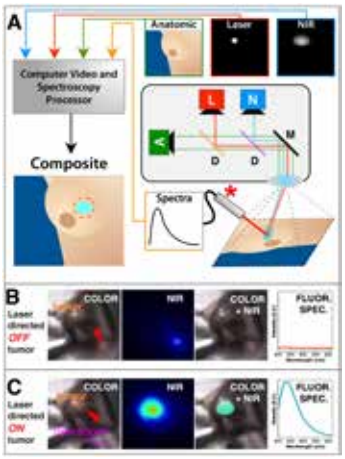
UNIVERSITY OF NEBRASKA MEDICAL CENTER
PRINCIPAL INVESTIGATOR: AARON M. MOHS, PhD

OVERVIEW

The overall goal of this project was to develop clinically applicable nanoparticle contrast agents and minimally invasive instrumentation to detect cancer and aid surgical resection. Through this project, Dr. Mohs aimed to develop and optimize activatable optical nanoparticles to be integrated into a flexible, miniaturized intraoperative probe to be combined with endoscopy to increase detection of tumor margins and residual tumor cells. This flexible spectroscopic endoscope with nanoparticle integration was validated in xenograft mouse models and in spontaneous canine tumors during surgery. Additional information about Dr. Mohs and his work can be found at http://www.unmc.edu/pharmacy/faculty/pharmaceutical-sciences/mohs.html and http://mohslab.weebly.com.

SCIENTIFIC ACHIEVEMENTS

The most relevant achievements of this award were the development of a series of polymeric nanoparticles (NPs) based on polysaccharide hyaluronic acid (HLA), a natural component of the cellular extracellular matrix which is overexpressed in many human tumors, and the translation into a spontaneous canine tumor model of an intraoperative imaging system designed by Dr. Mohs. The FDA-approved near infrared fluorescent dye indocyanine green (ICG) was entrapped in HLA-derived NPs termed NanoICG, a formulation shown to increase circulation time and facilitate delivery to tumor. It was hypothesized that NanoICG remains quenched until it is disassembled by hyaluronidases, such as HYAL-1 and HYAL-2, that are elevated in breast and prostate cancers, or when ICG is released and binds with serum proteins. These NPs showed increased tumor accumulation and produced stronger contrast enhancement compared to free ICG[1]. In collaboration with Dr. James Provenzale (Emory/Duke), and veterinary collaborators, Dr. Mohs further pursued development of his imaging system for studies of canines bearing tumors in a veterinary clinic setting. The group developed a wide-field imaging and spectroscopy system that was tested using indocyanine green[2,3]. In both mouse models and canine surgical samples, dye preferentially accumulated in tumor tissue, rather than surrounding normal tissue, resulting in sufficient contrast enhancement to differentiate between the two tissues. The ability to detect cancerous signal on the tumor margin would have great clinical utility, making these results very promising.



Schematic depiction (A) of fluorescence image-guided surgery system with color, near infrared, and spectroscopic views (B, C).

AWARD IMPACT ON CAREER PATH AND TRANSITION

The K99/R00 had a significant impact on Dr. Mohs transition from postdoctoral fellow to independent faculty member, by providing a framework in which he was able to obtain data as part of a project that would transition with him and work independently in a career training setting. The R00 component was very important during the start of his faculty career, as he could immediately work out a plan to hire research personnel, more easily recruit students, and quickly acquire data in support of his first R01 application.

1. Hill, T.K., et al. Indocyanine green-loaded nanoparticles for image-guided tumor surgery. Bioconjugate chemistry 26, 294-303 (2015).
2. Mohs, A.M., et al. An integrated widefield imaging and spectroscopy system for contrast-enhanced, image-guided resection of tumors. IEEE transactions on bio-medical engineering 62, 1416-1424 (2015).
3. Mohs, A.M., et al. Hand-held spectroscopic device for *in vivo* and intraoperative tumor detection: contrast enhancement, detection sensitivity, and tissue penetration. Analytical chemistry 82, 9058-9065 (2010).

Inhibition of Metastasis-initiating Cells by Chimeric Polypeptide Nanoparticles

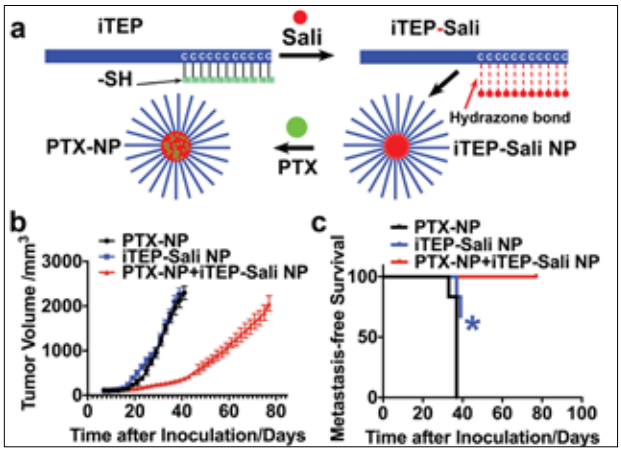
UNIVERSITY OF UTAH
PRINCIPLE INVESTIGATOR: MINGNAN CHEN, PhD

OVERVIEW

The overall objective of this project was to develop a nanoscale drug carrier system that exploits the invasive behavior of metastasis-initiating cells, a subset of cancer stem cells (CSCs), to prevent blood vessel-dependent cancer metastasis. Dr. Chen approached the project by using chimeric polypeptide nanoparticles (CP-NPs) to deliver an inhibitor (salinomycin) against this invasive sub-type in solid tumors by exploiting the enhanced permeability and retention (EPR) effect. This approach was intended to inhibit or prevent metastasis from the primary tumor. Additional information on Dr. Chen and his work can be found at http://nano.cancer.gov/about/meet/pathway_independence.asp#mchen and http://www.mchenlab.com.

SCIENTIFIC ACHIEVEMENTS

To develop a treatment for metastatic cancer, which is typically not responsive to standard chemotherapy, Dr. Chen devised and optimized CP-NPs that were able to encapsulate salinomycin or deliver salinomycin in a conjugate form, accumulate at tumors, and boost efficacy in reducing the number of CSCs in treated tumors in comparison to treatment with free salinomycin. To improve the translational potential of CP-NPs, he created a novel family of immune-tolerant elastin-like polypeptides (iTEP), and conjugated these polypeptides to salinomycin[1]. The NP-delivered salinomycin had an increased blood half-life and tumor accumulation compared to free salinomycin[2]. Particularly, the salinomycin that was loaded onto the NP through a cleavable bond had a longer and controlled *in vitro* release from the NPs, and enhanced CSC-elimination in an orthotopic breast tumor model in mice. The salinomycin-loaded NPs significantly inhibited both primary tumor growth and metastasis of the model. When combined with paclitaxel, a conventional chemotherapeutic, the salinomycin-loaded NP improved survival in mice bearing metastatic breast tumors.



Design schematic of iTEP-salinomycin (Sali) NP that loads salinomycin as a conjugate (a) and tumor growth and metastasis-free survival curves (b,c).

AWARD IMPACT ON CAREER PATH AND TRANSITION

The K99/R00 was an important factor in Dr. Chen's decision to continue research on cancer therapeutic nanocarriers. It facilitated a quick transition from postdoctoral fellowship to an independent research program and provided him with a broad and much needed platform for collaborations. Alliance membership enabled him to utilize this platform to make connections with many senior researchers in the cancer nanotechnology field, including those at his current institution. Dr. Chen now has several ongoing collaborative projects and grant applications with these senior researchers.

1. Cho, S., Dong, S., Parent, K.N. & Chen, M. Immune-tolerant elastin-like polypeptides (iTEPs) and their application as CTL vaccine carriers. Journal of Drug Targeting, 1-12 (2015).
2. Zhao, P., Dong, S., Bhattacharyya, J. & Chen, M. iTEP nanoparticle-delivered salinomycin displays an enhanced toxicity to cancer stem cells in orthotopic breast tumors. Molecular pharmaceutics 11, 2703-2712 (2014).

