NCI Alliance for Nanotechnology in Cancer

Accomplishments 2010 – 2013

Chapter 1 Program Overview

Background

Nanotechnology, the science and engineering of manipulating matter at the molecular scale to create materials and devices with novel chemical, physical and biological properties, has the potential to significantly improve the way we diagnose and treat cancer. Nanoscale materials inhabit the same size scale as biological materials, enabling unique interactions with cells and proteins that can be harnessed for efficient delivery of drugs and imaging agents to target sites in the body. Nanoscale features capable of highly sensitive, specific and versatile recognition of biological materials can also be integrated into devices for use in disease detection and characterization applications. To exploit nanotechnology's potential to improve cancer research and care, the National Cancer Institute (NCI) Alliance for Nanotechnology in Cancer program (Alliance) was launched in 2004 to promote the application of nanotechnology tools and approaches to basic and applied cancer research. The first phase of the Alliance, active from 2005 to 2010, was comprised of Centers of Cancer Nanotechnology Excellence (Centers), funded through U54 cooperative agreements; Cancer Nanotechnology Platform Partnerships (Platforms), funded through R01 research grants; multidisciplinary training centers funded jointly with the National Science Foundation (NSF) through NSF's Integrative Graduate Education Research Traineeship (IGERT) program; and the Nanotechnology Characterization Laboratory (NCL), an intramural laboratory located at the Frederick National Laboratory for Cancer Research (FNLCR, formerly NCI-Frederick). After the successful completion of the first phase of the program, as measured in terms of scientific output and translational outcomes, a second phase of Alliance awards was approved by NCI leadership for 2010-2015. The awards were made in response to a series of Requests for Applications, RFAs CA-09-012, -013, -014 and -015 .The second phase of the Alliance consists of a network of grants and cooperative agreements across the United States, shown in Figure 1. The Phase 2 Network contains nine Centers and twelve Platforms, with a greater emphasis on training reflected by the addition of seven Pathway to Independence in Cancer Nanotechnology K99/R00 awards and six Cancer Nanotechnology Training Centers (Training Centers) funded through the R25 mechanism. NCL continues to be an integral part of the Alliance Network and strategy. The Alliance maintains its network structure through program-wide meetings, working groups and collaborative Alliance Challenge projects. More information about the Alliance Network is given in Chapter 5, and descriptions of each award are given in Appendix A.

The Centers of Cancer Nanotechnology Excellence are the core of the Alliance Network and infrastructure and are the largest component of the program, measured by the number of supported scientists and projects and amount of funding. They are intended to advance nanotechnology discoveries into applications with cancer relevance and to aggressively develop nanotechnology for use in clinical oncology. The Centers are awarded through a cooperative agreement mechanism (U54), and Center members are expected to interact and collaborate with program staff and other Alliance members on relevant scientific and translational issues. Each Center is led by at least two Principal Investigators (PIs) – one of them with a clinical background and the other with a science/technology background. Center PIs serve on the Alliance Coordination and Governance Committee (CGC), which meets twice yearly to plan Alliance activities and consult on Alliance research and translation strategy. Each Center consists of 4-5 projects and one or more cores to support project researchers. Although Alliance funding is not used to support clinical trials, each Center is expected to bring at least one project to the clinical trial stage by the end of the five year funding period. Each Center includes in its funding an amount restricted for use in

Alliance Challenge Projects, collaborative research that strengthens links between or expands the Alliance Network.

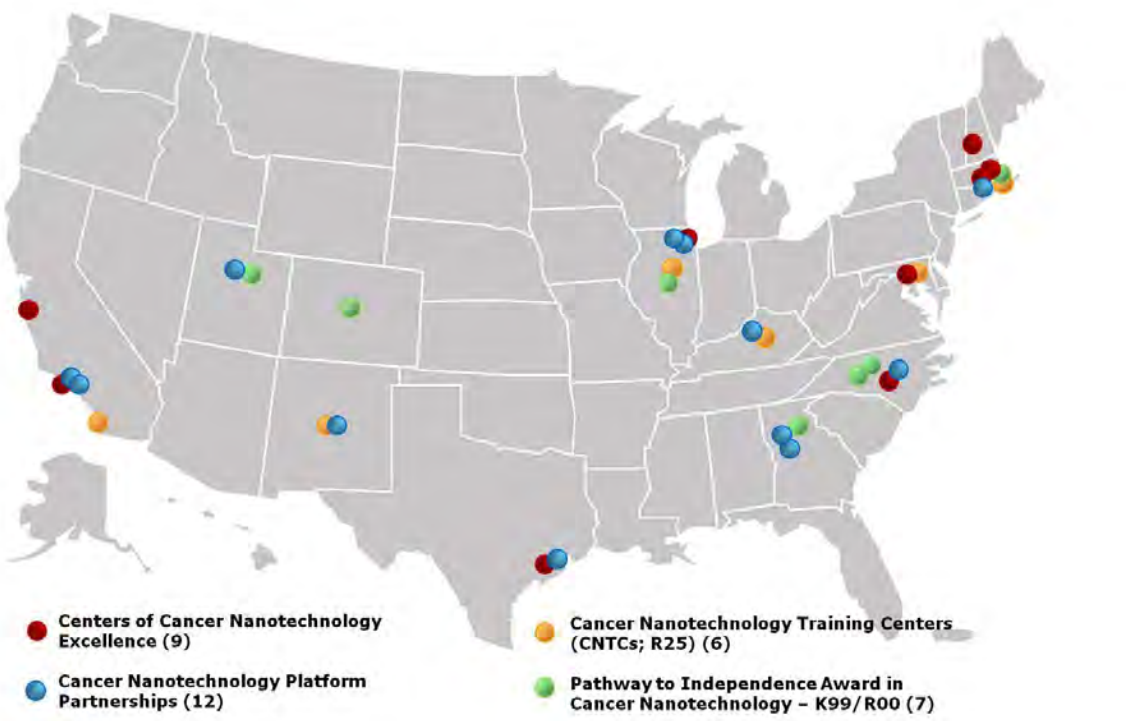


Figure 1. Map of Phase 2 Alliance awards

The Cancer Nanotechnology Platform Partnerships are individual, multi-disciplinary research projects that are also funded through a cooperative agreement mechanism (U01). Platform research focuses on different areas of basic or pre-clinical cancer research, but each Platform is expected to make discoveries that will generate new preventive, diagnostic or therapeutic approaches to cancer care. Platform investigators also participate in Alliance activities, including Challenge Projects paid for with restricted funds in the Platform awards, and each Platform PI serves one 18 month term on the Alliance CGC. Each Platform award is introduced below.

Alliance Training Centers are establishing innovative research education programs supporting the development of a cadre of investigators capable of pursuing cancer nanotechnology research. The training programs are focused on mentored laboratory based training in multi-disciplinary research projects, but each Training Center also develops seminars, workshops and short courses to teach the cross-cutting skills and knowledge necessary for successful research in cancer nanotechnology. The Training Centers also support career development activities for their participants, and participants are encouraged to join in Alliance activities. Many Training Centers host outreach events and symposia aimed at their host institutions or the wider cancer research or nanotechnology communities. They are awarded under the R25 mechanism used by NCI to fund cancer education and career development institutional training programs. Training Center members also participate in Alliance activities, and each Training Center PI serves one 18 month term on the Alliance CGC. An introduction to each Training Center is given below.