Tumor Targeting and Diagnostic Applications of Glycosylated Nanotubes

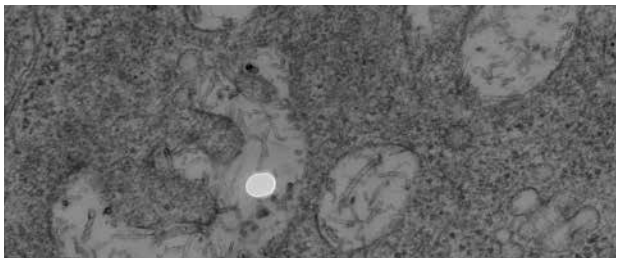
WAKE FOREST UNIVERSITY SCHOOL OF MEDICINE
PRINCIPLE INVESTIGATOR: RAVI N. SINGH, PhD

OVERVIEW

The primary goal of this award was to develop a new imaging tool based on multiwalled carbon nanotubes (MWCNTs) for the diagnosis and monitoring of advanced breast cancer. Dr. Singh hypothesized that MWCNTs targeted to glucose transporters would be taken up by cancer cells with high efficiency, and that such MWCNTs could be engineered to be effective imaging/diagnostic agents with multimodal applications in photothermal therapy of cancer. This award supported development of the MWCNT agent and studies to assess its blood compatibility and biodistribution. Additional information about Dr. Singh and his research can be found at http://nano.cancer.gov/about/meet/pathway_independence.asp#rsingh and http://www.wakehealth.edu/Research/Cancer-Biology/Ravi-Singh-Laboratory.htm.

SCIENTIFIC ACHIEVEMENTS

MWCNTs offer numerous advantages as agents for targeted molecular imaging, including increased sensitivity, multi-modal imaging capability, and combined imaging and therapeutic functionality. Dr. Singh synthesized and characterized MWCNTs for combined imaging and treatment of breast cancer, and studied the effect of surface chemistry on cell uptake and *in vivo* behavior. Cell binding and uptake studies using radiolabeled glycosylated nanotubes in the presence or absence of inhibitors of glucose uptake indicated that these tubes associate with cells in a glucose dependent manner[1]. Dr. Singh further found that oxidation of the nanotube surface is essential for reduced thrombogenicity following intravenous injection in mice[2]. *In vivo* tumor targeting studies indicated that glycosylated nanotubes optimized for stability in physiologic solutions exhibit improved accumulation in breast cancer xenografts compared to free sugars[1]. Dr. Singh also developed a dual mode imaging and photothermal agent based on MWCNTs that combines magnetic resonance contrast and near infrared absorptive properties for use in image guided drug delivery and photothermal therapy applications[3]. Notably, MWCNT-based photothermal therapy was shown to be effective at killing stem-cell like breast cancer cells thought to be responsible for treatment failure and disease relapse[4].



Transmission electron microscopy image of glycosylated multiwalled carbon nanotubes (MWCNTs) in breast cancer cells.

AWARD IMPACT ON CAREER PATH AND TRANSITION

Dr. Singh feels the K99/R00 award opened doors and eased the transition from post-doctoral to independent researcher. The award increased the visibility of his work, and the Alliance affiliation allowed creation of a broad network of potential collaborators. Further, Dr. Singh felt that his research and training would not have been possible without the award, as the supported work was outside the scope of his post-doctoral mentors' research programs. In this regard, he has leveraged his K99/R00 supported research project into three distinct projects for which he is currently pursuing new funding opportunities.

1. Fahrenholtz, C.D., Hadimani, M., King, S.B., Torti, S.V. & Singh, R. Targeting breast cancer with sugar-coated carbon nanotubes. Nanomedicine 10, 2481-2497 (2015).
2. Burke, A.R., et al. Determinants of the thrombogenic potential of multiwalled carbon nanotubes. Biomaterials 32, 5970-5978 (2011).
3. Ding, X., et al. Development of iron-containing multiwalled carbon nanotubes for MR-guided laser-induced thermotherapy. Nanomedicine 6, 1341-1352 (2011).
4. Burke, A.R., et al. The resistance of breast cancer stem cells to conventional hyperthermia and their sensitivity to nanoparticle-mediated photothermal therapy. Biomaterials 33, 2961-2970 (2012).

Nanotechnology Characterization Laboratory (NCL)

The Nanotechnology Characterization Laboratory (NCL; http://ncl.cancer.gov) is a formal interagency collaboration of NCI's Alliance for Nanotechnology in Cancer, with the U.S. Food and Drug Administration (FDA) and the National Institute of Standards and Technology (NIST). NCL was established in 2004 to fill a gap in preclinical characterization of nanomedicines and to accelerate the pace at which cancer-targeting nanomedicines are tested in clinical trials. Towards this goal, NCL has four main objectives: (1) Characterize nanoparticles using standardized methods; (2) Conduct structure activity relationship (SAR) studies to identify and characterize critical parameters related to nanomaterial ADME/Tox; (3) Facilitate regulatory review of nanotech constructs; and (4) Engage in educational and knowledge sharing efforts.

NCL is the only U.S. government laboratory with a focus on testing biomedical nanotechnology products, and over the past 10 years (2005-2015) has accumulated a tremendous knowledgebase about what does and does not work in nanomedicine design. As such, NCL's efforts have helped to drive private-sector investment in the development of nanomedicines. This past year, major pharmaceutical firms such as AstraZeneca invested in nanomaterials evaluated by NCL, and companies with NCL-tested nanomedicines now have over $1 billion in licensing agreements with the pharmaceutical industry. As a recognized leader in its field, NCL is bringing its resources to bear on areas it has identified as being of particular importance for the future of cancer nanotechnology research: these "gaps" in current understanding include surface characterization, nanomaterial immunotoxicity, nanomedicine metabolism, and nanotech co-formulation of synergistic chemotherapeutics. Recognizing the NCL's contribution to advancing nanomedicine and nanotechnology, several other countries, including the European Union, Australia, Chile, and China have begun to request proposals and dedicate funding to establishing versions of the NCL.

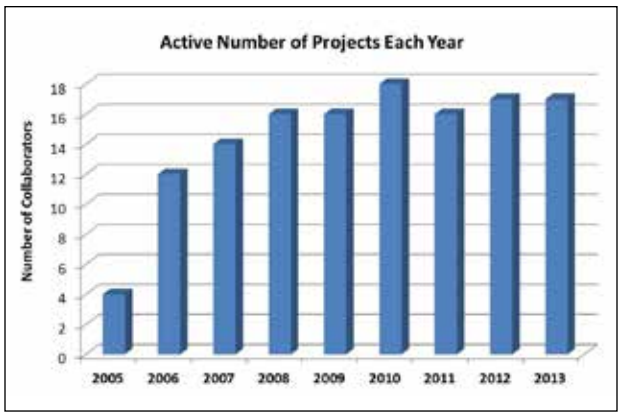
ACCOMPLISHMENTS

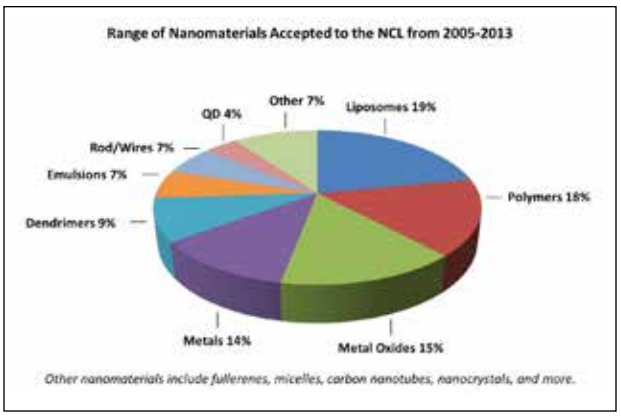
Characterize nanoparticles using standardized methods

Since NCL began accepting applications in 2005, NCL has characterized more than 300 different nanoparticles from over 100 different investigators. NCL averages 15 ongoing collaborations at any given time, characterizes an average of 75 nanoparticles per year, and conducts about 20 animal studies each year. The laboratory works with investigators from all backgrounds, including academia, small biotech companies, large pharmaceutical companies, and independent investigators. These collaborations have allowed NCL to elucidate trends relating physicochemical properties such as size and surface chemistry to nanoparticle behavior in biological systems, biodistribution, safety, and efficacy, resulting in 10 Phase I clinical trials for cancer nanotherapeutics. Additionally, NCL has standardized more than 40 *in vitro* assays for nanomaterial characterization and developed three ASTM standards.

Structure activity relationships studies

In addition to characterization projects, NCL also directs independent research projects and structure-activity relationship studies aimed at understanding the relationship between a nanoparticle's structure and its induced biological responses. NCL publishes the results of these studies to inform the nanotech and cancer research communities on the "lessons learned" from NCL characterization. Researchers can use the data from NCL SAR studies to inform their design of next-generation nanomedicines to "engineer out" undesirable properties like excessive uptake by the immune system or cytotoxicity.





Facilitate regulatory review of nanotech constructs

NCL interfaces with the FDA on a number of levels to ensure NCL's services are in line with current regulatory requirements. Senior FDA personnel participate on NCL's Scientific Oversight Committee (SOC) to review NCL-generated data to help ensure NCL's assays are capturing important aspects of characterization that are relevant for regulatory submission. NCL also interacts with FDA policymakers on national-level committees, such as those sponsored by the National Nanotechnology Initiative (NNI), and with FDA scientists and reviewers to address specific scientific challenges in nanomedicine. NCL has worked with four FDA centers and completed six projects on a variety of nanomaterial concerns. FDA and NCL also co-sponsor workshops, seminars, and meetings for standards development and characterization of nanomaterials.

Educational and knowledge sharing efforts

NCL has published more than 100 peer-reviewed manuscripts and top-level review articles, many in high-impact journals, and has also published book chapters and edited two books. In 2011, NCL developed a "Lessons Learned Workshop". This was an intensive 2-day workshop designed for all levels of nanotech researchers that highlighted many of the common shortcomings and mistakes NCL has seen from its over 100 collaborations. The series provided detailed talks on many different aspects of preclinical nanomedicine characterization, breakout sessions on select topics of interest, and case studies describing the various nanomaterial deficiencies encountered during NCL's three-tiered Assay Cascade characterization process.

In addition to publications and the Lessons Learned Workshop, NCL staff participate in scientific national and international conferences and give approximately 25 invited talks each year at scientific meetings, universities, companies, and more. NCL's scientists also contribute directly to the education of the next generation of nanotechnologists through a training course at the NIH, and provide educational opportunities to high school and college students through NCI and NIH internship programs. NCL staff also support the local community by serving as judges at science fairs, participating at high school career days, and serving as advisors for student research projects involving cancer nanotechnology.

COLLABORATIONS

62 RESEARCH COLLABORATIONS ACROSS THE ALLIANCE

65 TRANSLATION OF NANOTECHNOLOGY IN CANCER (TONIC): ACCELERATING TRANSLATION THROUGH PUBLIC-PRIVATE PARTNERSHIPS

67 NANOMATERIAL DATA SHARING: SUPPORT FOR NANOINFORMATICS AND DATABASES

Research Collaborations Across the Alliance

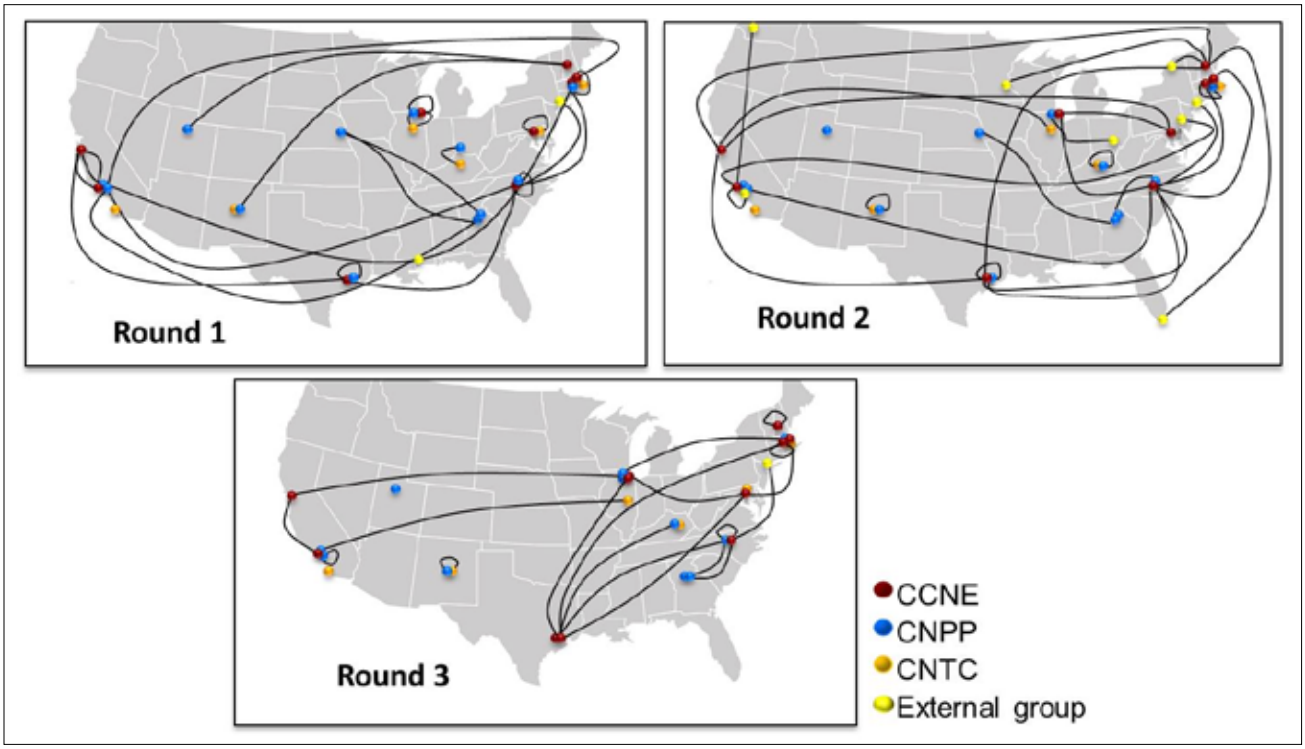
Phase II of the Alliance was organized as a transdisciplinary network of awards with the intention of promoting research collaboration, joint inquiry and discovery, and development of best practices. Alliance members were brought together at the annual Principal Investigators Meeting, semi-annual Coordination and Governance Committee meetings, and special purpose teleconferences to plan and initiate shared activities. Outcomes included topic-focused working groups, external partnerships (see "Translation Of Nanotechnology In Cancer (TONIC): Accelerating Translation through Public-Private Partnerships" on page 65), and trans-Alliance research collaborations.

Working groups were composed of Alliance members and representatives from NCI with shared interests in some aspect of nanomedicine research and development, such as formulation and characterization of nanoparticle drugs, proper choice of animal models for preclinical testing of nanomedicines, and gene therapy/RNA interference. Two working groups, Biotargeting and Imaging, authored publications to bring the combined insight of the Alliance to the broader research community. The Biotargeting perspective piece, "Biotargeted nanomedicines: Six tenets before you begin,"[1] addressed the biological barriers to effectively targeting nanomedicines to tumors, along with the translational difficulties, such as quality control and scale-up in manufacturing, that can prevent promising nanomedicines from moving into clinical application. In their article "Nanoparticles for cancer imaging: The good, the bad and the promise,"[2] the Imaging working group sought to share the challenges faced and lessons learned by Alliance members in developing nanoparticles as clinical imaging agents. The group provided an overview of the current status of nanoparticle imaging in cancer and identified the major factors that have prevented nanoparticle imaging agents from attaining regulatory approval and market acceptance.