Pathway to Independence Awards in Cancer Nanotechnology (K99/R00s) were made to late stage post-doctoral researchers to promote timely transition from the mentored to independent career stages. All seven Alliance K99/R00s awardees successfully transitioned to the R00 phase and are now junior faculty at research institutions across the U.S. They participate in all Alliance activities and are encouraged to collaborate with other Alliance members. The awards and associated R00 institutions are listed below.

Nanotechnology Characterization Laboratory

NCL is the only intramural component of the Alliance. NCL performs and standardizes the pre-clinical characterization of nanomaterials intended for cancer therapeutics and diagnostics developed by researchers from academia, government, and industry. NCL serves as a national resource and knowledge base for cancer researchers and aids the development and translation of nanoscale particles and devices for clinical applications. NCL contributors need not be funded through the Alliance program or NCI. A key activity of NCL is to use an assay cascade developed together with National Institute of Standards and Technology (NIST) and U. S. Food and Drug Administration (FDA) scientists to generate the necessary preclinical toxicology and pharmacology information to aid clinical translation and prospective submission of an Investigational New Drug (IND) application to FDA. This assay cascade characterizes a nanoscale device's physico-chemical attributes, *in vitro* biological properties, and *in vivo* compatibility. NCL will be discussed in more detail in Chapter 5.

Chapter 2: Program Goals

The Program Goals for the second phase of the Alliance span five areas, including scientific research, collaborative environment, translation, standards and resources, and training. These goals were set following consultation with the extramural community and NCI leadership. In 2008, NCI hosted a series of Strategic Workshops on Cancer Nanotechnology, in which leading academic and industrial participants in cancer and biomedical nanotechnology research came together to discuss challenges and opportunities in the field of cancer nanotechnology. The workshops identified a number of broadly defined areas in which significant progress was necessary to enable successful clinical adoption of new nanotechnology discoveries (Nagahara et al., 2010). The structure of the second phase of the Alliance program was designed to meet the needs identified by the workshops and to create an environment for research and translation in which the full potential of nanotechnology for cancer care is realized.

Summary of Program Goals

NCI sought to develop a program which does the following: meets the scientific milestones developed by the research community and the program office as outlined in the Strategic Workshops and Cancer Nanotechnology Plan (caNanoPlan; http://nano.cancer.gov/objects/pdfs/CaNanoPlan.pdf); sustains a cohesive network of researchers from different disciplines and institutions; promotes the translation of research discoveries to clinical use; and supports the training of students and postdoctoral fellows in nanotechnology and cancer biology. We further hoped this program would lead the field in the standardization of methods and practices for physico-chemical, *in vitro* and *in vivo* characterization of nanomaterials.

Scientific Goals

The Alliance is intended to support a diverse portfolio of basic and translational cancer nanotechnology research. The caNanoPlan was developed in 2010 to summarize significant areas in the field of cancer nanotechnology and to propose aggressive three, five and ten year goals for the field to drive progress. This document was developed largely through input from extramural investigators, independent of the funding plan for Phase 2 of the Alliance. In the three years since this roadmap was developed, researchers within the Alliance have made significant contributions towards meeting these goals in the course of pursuing the aims of their Alliance supported awards. This progress is detailed in Chapter 3 – Scientific Accomplishments.

Collaborative Environment

The development of Phases 1 and 2 of this program aimed to build a collaborative network of multidisciplinary researchers, both within institutions and between them. In addition to asking grant applicants to detail the multidisciplinarity of their existing teams, funds from the award were specifically earmarked for Pilot Projects within each Center with the intention of bringing additional researchers into the field. Across the Alliance program, interactions are supported through yearly Principal Investigator meetings, working groups, and Alliance Challenge Projects funded through award funds restricted for this purpose. These Challenge Projects are collaborative efforts between at least two institutions funded for a period of 18 months. Alliance network activities are detailed in Chapter 5.

Translational Research

The translation of research discoveries to clinical applications is an important goal for the program. Developing new, applied technology solutions towards cancer interventions with potential for clinical utility is an invaluable contribution to the general welfare. To this end, Alliance researchers are encouraged to leverage their Alliance award to raise additional resources for clinical testing and translation to the clinic. The Alliance program office also seeks to support Network members by facilitating interactions with representatives from industry and providing forums for exchange of best practices and advice on commercializing technology. One such endeavor is the TONIC (Translation of Nanotechnology in Cancer) consortium that has brought together leading academics, small businesses and large pharmaceutical companies to discuss leveraging of resources and intellectual capital in the precompetitive space. The efforts and successes in translational efforts from the program are highlighted in Chapter 4.

Standards and Resources

The Nanotechnology Characterization Laboratory (NCL), a joint effort between NCI, NIST and FDA, provides infrastructure support to the Alliance in the area of nano-characterization. In addition to performing pre-clinical characterization studies, the NCL also generates and promotes the adoption of standardized methods to characterize nanoparticles, facilitates regulatory review of nanotechnology constructs, and engages the research community in education and knowledge sharing efforts. NCL and the Alliance program office also support the creation, maintenance and adoption of public databases of nanomaterial properties and characterization protocols. The program office also participates in cross-agency initiatives to better leverage existing federal resources to promote commercialization of nanotechnology. Further details of these efforts can be found in Chapter 5.

Training

The Alliance supports six Training Centers, along with training and outreach components in each of the nine research Centers. The supported universities have been innovative in their approach to educating the next generation of researchers in the field of cancer nanotechnology and have also been creative in their outreach efforts to the general public. The training of graduate students and postdoctoral fellows in the Alliance has produced a cohort of accomplished young researchers, many of whom have been recognized for their excellence and moved on to faculty positions. More information about this aspect of the program can be found in Chapter 6.

Value of the Program

In summarizing the **accomplishments of the Alliance** in this write-up we hope to address the following wide-reaching questions with respect to meeting program goals:

- Have discoveries and translational efforts in nanotechnology made significant contributions to improvements in cancer research and clinical practice?
- What role was played by NCI initiatives in accelerating cancer nanotechnology discoveries and clinical translation?

We also hope to address the following more specific topical questions:

In relation to Alliance supported research:

- Does Alliance supported research address important issues in basic, translational and clinical cancer research?
- Are there gaps in the Alliance research portfolio? Are there gaps in cancer nanotechnology research that the Alliance could and should fill?
- How successful has the Alliance been in establishing and supporting an inter-disciplinary model of research? Does this model produce effective collaborations, and do these collaborations provide added value for discovery and translational research?
- What was the role of the Alliance program in the overall progress achieved in the field of cancer nanotechnology?
- Does the Alliance program appropriately balance support for discovery research in cancer nanotechnology and promotion of clinical translation of nanotechnology? Should this balance be modified or reconsidered in future NCI initiatives in cancer nanotechnology?

In relation to clinical translation and commercialization:

- How successful are Alliance researchers at clinical translation of their technologies? What role do the Alliance Network and its activities play in this success?
- How successful are Alliance efforts in fostering partnerships between academia and industry? What is the value of these partnerships? Which Alliance efforts have been most effective?
- Is the Alliance supporting development of standards and public datasets for nanomaterials and nanoscale devices and their widespread adoption? Is the Alliance improving access to information and data on nanomaterial properties and characteristics through public databases?

In relation to training in cancer nanotechnology:

- Do Alliance training programs support the creation of a cohort of multi-disciplinary researchers capable of applying nanotechnology tools to critical problems in cancer research and clinical oncology?
- Are the programs developed at Alliance Training Centers broadly applicable to other sectors of biomedicine?