The Alliance also engaged academic and industrial researchers external to the Alliance for discussions on areas of controversy or uncertainty in translational cancer nanotechnology research. At the onset of Phase II, the Alliance convened a meeting on Best Practices in Cancer Nanotechnology, which brought together experts from government, academia, and industry, and resulted in a workshop report outlining design, characterization, manufacturing, and regulatory issues to be considered when developing nanomedicines for cancer[3]. Similarly, the Alliance co-hosted a workshop on the enhanced permeability and retention (EPR) effect with TONIC members, which led to the formation of a clinical interest group and follow-up efforts within TONIC, as well as a workshop report[4].

Members of the Alliance network also engaged in collaborative research projects, many supported by funds set aside in each Center for Cancer Nanotechnology Excellence and Cancer Nanotechnology Platform Partnership award for Trans-Alliance Challenge Projects. Three rounds of the Challenge program encompassing 68 projects were held during the five year Phase II period, with an increasing number of external partners being brought into the network over time. There was an emphasis on technology and resource sharing in the projects pursued by awardees. Many of these projects led to lasting collaborations and peer reviewed publications.

A common theme in Alliance collaborations was the evaluation of complementary technologies and strategies used in combination to create more effective therapies. Members of the UNC and Texas Centers investigated the antitumor effects of combining metronomic doses of docetaxel in PLGA-PRINT nanoparticles, developed by UNC PI Dr. Joseph DeSimone, and anti-angiogenic mEZH2 siRNA delivered using chitosan nanoparticles, developed by Texas PIs Drs. Anil Sood and Gabriel Lopez-Berestein[5]. The study found the combination metronomic therapy to be effective at reducing tumor burden while also maintaining a



Map of Alliance Challenge Projects. Dots represent Alliance awards/external groups; lines indicate collaborative projects.

low toxicity profile. Benefits of the approach appear to result from both the properties of the nanomaterials and their intrinsic suitability for use with metronomic dosing, e.g., enhanced plasma exposure and burst drug release from PRINT nanoparticles.

Through a Challenge Project, the PRINT platform was also combined with a spray-assisted Layer-by-Layer (LbL) deposition process from the lab of Dr. Paula Hammond of MIT to create nanoparticle systems that are both scalable in manufacture and customizable in design[6]. The combined system allows precise control over the size, shape, and composition of nanoparticles through the PRINT process, with tightly controlled addition of functional coatings to the surface using the LbL process. Materials produced through this approach were well characterized and shown to have high uniformity and the potential for versatile applications. Dr. Hammond also worked with Dr. Julia Ljubimova of Cedars-Sinai Medical Center to apply the LbL process to enable controlled release of proteins from multilayer thin films of polymalic acid (PMLA), the material at the heart of the Cedars-Sinai platform award whose use in nanoparticle drug delivery was pioneered in Dr. Ljubimova's lab[7,8].

In keeping with the translational focus of the Alliance, questions of proper preclinical evaluation of nanotherapeutics were also pursued in collaborative projects. Thomas O'Halloran of Northwestern University and Meenakshi Upreti, a faculty member affiliated with the University of Kentucky Cancer Nanotechnology Training Center, evaluated a galectin-1 targeted nanobin formulation of arsenic trioxide and cisplatin in mice orthotopically implanted with tumor tissue analogs. These sophisticated three-dimensional co-culture spheroid tumor models incorporate color-coded tumor, endothelial,

and fibroblast cells to enable detailed evaluation of the effects of multimodal therapeutic strategies on the tumor microenvironment and the effectiveness of receptor targeting, two commonly used strategies in nanomedicine[9].

The Alliance also worked with other NCI networks to investigate the use of nanotechnology in drug and gene delivery. One very successful interaction was the use of administrative supplements from NCI to support collaboration between members of the Alliance and the Cancer Target Discovery and Development (CTD^2) program, which promotes translation of genomic characterization data into therapeutic targets. There was strong interest from both programs in the supplements, as CTD^2 members sought technologies to validate their extracted targets and Alliance members sought to apply their platforms to functionally annotated targets. Three project pursuing distinct approaches were funded—*in vitro* testing of a potential gene target for ovarian cancer, *in vivo* delivery of miRNA to test the contribution of key miRNA to synthetic lethality, and delivery of a peptide antagonist to KRas to inhibit KRas-Raf interactions implicated in pancreatic cancer progression.

From the beginning, the hope for the Alliance was that cancer nanotechnology development could be accelerated by enabling Alliance researchers to leverage network resources, including the skills and knowledge of fellow Alliance members. That hope has been fulfilled, and indeed the Alliance increasingly acts as a resource for other NCI networks and programs and the larger research community.

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2. Chapman, S., et al. Nanoparticles for cancer imaging: The good, the bad, and the promise. Nano today 8, 454-460 (2013).
3. Zamboni, W.C., et al. Best practices in cancer nanotechnology: perspective from NCI nanotechnology alliance. Clinical cancer research : an official journal of the American Association for Cancer Research 18, 3229-3241 (2012).
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5. Gharpure, K.M., et al. Metronomic docetaxel in PRINT nanoparticles and EZH2 silencing have synergistic antitumor effect in ovarian cancer. Molecular cancer therapeutics 13, 1750-1757 (2014).
6. Morton, S.W., et al. Scalable manufacture of built-to-order nanomedicine: spray-assisted layer-by-layer functionalization of PRINT nanoparticles. Advanced materials 25, 4707-4713 (2013).
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Translation Of Nanotechnology In Cancer (TONIC): Accelerating Translation through Public-Private Partnerships

The Alliance for Nanotechnology in Cancer established the Translation Of Nanotechnology In Cancer (TONIC) consortium in October 2011 with the mission of accelerating the translation and development of nanotechnology solutions for the early detection, diagnosis, and treatment of cancer. TONIC members aimed to combine their expertise to identify and evaluate the most promising technology candidates to develop a robust translational roadmap for the development of nanotechnology-based cancer products. In partnership with public, private, and academic sectors, TONIC provides Alliance researchers insight into industry needs for technology platforms and drug targets, promotes collaborations between Alliance investigators and industry partners on promising pre-competitive and late-stage programs, and serves as a sustained forum for nanotechnology idea exchange. The partnership further provides TONIC members the opportunity to interact with regulatory authorities and the Nanotechnology Characterization Laboratory (NCL) to promote the qualification, development, and regulatory acceptance of nanotechnologies in cancer. TONIC also encourages the sharing of consortium project results with the scientific community and independent verification opportunities to ensure data reproducibility and robustness.

Membership to the TONIC consortium remains free of charge, and for companies is limited to those that 1) have a successful track record of translating diagnostics and drug formulations and reaching their regulatory approval and, 2) are engaged in the development of nanotechnology-based formulations with application to imaging, diagnostics, and therapy. In addition, these companies are expected to have a corporate structure with centralized operations and the capability and resources to effectively move along translational efforts. Currently, membership includes 14 corporate partners, and three patient advocacy groups, with participation by NCL and the U.S. Food and Drug Administration. Advocacy groups are invited to promote research directed towards clinical translation and to increase community awareness of the TONIC mission.

ACCOMPLISHMENTS TO DATE

As an initial effort, TONIC arranged several teleconferences and face-to-face meetings both to introduce its members to the various Alliance-funded programs and to encourage discussions focused on gaps in the community's research portfolio, and nanotechnology specific concerns in drug delivery and other applications. These initial interactions led to the identification of the enhanced permeability and retention Effect (EPR) as an important area for the group to focus on, leading to an NCI hosted workshop on EPR in October 2012. The main focus of this workshop was to gain a better understanding of EPR characteristics impacting the use of nanoparticles in the clinic. This resulted in a report published in Cancer Research that outlined the key gaps that exist between the experimental evidence of EPR in animal models and humans, and ways to address these gaps[1].