Chapter 3 Scientific Accomplishments

The caNanoPlan was originally a document produced by NCI program staff in 2004 as an assessment of the current status and expected short and middle term outcomes in major areas of research and translational activity in the cancer nanotechnology field. As the first phase of Alliance awards was nearing the end of its five year cycle, the NCI program staff began to put together a revised edition of the caNanoPlan. Although collated and edited by NCI program staff, the caNanoPlan 2010 was largely written by researchers, educators and experts in cancer nanotechnology research and commercialization. It was meant to provide a vision for the entire field of cancer nanotechnology in the United States, not just the subset of activities supported by the NCI Alliance program, so perfect concordance between Alliance outcomes and caNanoPlan measures should not be expected. In some areas, such as cancer prevention, the awards comprising the second phase of the Alliance have only minimal effort or involvement. For other areas, such as siRNA and miRNA based therapeutics and diagnostics, Alliance progress is necessarily conditional on advances in cancer biology made outside the program. However, given the major role the Alliance plays in cancer nanotechnology (both in dollars spent and involvement of key institutions, companies and researchers) we feel performance measured against caNanoPlan milestones is a reasonable standard for how well the Alliance is fulfilling its mission to rapidly advance new nanotechnology discoveries and speed their transformation into cancer-relevant applications in clinical practice. For each focus area of the caNanoPlan, three, five and ten year milestones from 2010 were given in the caNanoPlan; a table summarizing Alliance performance towards meeting the shorter term caNanoPlan milestones is given as Appendix B of this book.

The following sections provide an overview of how well the Alliance is supporting the research and translational activities identified in the caNanoPlan and by the 2008 Strategic Workshops in Cancer Nanotechnology as being crucial for exploiting the full potential of cancer nanotechnology. The summaries of Alliance research in the following chapter represent a significant portion of the research funded by the Alliance program and the related translational efforts in cancer nanotechnology that have reached publication stage. We believe the overview provided by this book captures the breadth and depth of Alliance research, while highlighting those contributions that we expect to make the largest impact on cancer research and care. We feel Alliance research has made significant progress towards answering the questions posed and overcoming the obstacles identified during the planning stages of the Program. However, a book of this length cannot contain all the projects ongoing in the Alliance awards, or even fully detail those projects that are contained within this chapter. In the interest of space and brevity, some important and valuable contributions have been omitted, particularly in areas in which the Alliance is heavily invested and there are numerous projects from which to choose. A database of all Alliance publications is available from the program office, and questions about any research not presented in this book are welcomed by the program office.

We would also like to note that numerous funding sources contribute to the large and extensive research programs run by many Alliance PIs, and that some of the research described below is supported not just by the Alliance, but also by other NCI or National Institutes of Health (NIH) initiatives, other agencies within the federal government, regional, state and local governments, international governments, or by private sources. Many of the most exciting results described in this book build on earlier work from the first round of Alliance funding or on other sources of support, including NCI programs. We have attempted to properly credit other sources of support when appropriate.

Understanding Nanoparticle Behavior *In Vivo*

The Alliance was founded on enthusiasm about the potential of nanotechnology to transform cancer therapy and diagnostics by enhancing existing approaches and enabling entirely new strategies for cancer care. Nanoscale materials function at the same size as biological materials and exhibit size-dependent interactions with cell and tissue components and structures, including efficient intracellular uptake and transcytosis across biological barriers. At the same time, nanoparticles are large enough to carry significant quantities of drugs or imaging agents, their compositions can be well controlled and their surfaces can be decorated with biologically or chemically active agents. These characteristics mean nanomaterials can be engineered for systemic delivery of therapeutic or diagnostic agents, with advantageous solubility, bioavailability and drug release profiles.

The development of safe and reliable nanoparticle platforms is a critical enabler to emerging nanomedicine-based therapies. A variety of different nanoparticle platforms have been explored as potential delivery vehicles for new cancer therapies and diagnostics: polymers, liposomes, micelles, emulsions, metal, metal oxide, dendrimers, fullerenes, quantum dots, and carbon nanotubes. Not only have the types of nanomaterials in use broadened widely, but researchers have discerned how to functionalize nanoparticles, characterize complex multifunctional conjugates, understand effects on biodistribution and toxicity, and begin to define trends.

In vitro and *in vivo* analysis of nanomedicines has allowed for elucidation of some very important trends in nanoparticle biocompatibility. As detailed in Figure 2 (McNeil, 2009), it has been ascertained that a nanoparticle's physicochemical properties directly influence its biocompatibility. More specifically, size, surface charge and hydrophobicity are key factors influencing biocompatibility and biodistribution. A significant amount of research has gone into determining fairly rigid thresholds for nanoparticle size and its correlation with in vivo circulation characteristics and clearance routes. For example, a nanoparticle less than about 8 nm will be excreted through the kidneys, and nanoparticles greater than about 200 nm will be taken up by the organs of the mononuclear phagocyte system (MPS), e.g. liver, and spleen. Of course, size is not the sole contributing factor in determining the clearance route; particle charge can also play a role, especially as the size nears the periphery of the ranges noted above. It is also fairly well established that nanoformulations with a net positive zeta potential (which is related to surface charge) are cytotoxic. Hydrophobicity also plays a crucial role in biocompatibility and influencing the clearance route. Just as larger (>200 nm) nanoparticles, very hydrophobic molecules will often accumulate in organs of the MPS system. Hydrophobicity is often tuned through PEGylation, or similar modification, of the nanoparticle surface. Careful scrutiny is required to ensure the nanoparticle contains the proper amount of surface coating and that it remains intact over periods of storage and during administration. Better understanding of nanomaterials properties and structure activity relationships will translate into more successful design of nanoconstructs capable of safe and efficacious delivery.

Although some general trends in behavior as a function of these properties have been established through the work of NCL and others, further studies utilizing standardized characterization techniques for the *in vitro* and *in vivo* properties of nanoparticles is needed. The importance of such standardized studies is evidenced by NCI's significant investment in NCL and the focus Alliance investigators place on careful nanomaterials characterization. Every Center has at least one core devoted to physico-chemical characterization or the study of nanoparticle biodistribution or pharmacokinetics, with the Stanford, Texas and University of North Carolina (UNC) Centers particularly active in these areas.