The participants of the ERP workshop strongly recommended the formation of a working group to establish translational and clinical procedures for integrated clinical trials involving nanotherapeutic constructs and accompanying imaging approaches. Such translational studies and clinical trials would enable further understanding and predictability of EPR function in a tumor and its primary or metastatic sites, and could be critical for the development of future effective nanodrugs. Subsequently, in January 2014, a nanodrug clinical working group was formed consisting of leaders in the cancer nano-technology research field and TONIC industry members with particular interest in advancing nanotechnology platforms to the clinic. This group has since defined strategic preclinical and clinical study designs to address the EPR effect question; an overview of these strategies was reported in The Lancet[2]. The working group is currently evaluating various funding

mechanisms to conduct the proposed work, and is exploring plans to expand its scope to serve as a resource that can help new investigators in preclinical and clinical trial designs.

Additionally, TONIC has organized several meetings and presentations at various venues over the past three years to educate the pharmaceutical industry and enhance awareness of nanotechnology platform opportunities in developing cancer solutions. It continues to participate in the Annual Alliance Principal Investigators' meetings to promote networking and collaborations between industry and academic groups, and encourages the evaluation of external opportunities and platforms. The consortium has been credited with facilitating interactions with NCL for TEVA (http://www.tevapharm.com), AstraZeneca (http://www.astrazeneca-us.com/home), and two TONIC members. TEVA and NCL signed an agreement to initiate a collaborative study. Cytimmune (http://www.cytimmune.com) credits TONIC for facilitating a research agreement with AstraZeneca to create a new nanomedicine using an AstraZeneca proprietary drug mounted on Cytimmune's PEGylated TNF gold nanoparticle platform. Moving forward, TONIC will continue to take advantage of new opportunities to accelerate the consortium's mission of translating nanotechnologies to the clinic, and enhance academic-industrial partnerships.

1. Prabhakar, U., et al. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. Cancer research 73, 2412-2417 (2013).
2. Gabizon, A., et al. Cancer nanomedicines: closing the translational gap. Lancet 384, 2175-2176 (2014).

Nanomaterial Data Sharing: Support for Nanoinformatics and Databases

The "Omics" communities (e.g., genomics and proteomics) pioneered the development of databases and data deposition guidelines to facilitate data reproducibility and integration across datasets. For nanotechnology, lack of access to high quality research data is a known challenge, further complicated by the diversity and growing number of nanomaterials. NCI's Office of Cancer Nanotechnology Research (OCNR) recognizes the importance of data sharing to enable new knowledge discovery and the rational design of nanomaterials to ultimately aid the development of optimized diagnostics and therapeutics. To address the challenges in nanotechnology data sharing, NCI supports several nanoinformatics efforts at the federal and community levels as part of the Alliance for Nanotechnology in Cancer (Alliance) program. Described below are some of these efforts and accomplishments during the past two Alliance phases.

CANCER NANOTECHNOLOGY LABORATORY DATA PORTAL

As part of the first phase of the Alliance program (2005-2010), the cancer Nanotechnology Laboratory data portal (caNanoLab; https://cananolab.nci.nih.gov) was initiated in 2006 as a collaborative effort between the NCI Center for Biomedical Informatics and Information Technology and OCNR. caNanoLab is a web-based portal designed to capture nanotechnology data and serves as a data repository that allows researchers to submit and retrieve information on well-characterized nanomaterials including compositions; physico-chemical, *in vitro*, and *in vivo* characterizations; associated publications; and protocols. It was originally designed to capture information about a nanomaterial sample to fulfill the characterization requirements for federal regulatory review of nanomaterial-based investigational diagnostic devices, imaging agents, and new drugs[1,2]. Over the past nine years, caNanoLab has become an established resource designed to address the needs of the cancer, biomedical, and nanotechnology communities. As of October 2015, the portal has information on 1,106 curated nanomaterial samples, 46 protocols, and 1,904 publications that are available for public use. The majority of these data have been curated from publications by an in-house curator; however, individual users can submit data via web-based forms and an established workflow. Visibility of data can be customized by submitters to range from private, sharable within a collaboration group, or open to the public. caNanoLab can also be used for discovery purposes by searching the results of all the publicly available data using webform-based queries. These results can be downloaded in spreadsheet-based reports for additional analyses. For those interested in maintaining a local instance of caNanoLab, the caNanoLab web application software is open source and available for download and local installation from GitHub (https://github.com/NCIP/cananolab).

As part of the Phase III Alliance program, awardees are now required to share nanomaterial data through caNanoLab as a Term and Condition of Awards issued under RFA-CA-14-013 (http://grants.nih.gov/grants/guide/rfa-files/RFA-CA-14-013.html) and PAR-14-285 (http://grants.nih.gov/grants/guide/pa-files/PAR-14-285.html). Although not yet a common requirement for other nanomaterial-related funding opportunities, OCNR expects this award condition will encourage sharing and submission of nanotechnology data directly by investigators, which is one of the most challenging aspects of data curation. To aid in this process, caNanoLab made website enhancements such as the addition of a "My Workspace" feature and navigation changes to improve individual user data submission (caNanoLab 2.0 release in December 2014; upcoming caNanoLab 2.1 release). Also, the caNanoLab team has been working with journals to encourage investigators to submit data during the manuscript submission process, and promote usage of databases.
For example, caNanoLab is one of several approved data repositories (http://www.nature.com/sdata/data-policies/repositories) for Nature Publishing Group's data descriptor journal, Scientific Data, which requires the deposition of

datasets associated with submitted manuscripts, and is also a recommended repository for PLOS journals (http://journals.plos.org/plosone/s/data-availability#loc-recommended-repositories). The scientific publisher Elsevier implemented reciprocal linking between datasets housed in caNanoLab and the associated published research article (http://www.elsevier.com/books-and-journals/content-innovation/data-base-linking#supported-data-repositories). This linkage is in place for 10 journals with plans for additional Elsevier journal linkages. Most recently, the caNanoLab team added caNanoLab as a PubMed LinkOut resource to directly link Pubmed articles with data curated into caNanoLab (http://www.ncbi.nlm.nih.gov/projects/linkout/journals/htmllists.cgi?type_id=9).

NANOMATERIAL REGISTRY

With support from the National Institute of Biomedical Imaging and Bioengineering and NCI, the Nanomaterial Registry was launched in 2011 to serve as a web-based tool that archives research data on nanomaterials and their biological and environmental implications (https://www.nanomaterialregistry.org)[3]. All data housed in the Registry is systemically curated and reviewed using a set of minimal information about nanomaterials (MIAN) developed by the Registry team to create criteria for curation and comparisons of nanomaterials that are curated into the Registry. Currently, the Registry holds 2,031 nanomaterial records from various environmental and biological data sources such as caNanoLab, individual partnerships, the patent literature, and manufacturer catalogs. Registry features include the ability to search, compare nanomaterials side-by-side, and search for similar nanomaterials, as well as compliance ratings for each nanomaterial based on the quality and quantity of data, and a data export function. Most recently, the Registry team worked on integrating modeling and simulation tools through a portal from the Registry to nanoHUB (http://www.nanohub.org), an online simulation resource for nanotechnology. Through an interface on the NanoHUB site, all nanomaterial records in the Registry are periodically exported to NanoHUB. NanoHUB imports these data records and offers them to users for filtering, searching, and downloading for use in simulations and predictive modeling (https://nanohub.org/groups/nanomaterialregistry). Currently, the Registry is working on refining a Data Visualization Dashboard, and data submission tools to allow submission directly by users.