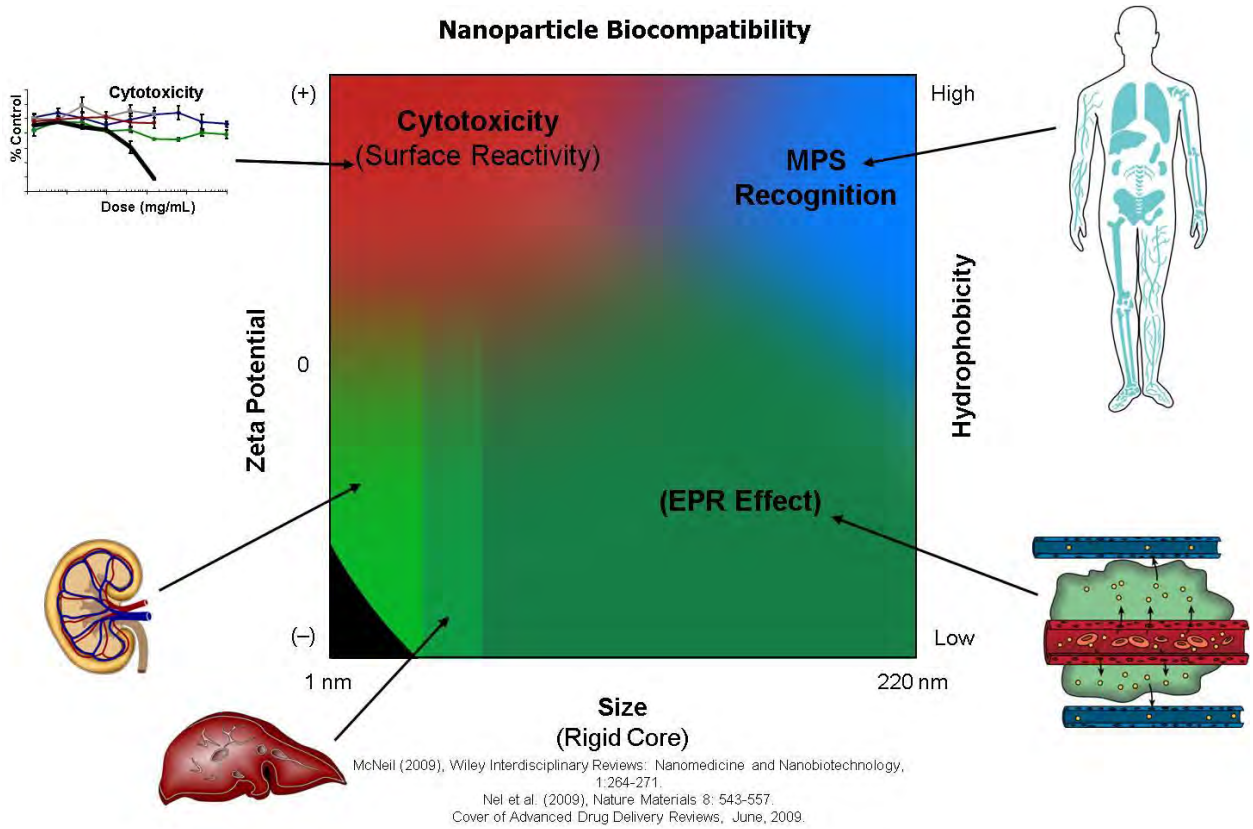


Figure 2. This phase diagram qualitatively shows trends the NCL has observed in relationships between the independent variables of particle size, particle zeta potential (surface charge), and hydrophobicity with the dependent variable of biocompatibility. Biocompatibility includes route of uptake and clearance (shown in green), cytotoxicity (red), and MPS or reticuloendothelial system (RES) recognition (blue). The physical characteristics of a nanoparticle determine its biocompatibility and ultimately its safety and efficacy as an anti-cancer drug or diagnostic. Image courtesy of NCL.

Alliance researchers also recognize that characterization of nanoparticles after synthesis is not enough, and that more studies should be focused on methods to optimize the design of nanoparticle agents for proposed uses. Optimizing nanoparticle design requires the detailed understanding of the effect of nanoparticle size, shape, composition and surface chemistry on biodistribution, cellular internalization and drug release gained by careful characterization studies. Alliance supported research is leading to a growing knowledge base and understanding of nanoparticle-host interactions that should result in more streamlined development in the future. For example, there is a growing body of predictive modeling data to support the dependence of efficient delivery to the microenvironment and cellular internalization on nanoparticle shape and surface characteristics. This further suggests that rational design of nanomaterials will be necessary for more effective transport across biological barriers encountered through different modes of delivery (e.g. inhalation, oral, intravenous or intraperitoneal). Alliance driven research on the underlying mechanisms of *in vivo* nanoparticle behavior is also being used to improve the reproducibility and effectiveness of drug release *in vivo*.

Rational Nanoparticle Design and Predictive Modeling

Researchers in the Texas Center combine experimental studies in multiple projects with computational modeling expertise in the Biomathematics Core to predict nanoparticle-host

interactions and inform nanoparticle design. The effects of the *in vivo* environment on the nanoporous silicon particles pioneered by PI Mauro Ferrari and widely used by Center researchers, and the dependence of vehicle degradation and therefore drug release rates on nanoparticle size, porosity, and drug loading, are being studied (Godin et al., 2012, Yokoi et al., 2013). The results are being used to design strategies for controlled drug release in clinically relevant models (Shen et al., 2013). Paolo Decuzzi, leader of the Biomathematics Core, is studying the determinants of nanoparticle biodistribution and tumor delivery for these particles. These investigations require collaboration between clinicians, materials scientists and mathematicians that would be difficult if not impossible to form and sustain without the cohesion and integrated support provided by the Center.

Important findings about the rational design of nanoparticles have come from these collaborations. Decuzzi and his colleagues recently completed a study examining how alteration in the shape of nanoparticles can improve their targeting to diseased microvasculature (Adriani et al., 2012). Many diseases, including cancers, have vascular abnormalities which would be excellent targets for nanoparticle treatment. However nanoparticle delivery to the vasculature is highly dependent on the shape of the nanoparticle and its hydrodynamic properties. Previous modeling by the Texas team determined that non-spherical geometries worked best for vascular targeting. They advanced this work by generating mesoporous silica disk and rod shaped particles of different sizes, shown in Figure 3, and performing *in vitro* experiments and computer modeling to look into the hydrodynamic forces the particles undergo to explain differences in particle behavior.

The particles, all ~65% porous with pores of about 30 nm, were designed with sizes that allow them to safely pass through the vascular system while maintaining a large enough surface area to adhere to vessels. The particles were tested in conditions simulating flow conditions for healthy and diseased vessels. All preferentially adhered to the substrate in the simulated diseased condition (low wall shear rate), but disks showed greater adherence than rods. Computational modeling revealed that for both rods and disks the hydrodynamic drag and torque were less than that for spherical particles of an equivalent volume, while disks had an increased likelihood of drifting to the edges of the flow and interacting with the vessel surface. Based on these results Decuzzi's group hypothesized that the greater preference for disk adherence to vessel walls than for rods is due to the rods having a reduced ability to move out of the bulk flow and make the necessary contact with the substrate to form adhesion interactions, compared to the disks.

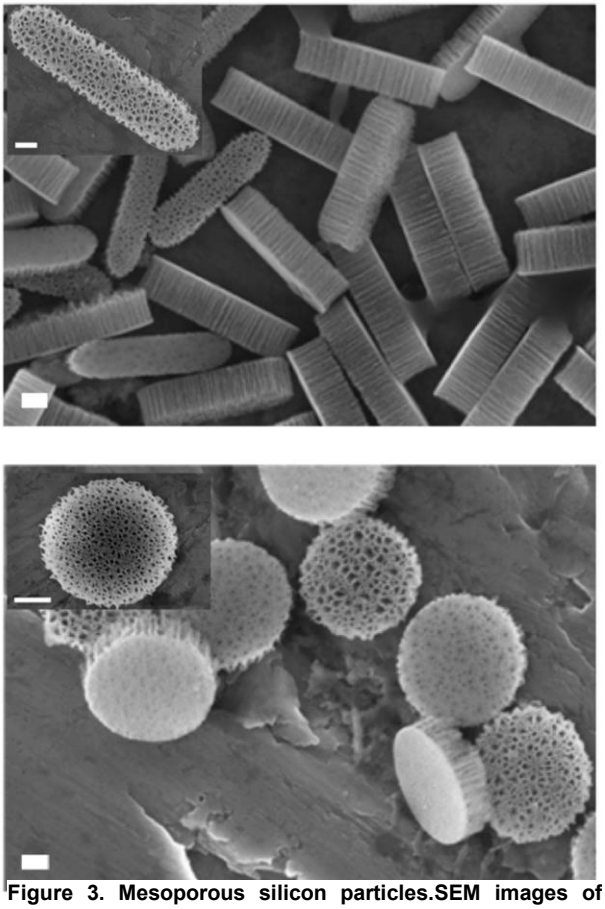


Figure 3. Mesoporous silicon particles.SEM images of 1800×400 nm rods (top) and 1000×400 nm disks (bottom). Scale bars 200 nm. Image courtesy of P. Decuzzi.

The models developed by Decuzzi's group represent a generalized approach to rational nanoparticle design that could enable more efficient development and deployment of new delivery vehicles. Decuzzi chairs the Alliance Working Group on Nanoparticle Biodistribution and advocates for data sharing across the Alliance to feed into computational models of biodistribution. He has also begun

collaborations with William Zamboni of the Carolina Center and Brian Rutt of Stanford to combine nanoparticles and techniques to build a better understanding of the mechanisms underlying nanoparticle transport in the body. Together this group advocates for more rational design of nanoparticles based on predictive modeling and detailed *in vivo* investigation to improve the relevance of preclinical nanomedicine studies to clinical application.

Alliance Highlight – the Carolina Center and Nanoparticle Pharmacology

To drive nanomedicine towards clinical application, members of the Carolina Center are also striving to understand the interactions between nanoparticles and hosts and how these interactions shape nanoparticle biodistribution, pharmacokinetics (PK) and pharmacodynamics (PD). The Center was started eight years ago with a focus on smart nanoparticles, largely driven by the invention of the Particle Replication in Nonwetting Templates (PRINT®) technique for particle synthesis by Center lead Joseph DeSimone. PRINT is a method for producing polymer nanoparticles with tightly controlled shape, size distribution, and composition (Perry et al., 2011, Rolland et al., 2005). As shown in Figure 4, materials are formed in polymer molds in a roll-to-roll process that fabricates a large number of particles with very high precision, and their surfaces can be preferentially treated prior to release from the mold. From the beginning, the Center's strategy has been to integrate innovative animal models and in depth analytical and PK/PD studies in nanoparticle development. The Phase 2 Center maintains this strategy, utilizing sophisticated Animal Imaging and Analytical and PK core facilities to enable reproducible studies on multiple nanoparticle types. DeSimone has leveraged the Center award to garner a $1M commitment from the UNC Lineberger Cancer Center for studies in support of an IND application to FDA for the lead candidate to arise from the Center's research program.

DeSimone is collaborating with Analytical and PK Core Director William Zamboni to investigate the role of size, shape, drug loading and surface properties in determining nanoparticle PK and tumor uptake (Chu et al., 2013). Using a mouse model of ovarian cancer, they specifically analyzed monodisperse, cylindrical poly(lactic-co-glycolic acid) (PLGA) particles of two sizes, 80 x 320 nm and 200 x 200 nm, made using the PRINT process. The two types of particles have similar hydrodynamic radii as measured by dynamic light scattering, providing a good model set to isolate the effects of shape on *in vivo* behavior. The group was particularly interested in the behavior of the 80 x 320 nm particles, which they suspected would be able to pass through smaller pores in the vasculature, despite their greater length, and have preferable PK. Delivery of docetaxel loaded nanoparticles into tumor bearing mice resulted in greatly increased (~20x) plasma exposure compared to free docetaxel, and correspondingly greater tumor exposure. Differences in nanoparticle and docetaxel accumulation in tumors and comparisons of the plasma concentrations over time for the two particle types suggested differing tumor accumulation and drug release profiles. Their results were consistent with more rapid *in vitro* release of drug by the 80 x 320 nm particles, possibly due to a higher surface area to volume ratio, and suggest that modifying the 80 x 320 nm particle formulation to decrease the drug release rate could increase docetaxel exposure in tumors. This is desirable because the 200 x 200 nm particles had greater off-target lung, liver and spleen accumulation.

They also used the PRINT particles as a model system to study the effect of PEG surface density on protein binding, macrophage association, biodistribution and PK (Perry et al., 2012). Nanoparticles intended for *in vivo* use must be surface coated to prevent aggregation and to slow otherwise very rapid uptake and removal from circulation by the mononuclear phagocytic system (MPS). However, the effect these surface coatings have on biodistribution and cellular uptake is largely unknown. This is to some extent due to a lack of well-developed and widely accepted ways to quantify or characterize surface

coating parameters, such as polymer density or conformation. The Carolina group determined from the literature that a long half-life is typically associated with dense “brush” PEG coatings as opposed to sparser PEGylation, which produces a "mushroom" shape to the PEG chains. They then synthesized 80 x 80 x 320 nm PRINT nanoparticles with high or low PEG density surface coatings, with PEG in brush or mushroom conformations, respectively. Consistent with the literature, protein binding decreased with increased PEG density, although they saw protein rejection at lower PEG densities than previously reported. This pattern extended to macrophage uptake experiments and particle circulation observed using intravital microscopy. Their results suggest that predictive screening methods can quickly assess the circulation fate of PEG coated PRINT nanoparticles and provide insight into the PEG density necessary to facilitate long-circulation. Zamboni is extending this approach through Alliance Challenge Project collaboration with Paolo Decuzzi, in which Decuzzi will develop models of the interaction between nanoparticles and the MPS, based on data collected by high throughput screens in Zamboni’s lab.