NATIONAL CANCER INFORMATICS PROGRAM (NCIP) NANOTECHNOLOGY WORKING GROUP

Members of the caNanoLab and Nanomaterial Registry teams are engaged in many activities to better serve the needs of the nanotechnology research community. The teams actively work with databases, community-based programs, and federal initiatives such as the National Nanotechnology Initiative's Signature Initiative on Nanotechnology Knowledge Infrastructure (http://www.nano.gov/NKIPortal) and the National Cancer Informatics Program Nanotechnology Working Group (NCIP Nano WG; https://wiki.nci.nih.gov/display/ICR/Nanotechnology+Working+Group), to develop data exchange standards and deposition guidelines for data submission and sharing. In particular, the NCIP Nano WG, which was established during the first Alliance phase, prioritizes enabling the exchange of information across the nanotechnology community. Consisting

of researchers from academia, government, and industry, much of the group's focus has been on the collaborative development and dissemination of data standards. Efforts in this area include development and enhancement of the Nanoparticle Ontology for cancer nanotechnology research (NPO; www.nano-ontology.org) and ISA-TAB-Nano (https://wiki.nci.nih.gov/display/ICR/ISA-TAB-Nano)[4,5]. ISA-TAB-Nano is a spreadsheet-based format for representing and sharing information about nanomaterials and is currently used by NCI, the NBI Knowledgebase (http://nbi.oregonstate.edu), and the EU NanoSafety Cluster (http://www.nanosafetycluster.eu) to enable interoperability between databases. Most recently, the NCIP Nano WG established a subgroup focused on developing guidelines for data curation (Nanomaterial Data Curation Initiative, https://nciphub.org/groups/nanotechnologydatacurationinterestgroup). The subgroup is in the process of writing a series of consensus papers to provide an overview of current curation practices and recommendations. Two of these have been published so far in the Beilstein Journal of Nanotechnology[6,7].

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4. Thomas, D.G., et al. ISA-TAB-Nano: a specification for sharing nanomaterial research data in spreadsheet-based format. BMC biotechnology 13, 2 (2013).
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7. Powers, C.M., et al. Nanocuration workflows: Establishing best practices for identifying, inputting, and sharing data to inform decisions on nanomaterials. Beilstein journal of nanotechnology 6, 1860-1871 (2015).

ALLIANCE RESOURCES

71 MATERIALS AND COMPOUNDS

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Below is a list of materials and compounds, protocols, animal models, software, devices, and services developed by members of the Alliance Phase II program that are available to the broader research community. If interested in learning more, please visit the relevant publications and/or website links.

MATERIALS AND COMPOUNDS

Material or Compound	Description/Application(s)	Award/Investigator(s)	Relevant Publication(s)/ Link(s)
Magnetic nanoparticles (MNPs) for hyperthermia treatment	MNPs consisting of small (2-5nm) single crystals of gamma-Fe_2O_3 with saccharide chains implanted in their crystalline structure, forming 20-40 flower-like aggregates; can produce a significant amount of heat at field strengths as low as 100Oe at 99-164 kHz. Size: 110-120 nm.	Dartmouth-CCNE/Baker	[1]
1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) nanoliposomes	Neutral liposomes used for *in vivo* siRNA delivery. Size: 30-40 nm.	Texas-CCNE/Lopez-Berestein	[2]
Chitosan nanoparticles	Arg-Gly-Asp (RGD) peptide-labeled chitosan; tumor targeted delivery system for siRNAs. Size: ~200 nm.	Texas-CCNE/Sood	[3]
Gold nanoshells	Nanoparticles that convert near-infrared light to heat for use in photothermal cancer therapy; can be conjugated to gadolinium for imaging, cancer diagnosis. Size: ~150 nm.	Texas-CCNE/West	[4]
Multistage vector delivery system	Nano-carriers with first-stage mesoporous silicon particle carriers that allow for the load and release of second-stage nanocarriers (e.g., DOPC nanoliposomes). Size: 1-2 µm.	Texas-CCNE/Ferrari	[2]
In cellulo self-assembling nanoparticles for MRI	Redox activated Gd-based MRI probes with 60% increase in r1 relaxivity upon activation from its reduced form. Size: 100-200 nm.	Stanford-CCNE/Gambhir	[5]
In cellulo self-assembling nanoparticles for optical imaging	Caspase-sensitive nano-aggregation, biocompatible fluorescent probe with near-infrared (NIR) spectral properties; can undergo triggered self-assembly through condensation chemistry *in vivo* Size: ~150 nm.	Stanford-CCNE/Gambhir	[6]
Gold-silica-based Raman imaging agents (SERS)	Multimodal contrast agents based on fluorescent surface-enhanced Raman scattering gold-silica nanoparticles for endoscopic and multiplexed molecular imaging. Size: ~120 nm.	Stanford-CCNE/Gambhir	[7,8]
Poly(2-oxazoline) polymeric micelles, POx	Polymeric drug carrier/delivery system for loading and solubilization of hydrophobic drugs. Size: < 100 nm.	Univ. North Carolina-CNPP/Kabanov	[9-12]
Nanodiamond particles	Multimodal imaging/therapeutic nanoparticles composed of carbon. Size: 2-8 nm.	Northwestern-CCNE/Mirkin	[13-16]
Silica-coated zinc ferrite nanocubes	Magnetic nanoparticles suitable for magnetoresistive immunoassay labels. Size: ~130 nm.	Utah-CNPP/Porter	[17,18]
Redox-responsive gelatin	A biocompatible and biodegradable model material used to design multimodal delivery platforms for nucleic acids and small molecules.	Northeastern-CCNE/Torchilin	[19]

Material or Compound	Description/Application(s)	Award/Investigator(s)	Relevant Publication(s)/ Link(s)
Pro-apoptotic, anti-proliferative and migration-suppressive compound	A PIT-1 small molecule inhibitor series: non-lipid inhibitors of PIP3-dependent signaling events; compounds exhibit antitumor efficacy *in vitro* and *in vivo* when loaded into polyethylene glycol-phosphatidylethanolamine (PEG-PE) micelles alone and in combination with pro-apoptotic molecules. Micelle size: 16-20 nm.	Northeastern-CCNE/Torchilin	[20,21]
Monodisperse mesoporous silica nanoparticles	Mesoporous silica nanoparticles with varied surface chemistry properties. Size: 25-300 nm.	Univ. New Mexico-CNPP/William	[22]
Ultra small magnetic iron oxide nanoparticles	Sub 5 nm magnetic iron oxide nanoparticles with dual T1 and T2 switchable MRI contrasts, improved intratumoral delivery and alternative renal clearance. Size: < 5 nm.	Emory-CNPP/Yang/Mao	[23]
Theranostic iron oxide nanoparticles	Iron oxide nanoparticles conjugated or co-encapsulated with chemotherapeutics (gemcitabine and doxorubicin) in polymers, or conjugated to siRNA, and targeted to various cancers (e.g., pancreatic, ovarian, breast). Size: 60-100 nm. Core size: 10 nm, hydrodynamic size of theranostic nanoparticles: 20-45 nm.	Emory-CNPP/Yang/Mao	[24-27]
Anti-fouling polymer	Block copolymer poly(ethylene oxide)-block-poly(γ-methacryl oxypropyltrimethoxysilane) (PEO-*b*-PγMPS); coating to reduce nonspecific, off-target uptake of nanoparticles.	Emory-CNPP/Yang/Mao	[28,29]
Immune tolerant elastin-like polypeptide iTEP materials	Immune compatible, functional materials that can be used in medical products.	Chen K99/R00	[30]
Chimeric polypeptide nanoparticle (CP-NP)	Recombinant chimeric polypeptides that conjugate hydrophobic molecules and self-assemble in nanoparticles.	Chen K99/R00	[31]
Quantum dot (QD)-based nanoprobes	Brightness-equalized multicolor QDs, nonblinking QDs, compact QDs, and QD coatings.	Smith K99/R00	[32]
Theranostic nanoconstructs	Polymeric nanoparticles, liposomes and their hybrid nano-constructs encapsulating drugs and imaging agents, with targeting ligands attached.	Rai K99/R00	