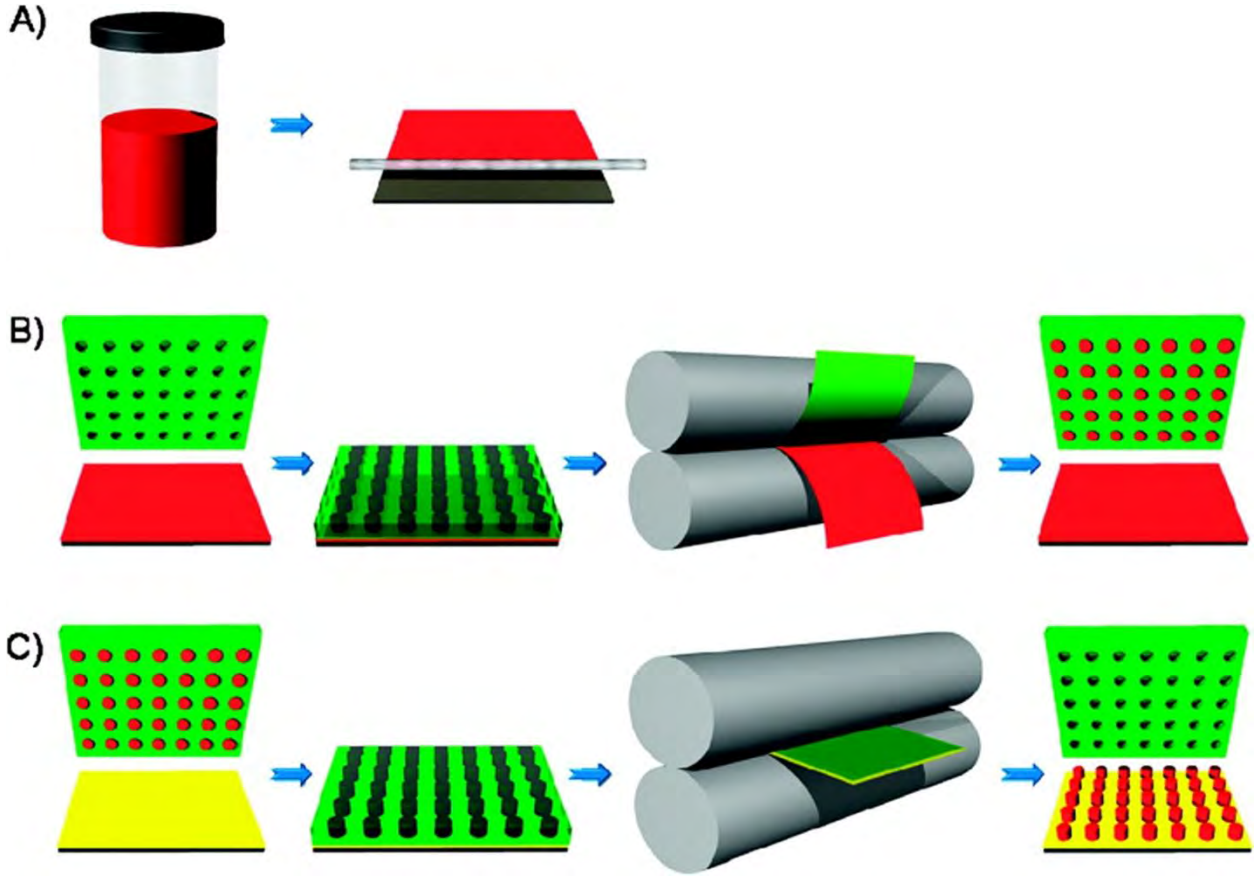


Figure 4. The PRINT® Process. (A) Delivery Sheet Casting: PLGA and docetaxel are dissolved in DMF and DMSO (4:1 solvent ratio) to create a true solution (red). A mayer rod is then used to draw a film from this solution on a PET substrate. The solvent is removed under heat generating a solid state solution film referred to as the delivery sheet, as it will deliver the composition to the mold. (B) Particle fabrication: a perfluoropolyether elastomeric mold (green) in brought into contact with a PLGA (red) film, passed through a heated nip (gray) and split. The cavities of the mold are filled. (C) Particle harvesting: a filled mold is brought into contact with a high energy film or excipent layer (yellow) and passed through the heated nip without splitting. After cooling the mold is removed to reveal an array of particles on the high energy film or excipent layer. Reprinted with permission from (Enlow et al., 2011) . Copyright 2011 American Chemical Society.

Zamboni is an interesting case study as both beneficiary and nexus of Alliance Network collaboration. He has been supported in his efforts to extensively characterize the biodistribution of and perform PK/PD

studies on nanotherapeutic delivery systems since he received a Pilot award from the Carolina Center during the first phase of Alliance funding. Zamboni's studies have focused on liposomes, a relatively mature advanced drug delivery technology. He has developed a complex picture of the liposomal, host and treatment factors that affect PK/PD (Song et al., 2012). He now characterizes several of the other drug delivery platforms in the UNC Center and across the Alliance, including liposomal formulations from the lab of Platform PI Mansoor Amiji, and is active in encouraging best practices in *in vivo* characterization of nanoparticle behavior. He was invited to share his data and expertise on nanotherapeutic biodistribution and PK/PD at the Alliance and TONIC organized workshop on the enhanced permeability and retention (EPR) effect, in which leaky and malformed vasculature combine with dysfunctional lymphatics in a tumor to allow preferential accumulation of large molecules and nanoparticles. He was a co-author on the resulting workshop report (Prabhakar et al., 2013).

The Phase 1 Center was the first major NIH funding for DeSimone, an accomplished inventor and 2008 Lemelson-MIT Prize winner. Building on this initial funding, DeSimone and his startup company Liquidia have moved to the forefront of nanomedicine. DeSimone was the recipient of an NIH Director's Pioneer Award in 2009, and he and Liquidia are using PRINT particle to deliver new classes of therapeutics and vaccines, including siRNA (Dunn et al., 2012, Hasan et al., 2012) and acid catalyzed pro-drugs (Parrott et al., 2012) for cancer therapy. DeSimone's Alliance funding has been used to study delivery of gemcitabine attached to an acid sensitive bifunctional silyl ether group. This complex, which they termed asymmetric bifunctional silyl ether (ABS), was then used to make cylindrical nanoparticles with 20% weight ABS prodrug through the PRINT process. They investigated three versions of their prodrug, differing in the bulk of the alcohol substituent on the silicon atom, i.e., diethyl gemcitabine ABS, diisopropyl gemcitabine ABS and di-tert-butyl gemcitabine ABS. They found that release of gemcitabine was dependent on the bulk of the alkyl group, with very slow release ($t_{1/2}$=6995 hours) observed with *tert*-butyl substitution and lower release at neutral pH compared to acidic conditions. Cell viability studies indicated the toxicity of the prodrugs decreased with increasing bulk of the alkyl group, a result they attributed to the time required for cellular internalization and pro-drug degradation. These combined results are proof of principle that a prodrug formulation can be designed with little toxicity in healthy tissue, but sufficient drug release in the tumor for therapeutic efficacy. Modification of the silyl ether prodrug in their formulation controls the drug release rate and toxicity, enabling tunable treatment regimen design. Regimen design with PRINT particles is also being investigated in an Alliance Challenge Project on metronomic therapy for ovarian cancer with the Texas Center.

Improving Drug Delivery

UNC Platform PI Alexander Kabanov is also seeking to understand and control drug release from nanoparticles. He and his collaborators have developed a simple way to enhance drug release from liposomes in tumors through administration of Pluronics, block copolymers based on ethylene oxide and propylene oxide, following treatment with Doxil®, a liposomal formulation of doxorubicin (Zhao et al., 2013). Doxil is deposited at the periphery of tumor sites through the EPR effect and often has trouble penetrating the tumor. Attempts to improve liposome penetration by increasing tumor matrix permeability using collagen synthesis inhibitors often result in increased toxicity of the drug in normal tissues and have also been found to lead to enhanced risk of tumor progression and metastases. There have also been reports that the encapsulated drug is trapped in the interstitial space around tumor cells and remains inactive until it is released from the liposome. To overcome these difficulties, Kabanov's group administered Doxil, waited up to 48 hours to allow accumulation in the tumor (waiting longer resulted in significant Doxil diffusion from the site), and administered Pluronics. The copolymers also accumulate in tumor tissues, where they encounter the Doxil and incorporate into its liposomal membrane, increasing its

permeability and allowing the doxorubicin to escape. Since drug release occurred in tumor vessels, the increase in tumor drug loading comes with no additional free drug entering circulation. This work indicates that the copolymer may be a useful adjuvant to Doxil-based chemotherapy and points to a new strategy for improved liposomal drug release in general. Kabanov is testing the general applicability of the Pluronic strategy through an Alliance Challenge Project with Zamboni and members of the Texas Center. Investigators at Texas will evaluate if the strategy improves the efficacy of a tumor targeted liposomal siRNA formulation in ovarian and breast cancer models, and Zamboni will characterize the PK and biodistribution of the combination therapy.

Pluronics are also a key component of a polymeric nanoparticle platform designed to penetrate biological barriers known to impede drug delivery, including the brain extracellular space and mucus coated interfaces (Nance et al., 2012, Yang et al., 2011b). The platform was developed by Justin Hanes of the Johns Hopkins University Center. Building on Hanes's earlier work using dense coatings of low molecular weight PEG to enable nanoparticle penetration through thick mucus layers, his group coated 100 nm polystyrene particles with PEG or carboxylic acid and tracked movement through *ex vivo* tissue and in a mouse model. They observed the PEG coated nanoparticles transversing the brain extracellular space, despite earlier estimates of its pore size smaller than 70 nm. They posit that the dense, neutral PEG coating leads to a fluid interaction between nanoparticle and extracellular matrix, enabling transport across the matrix. Their results suggest larger nanoparticles, with concomitantly larger drug loading capacity, than previously thought can be used for treatment of brain disease. Hanes' group also developed a non-covalent coating process to create mucus penetrating particles composed entirely of materials generally regarded as safe by FDA. The use of non-covalent attachment avoids the creation of new chemical entities subject to extensive regulatory review. Using different molecular weight Pluronics to coat PLGA nanoparticles, Hanes observed a critical dependence of particle motion through mucus on copolymer molecular weight. Particles coated with higher molecular weight Pluronic were more effective at mucus penetration, suggesting the large size compensated for the lack of surface attachment in shielding the nanoparticle surface. This is a versatile approach that can be used with cores of a variety of generally regarded as safe materials, suggesting wide applicability for these particles.

Nanotherapeutic Delivery Systems

Improved efficacy and decreased toxicity for chemotherapeutics were the first goals of cancer nanotechnology to be well articulated, starting with the FDA approvals of Doxil almost twenty years ago and of Abraxane® ten years later. While Doxil and Abraxane's size and lack of toxic excipients mainly account for their favorable properties compared to free forms of the drugs doxorubicin and paclitaxel, judicious selection and design of materials is enabling enhanced performance by newer delivery systems. Improvements include increased payload concentration, better stability *in vivo*, more efficient delivery across biological barriers and increased accumulation in tumor tissue. The clinical potential for nanotherapeutic delivery systems that enhance the efficacy and therapeutic index of existing drugs, resurrect failed therapeutics, or deliver new anti-tumor macromolecules such as peptides, siRNA, proteins, and small molecule inhibitors is enormous. Alliance researchers are advancing delivery systems to meet these needs in the clinic (Weiss et al., 2013, Hrkach et al., 2012, Tabernero et al., 2013). These studies will be discussed in Chapter 4. In this section we provide a survey of Alliance supported platforms, given in Table 1, and highlight examples in which material design has enabled delivery of previously untenable agents.

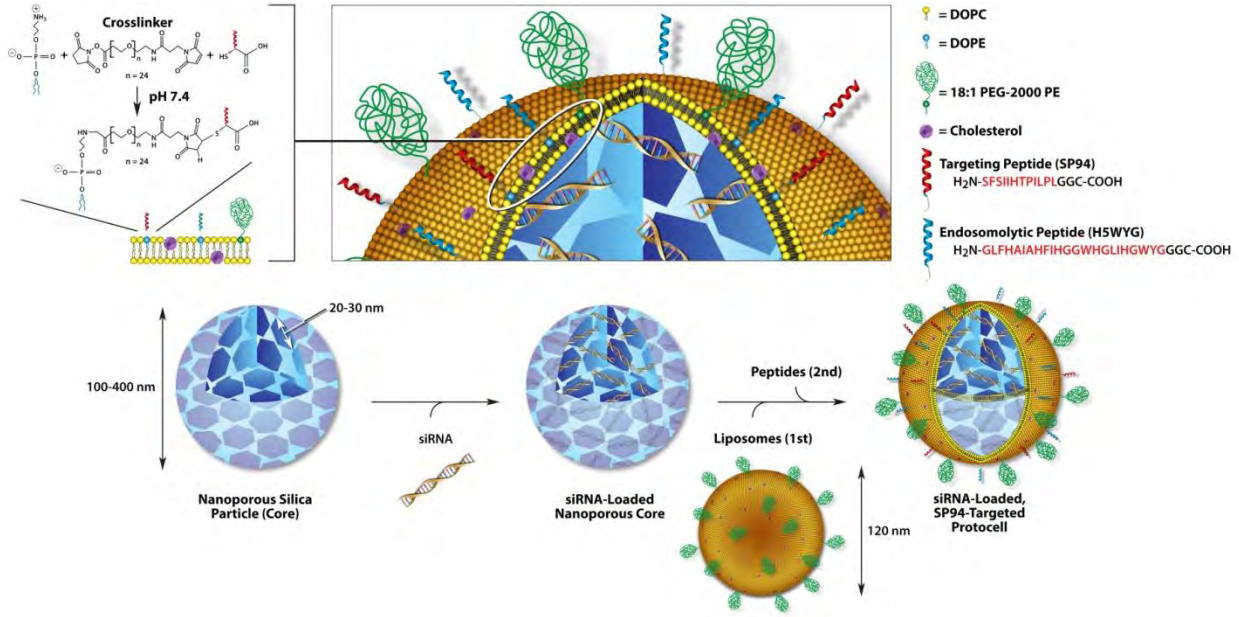


Figure 5. Schematic depicting the process used to synthesize siRNA-loaded mesoporous silica nanoparticle-supported lipid bilayers (protocells). To form protocells loaded with therapeutic RNA and targeted to hepatocellular carcinomas (HCC), mesoporous silica cores modified with an amine-containing silane (AEPTMS) were first soaked in a solution of small interfering RNA (siRNA). Liposomes composed of DOPC, DOPE, cholesterol, and 18:1 PEG–2000 PE (55:5:30:10 mass ratio) were then fused to siRNA-loaded cores. The resulting supported lipid bilayer (SLB) was modified with a targeting peptide (SP94) that binds to HCC and an endosomolytic peptide (H5WYG) that promotes endosomal/lysosomal escape of internalized protocells. Peptides, modified with glycine–glycine (GG) spacers and C-terminal cysteine residues, were conjugated to primary amines present in DOPE moieties *via* a heterobifunctional cross-linker ($SM(PEG)_{24}$) with a 9.5 nm polyethylene glycol (PEG) spacer. The SP94 and H5WYG sequences are given in red. Reprinted with permission from (Ashley et al., 2012). Copyright 2012 American Chemical Society.