PROTOCOLS

Protocol	Description/Application(s)	Award(s)/Investigator(s)	Relevant Publication(s)/ Link(s)
Synthesis of polymeric hyaluronic acid (HA)-based nanoparticles	Synthesis of dextran coated HA-PEI/HA-PEG, CD44-targeted nanoparticles loaded with siRNA or chemotherapeutic drugs; ligands target multi-drug resistant ovarian cancer. Size: ~200 nm.	Northeastern-CNPP/Amiji	[33]
Development of multi-drug resistant cell lines	Development of multi-drug resistant breast tumor cell lines under hypoxic conditions for establishing tumor xenografts.	Northeastern-CNPP/Amiji	[34]
Method for quantifying number of Ab fragments per IONP	Quantification of number of Ab fragments per NP using commercial bicinchoninic acid assay and correcting for the NPs using standard curves from appropriate controls.	Dartmouth-CCNE/Griswold, Project 1	[9,35]
Dual tracer receptor density imaging	Dual-tracer approaches to quantify cell-surface cancer receptor concentrations in primary tumor using planar fluorescence imaging and fluorescence tomography. Can localize cancer biomarkers and quantify; tumor burden.	Dartmouth-CCNE/Pogue, Project 2	[36-39]
Production of Fab antibody fragment	Engineering, production, and purification of Fab antibody fragments derived from clinically validated IgG antibody therapies.	Dartmouth-CCNE/Griswold, Project 1	[9,35]
Thioaptamer selection	Two-step selection strategy for identifying thiophosphate modified aptamers.	Texas-CCNE/Gorenstein	[40]
Next generation X-aptamers	Bead-based selection method for attachment of ligand binding moieties onto next-generation aptamers to enhance binding affinity.	Texas-CCNE/Gorenstein	[41]
Assembly of RNA nanoparticles	Assembly and evaluation of RNA nanoparticles through hand-in-hand, foot-to-foot, and stable branched RNA junction interactions. Size: varied sizes.	Kentucky–CNPP /Guo	[42-44]
Large scale purification of RNA nanoparticles	Method for large-scale purification of RNA nanoparticles through density gradient and rate-zonal ultracentrifugation.	Kentucky–CNPP /Guo	[45]
Time dependent *in vivo* fluorescence imaging	Assessment of nanoparticle biodistribution in intact living mice.	Rice-CNPP/Joshi	http://www.mcw.edu/radiology/faculty/Amit-Joshi.htm
Synthesis of gold-silica plasmonic nanoparticles ("nanomatryoshkas")	Synthesis of nanoparticles consisting of gold core, porous silica spacer layer, and thin gold outer shell layer; for use in photothermal therapy and nanoparticle tracking. Size: ~90 or ~150 nm.	Rice-CNPP/Halas	[46,47]
Spherical nucleic acid (SNA) nanostructures	Inorganic (gold, etc.) or hollow nanoparticles surrounded by densely functionalized, highly oriented oligonucleotides; applicable as therapeutic, diagnostic. Size: 13-50 nm.	Northwestern-CCNE/Mirkin	[48,49]
Magnetic nanostructures (MNS)	Spherical hybrid magnetic nanostructures-thermoresponsive hydrogels with metal core; use in multimodal imaging, therapeutic. Size: ~200 nm.	Northwestern-CCNE/Dravid	[50]

Protocol	Description/Application(s)	Award(s)/Investigator(s)	Relevant Publication(s)/ Link(s)
Nanostructured matrices for cancer cell biology	New technology for causing any desired mixture of expressed proteins to self-assemble into nanofibers and gels; useful for cell culture.	Northwestern-CCNE/Collier	[51]
Multifunctional cantilever-free scanning probe arrays coated with multilayer graphene	A high throughput procedure for coating scanning probes with multilayer graphene films for benchtop nanofabrication.	Northwestern-CCNE/Mirkin	[52]
Self-Assembling peptide amphiphile	Peptide configured to inhibit angiogenesis.	Northwestern-CCNE/Stupp	[53]
Effective antibody screening method	Multiplexed surface-enhanced Raman scattering based immunoasssays using gold nanoparticles.	Utah-CNPP/Porter	[17,54]
Magnetovaccination	Devised to visualize APC mediated transport of antigen to the draining lymph node for evaluating vaccine responses.	Hopkins-CCNE/Bulte	[55]
Mucus penetrating nanoparticle & targeting ligands	A strategy to overcome airway mucus, a primary barrier to inhaled nanoparticle-based therapies.	Hopkins-CCNE/Hanes	[56]
Synthesis of gold nanorod photothermal contrast agents	Synthesis of highly elongated gold nanocrystals for plasmonic photothermal therapy (PPTT). Size: 28 nm by 8 nm.	Emory-CNPP/ El-Sayed	[57,58]
Micelle nanoparticle targeting approach involving phage display	A nanocarrier targeting approach involving the fusion of micellar nanoparticles with target-specific phase coat protein obtained from landscape phage display libraries.	Northeastern-CCNE/Torchilin	[59,60]
Preparation of PEG-PE micelles combined with pro-apoptotic molecules	Preparation of PEG-PE micelles combined with TRAIL inhibitors, which have shown efficacy in a TRAIL-resistant tumor model. Micelle size: 16-20 nm.	Northeastern-CCNE/Torchilin	[20,21]
Synthesis of pH-, redox-, hypoxia- and MMP2-sensitive polymeric micelle conjugates	Synthesis of micelle carriers that enable co-delivery of hydrophilic siRNA and hydrophobic drugs in response to changes in pH, oxidation state, oxygen levels, and enzymatic activity. Size: 15-25 nm.	Northeastern-CCNE/Torchilin	[61]
Liposomal doxorubicin combination therapy involving thermal ablation	A protocol for adjuvant therapy of intravenous liposomal doxorubicin to radiofrequency tumor ablation in animal models.	Northeastern-CCNE/Goldberg	[62]
Synthesis of targeted liposomal formulations of SYK P-site inhibitor C-61	Synthesis of CD19-antigen specific nanoscale liposomal formulation of the SYK P-site inhibitor C61 for systemic therapy of acute lymphoblastic leukemia (ALL). Size: ~140 nm.	Children Hospital LA-CNPP/Uckun	[63]
Dot blot technique for evaluation of nanoparticle stability	A method to assess the stability of PEGylated nanoparticles decorated with monoclonal antibody in human serum.	Children Hospital LA-CNPP/Uckun	[63]

Protocol	Description/Application(s)	Award(s)/Investigator(s)	Relevant Publication(s)/ Link(s)
Magnetic resonance imaging protocol for iron oxide nanoparticles	T2-weighted and susceptibility weighted relaxometry mapping for iron quantification; Ultrashort echo time imaging method for turning IONP to positive contrast and delineating the quantitative relationship between T1 and T2 contrast and accumulation of iron oxide nanoparticles in tumors and tissues.	Emory-CNPP/Yang/Mao	[64,65]
Sugar-functionalized CNTs	Protocols for synthesis and purification of scaled-up production of sugar-functionalized CNTs using both self-assembly and covalent conjugation procedures.	Singh K99/R00	[66]

ANIMAL MODELS

Animal Model	Description/Application(s)	Award/Investigator(s)	Relevant Publication(s)/ Link(s)
Multi-drug resistant tumor models	Xenograft multi-drug resistant (MDR) ovarian tumor model in nude female mice.	Northeastern-CNPP/Amiji	[33]
SKOV3, OVCAR8 and A459 Orthotopic and Subcutaneous Models	Orthotopic and subcutaneous tumor models using SKOV3 & OVCAR8 ovarian adenocarcinoma and A549 lung adenocarcinoma cells.	Northeastern-CNPP/Amiji	[67,68]
Acquired drug resistance model development by administration of cancer therapeutics	Subcutaneous SKOV3 model administered with paclitaxel for up to 3 weeks to induce drug resistance.	Northeastern-CNPP/Amiji	[69]
$Kras^{LSL}$-G12D/+, $p53^{fl/f}$ genetically-engineered mouse (KP-GEM) model of non-small cell lung cancer (NSCLC)	Genetically-engineered NSCLC model.	Northeastern-CNPP/Amiji	
Orthotopic non-small cell cancer model	Luciferase-expressing human lung adenocarcinoma cell line A549-luc-c8 injected into the lung parenchyma of female nude mice.	Univ. North Carolina-CCNE/Mumper	[70] Contact: Russ Mumper, mumper@email.unc.edu
Mouse models of ovarian and pancreatic cancers	Orthotopic tumor models in immune-deficient and immune competent mouse models.	Texas-CCNE/Zhang, Cui	[71,72]
Colorectal cancer metastasis model (with Evers lab)	Mouse model of colorectal cancer metastasis to liver and lung.	Kentucky –CNPP/Guo	[73,74]
Patient Derived Xenograft (PDX) glioma models	A mouse model that recapitulates phenotypic and genotypic complexities of human high grade glioblastoma multiforme.	Northwestern-CCNE/Stegh	[75,76]
Cell line derived xenograft models of metastatic breast cancer	Includes models based on HeyA8, a robust metastatic breast cancer model and a lung metastatic variant of MDA-MB-231 called 231-LM2-4.	Northwestern-CNPP/O'Halloran	[77]

Animal Model	Description/Application(s)	Award/Investigator(s)	Relevant Publication(s)/ Link(s)
Pancreatic cancer model	Animal models of human pancreatic cancer developed by University of Utah Pancreas Cancer Research Program from 11 PDAC cell lines and >30 patient-derived xenografts.	Utah-CNPP/Porter	http://healthcare.utah.edu/huntsmancancerinstitute/research/disease-oriented-research-teams/pancreatic-cancer-dot.php
Subcutaneous pancreatic tumor model	An animal model developed via subcutaneous inoculation of SCID beige mice with Panc-1 human pancreatic adenocarcinoma cells.	Northeastern-CCNE/Torchilin	[19]
Human leukemia xenograft clones	Xenograft clones that can result in fatal leukemia in NOD/SCID mice.	Children Hospital LA-CNPP/Uckun	[78,79]
Chemo and radiation resistant leukemia models	NOD/SCID mouse xenograft models for chemotherapy-resistant and radiation-resistant leukemia.	Children Hospital LA-CNPP/Uckun	[78,79]
Avian embryo *in vivo* screening model	For use in determining nanoparticle vascular flow, stability, circulation time, non-specific interactions, toxicity, target cell binding specificity, internalization, and cargo release can all be monitored in real time (in millisecond intervals) and at light microscopy level resolution.	Univ. New Mexico-CNPP/Willian	[22]
Orthotopic human pancreatic cancer model	Nude mice bearing orthotopic tumor xenografts derived from the MIA PaCa-2 human pancreatic cancer cell line.	Emory CNPP/Yang	[25,26]
Orthotopic human pancreatic cancer model	Nude mice bearing orthotopic pancreatic cancer xenografts derived from human patient tissues (PDX).	Emory CNPP/Yang	[25,26]
Orthotopic mouse pancreatic cancer model	Orthotopic mouse pancreatic cancer model derived from a mouse PANC02 cell line inC57BL/6 mice.	Emory CNPP/Yang	
PDX-1-CRE, LSL-$Kras^{G12D}$	Kras transgenic mouse pancreatic cancer model.	Emory CNPP/Yang	

SOFTWARE AND DEVICES

Software/Device	Description/Application(s)	Award/Investigator(s)	Relevant Publication(s)/ Link(s)
Magnetic nanoparticle imaging system	An array of digitally controlled drive coils and compensated fluxgate magnetometers enables magnetic susceptibility imaging of magnetic nanoparticles.	Dartmouth-CCNE/Pilot Project, Diamond	[80]
Quantitative model of enhanced permeability and retention (EPR) effect	A pharmacokinetic model to quantitatively assess the influence of EPR effect on the uptake of drug into solid tumor.	Hopkins-CCNE/Searson	[81]
In vitro functional vessel platform	An *in vitro* setting for the evaluation of extravasation and efficacy of drug delivery system.	Hopkins-CCNE/Wirtz	http://inbt.jhu.edu/apps/toolrepository
Pharma-Designs	Collaboration software for accelerated translation of nanomedicines.	Northeastern-CCNE/Torchilin	http://www.nemucore.com

SERVICES

Service	Description/Application(s)	Award/Investigator(s)	Relevant Link(s)
PRINT particles	Innovative molding technology for mass production of nanoparticles with well-controlled properties.	Univ. North Carolina-CCNE/Desimone	Contact: Joseph DeSimone, desimone@unc.edu
In situ hybridization of ncRNAs, X-aptamer selections, and X-aptamer imaging agent-nanoparticle formulations	Provided by the Targeting Core of this CCNE.	Texas-CCNE	http://www.mdanderson.org/education-and-research/departments-programs-and-labs/programs-centers-institutes/texas-center-for-cancer-nanomedicine/index.html
Biomarker detection with porous silicon microchips	Provided by the Nanoengineering Core of this CCNE.	Texas-CCNE	http://www.mdanderson.org/education-and-research/departments-programs-and-labs/programs-centers-institutes/texas-center-for-cancer-nanomedicine/index.html
Nano Characterization and nanofabrication Cores	Provides resources for fabrication and analysis of micro- and nanostructures.	Stanford-CCNE/Gambhir	http://mips.stanford.edu/grants/ccne-t/resources.html

Service	Description/Application(s)	Award/Investigator(s)	Relevant Link(s)
Bio-repository	Collects and store samples from both mouse cancer models and human patients.	Stanford-CCNE/Gambhir	http://cancer.stanford.edu/research/core/Tissue_Procurement.html
Nanomedicines Characterization Core (NCore)	Open resource; functions on a fee for service basis for users from academia as well as from industry, government labs, and other institutions.	Univ. North Carolina-CNPP/Kabanov	http://ncore.web.unc.edu
Translational nanoformulation (TransNanoForm)	A core facility that supports safe and effective translation of new drug candidates into clinical trials through advanced formulation techniques.	Univ. North Carolina-CNPP/Kabanov	http://nform.web.unc.edu
Atomic and Nanoscale Characterization Experimental Center	Characterization/analytical facilities for nanostructures, biological structures, and systems.	Northwestern-CCNE/Dravid	www.nuance.northwestern.edu
Center for Advanced Molecular Imaging	Facilities for *in vivo* imaging of small animals; provides range of imaging modalities including magnetic resonance image and whole body bioluminescence/fluorescence imaging.	Northwestern-CCNE/Mirkin	www.cami.northwestern.edu
BioNanoprobe LS-CAT	Advance Photon Source—only x-ray microscope in the world that has 30nm resolution with cryo and tomographic capabilities.	Northwestern-CCNE/Woloschak	https://ls-cat.org/bnp.html
Pancreatic cancer primary patient xenograft archive & specimen library	Based on collaboration with Pancreas Cancer Research Program (PCRP) at the U. Utah. PCRP has enrolled 584 patients and has a collection of 1450 serum and plasma samples from patients.	Utah-CNPP/Porter	http://healthcare.utah.edu/huntsmancancerinstitute/patient-care/clinics-and-care-teams/high-risk-cancer-research-clinics-and-studies/pancreas-cancer-research-program
MIT Electron Microscopy and X-ray Core Facilities	MIT has available three high transmission resolution electron microscopes for TEM imaging, electron diffraction and elemental mapping, JEOL 2010, JEOL 2010 F, and JEOL 2200F cryo microscope. The X-ray diffraction facilities include 4 Rotating Anode Generators, 3 conventional Sealed X-ray Tube Generators and diffractometers.	MIT/Harvard CCNE/Langer-Weissleder	http://web.mit.edu/cmse/facilities/electron.shtml; http://web.mit.edu/cmse/facilities/xray.shtml
MIT Nanostructures Laboratory (NSL)	Develops techniques for fabricating surface structures with feature sizes that range from nanometers to micrometers. The NSL includes facilities for lithography liftoff, electroplating, sputter deposition, and e-beam evaporation.	MIT/Harvard-CCNE/Langer-Weissleder	http://nanoweb.mit.edu
Center for Molecular Imaging Research (CMIR)	Develops novel technologies for *in vivo* sensing and imaging of molecular events. CMIR provides advanced chemistry resources to synthesize molecular imaging probes, and a wide range of cutting edge, dedicated imaging equipment.	MIT/Harvard-CCNE/Langer-Weissleder	http://cmir.mgh.harvard.edu

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