The advantages of nanoparticle drug delivery are drawing established cancer researchers into the field. **Cheryl Willman, Director of the University of New Mexico (UNM) Cancer Center, is working with C. Jeffrey Brinker, a materials scientist, to develop multicomponent silica "protocells" for drug delivery to cancer cells (Ashley et al., 2011b, Ashley et al., 2012).** The protocells combine the most favorable properties of nanoporous nanoparticles and liposomes by fusing liposomes to high-surface-area spherical nanoporous silica particles, as shown in Figure 5. The core suppresses large scale bilayer fluctuations in the liposome, and the supported lipid bilayer is PEGylated and functionalized with targeting and fusogenic peptides for endosomal escape. The fluidity of the supported layer allows efficient and highly selective targeting with minimal numbers of attached peptides, as the liposome shell can rearrange during cell surface interactions. This property is unique to a nanoporous core with a fluid shell, as solid cores would fix the liposome and prevent peptide recruitment at the cell surface and peptides cannot move across a gel-like layer. The researchers report that the targeted protocells have a more than a 10,000-fold greater affinity for hepatocellular carcinoma cells than for healthy hepatocytes or other normal cells. This is a more than 100x improvement over comparable liposomes. The nanoporous core is also amenable to loading with small molecule drugs, siRNA, protein, and/or nanoparticles, and has a large capacity (10-100 fold more loading than comparable liposomes) for each owing to its large surface area. This allows a single drug loaded protocell to kill a drug resistant hepatocellular cancer cell, as opposed to the 10^6 comparison liposomes necessary to do the same. Alliance funding is supporting a growing community of cancer nanotechnology researchers at UNM, where nanotechnology is now a focus area for the Cancer Center. UNM also hosts an Alliance Training Center, led by Janet Oliver, and has recently

recruited Renata Pasqualini and Wadih Arap, project leads in the Texas Center, as faculty in the UNM Cancer Center.

Platform PI Fatih Uckun of the Children's Hospital of Los Angeles is using Alliance support to develop a liposomal formulation for a SYK inhibitor that is highly potent and selective but also poorly water soluble and not clinically viable due to potentially life-threatening off-target effects (Cely et al., 2012, Uckun et al., 2013). The pentapeptide mimic 1,4-bis(9-O-dihydroquinidinyl)phthalazine hydroquinidine 1,4 phathalazinediyl diether ("compound 61" or C61) targets the substrate binding groove of SYK kinase rather than the ATP-binding pocket, a unique and potentially more selective approach to kinase inhibition (Uckun et al., 2010). Clinical application of this promising compound has been limited by off-target side effects owing to its quinine-like chemical structure, especially the development of acute, severe intravascular hemolysis and shock with secondary kidney failure and seizures at moderate-to-high dose levels. To bypass these effects, liposomal formulations of C61 were prepared, and entrapment of C61 within the interior space of the liposomal nanoparticles was achieved using a pH gradient procedure. The liposomal formulation was shown to induce apoptosis in SYK+ but not SYK- leukemia/lymphoma cells. *In vivo* PK studies on a mouse model of B-precursor acute lymphoplastic leukemia showed plasma concentrations of C61 more than 100-fold higher than those needed to cause apoptosis in leukemia cells could be achieved at non-toxic doses in the liposomal formulation. By challenging mice with untreated or C61 liposome treated xenograft cells, they also showed that treatment with C61 liposomes abrogated the ability of the xenograft cells to engraft and initiate leukemia in NOD/SCID mice, evidence that the liposomes are destroying leukemic stem cells.

Relatively new to nanomedicine when he joined the Alliance in 2010, with only one prior publication in the field, Uckun has rapidly expanded his use of the technology and published five papers about nanoparticle drug delivery since then. He has been an active member of the Alliance, as an author of the Biotargeting Working Group perspective piece on targeting (Goldberg et al., 2013) and an invited speaker at Alliance sponsored meetings. He has used Alliance Challenge Projects to investigate new applications and formulations for C61:as a radiosensitizer with Andrew Wang of the UNC Center and in polylactide nanoparticles formulations with Jianjun Cheng of the University of Illinois Urbana-Champaign (UIUC).

Platform	Cargo	Unique Features	Development Stage
Leukolike vectors	Doxorubicin	• Leukocyte membrane coated nanoparticles • Synthetic cell nanoparticle	Proof of principle (Parodi et al., 2013)
DNA nanoparticles	siRNA	• Stoichiometric and reproducible • Thermodynamically stable	Proof of principle (Lee et al., 2012)
Bacteriophage MS2 virus like particles	siRNA, small molecule drugs, protein toxins	• Precise control of capsid surface chemistry	Proof of principle (Ashley et al., 2011a)
Silica protocells	siRNA, docetaxel	• High density drug loading • Fluid coating on solid core for efficient targeting	Targeted delivery in cell lines (Ashley et al., 2012, Ashley et al., 2011b)
Gold nanostars	AS1411 DNA aptamer	• Targeted delivery and cargo trafficking to cell nucleus	Established targeted delivery *in vitro* (Dam et al., 2012)
Polymeric NP	Wortmannin	• Resurrection of failed compound	Established *in vitro*; preliminary efficacy work in animals (Karve et al., 2012)
High density lipoprotein		• Nanoparticle is active ingredient (cholesterol)	Proof of principle; preliminary efficacy in animal models (Yang et al., 2013b)
pRNA nanoparticles	siRNA	• Stoichiometric, reproducible • Thermodynamically stable	Full physico-chemical characterization; Biodistribution, PK/PD studies (Shu et al., 2013)
Liposome	Pentapeptide mimic compound 61 (C-61) (anti-SYK)	• Site selective kinase inhibitor • Resurrection of failed compound	Efficacy in animal models; biodistribution, PK/PD studies (Cely et al., 2012)
Polymer coated IONP	Gemcitabine, siRNA	• Theranostic activity • Targets pancreatic cancer stroma	Efficacy in animal models; Biodistribution, PK/PD studies (Cho et al., 2013, Lee et al., 2013)
Poly (2-oxazoline) Micelles	paclitaxel, docetaxel, 17-allylamino-17-demethoxygeldanamycin, etoposide, bortezomib	• Versatile platform • High drug concentration • Potential PEG replacement	Physico-chemical characterization and *in vitro* efficacy (Han et al., 2012)

Platform	Cargo	Unique Features	Development Stage
Silicon multi-stage vectors	siRNA, docetaxel liposomes	• Controlled release • Vehicle acts as *in vivo* reservoir	Efficacy in animal models, Biodistribution, PK/PD for vector platform (Shen et al., 2013)
Thioaptamer targeted liposome		• Aptamer against E-selectin as targeting ligand	Biodistribution and targeting efficiency in animals (Mann et al., 2011)
DOTAP-DOPE-apolipoprotein nanoparticle	Cytochrome C	• Intracellular targeting	Efficacy in animal models (Kim et al., 2012)
Hollow gold nanospheres	Doxorubicin	• Combined photo- and chemotherapy	Efficacy in animal models (You et al., 2012)
Spherical nucleic acid gold nanoparticles	siRNA	• Topical application • Transdermal delivery	Efficacy in animal models (Zheng et al., 2012)
Heparin-folate-paclitaxel conjugate	Paclitaxel	• Effective treatment for MDR cancer	Efficacy in animal models (Wang et al., 2011)
Polymer nanobins	Arsenic trioxide	• Ferto-protective chemotherapy	Efficacy in animal models (Ahn et al., 2013)
Polysilsesquiox-ane nanoparticles	Oxaliplatin	• Metal-organic framework chemistry • Triggered drug release	Efficacy in animal models (Della Rocca et al., 2011)
Mucic acid polymer conjugate	Camptothecin	• Herceptin-polymer complex	Efficacy in animal models (Han and Davis, 2013)
PMLA nanoparticle	Morpholino antisense oligo	• Targeting across blood-brain-barrier • pH controlled drug release	Efficacy in animal models; Biodistribution, PK/PD studies (Huang et al., 2012b)
Polycaprolacton e nanoparticles	Paclitaxel, lonidamine	• Effective combination therapy for MDR cancer	Efficacy in animal models; Biodistribution, PK/PD studies (Milane et al., 2011b)
PRINT® PLGA NPs	Gemcitabine, siRNA	• Highly reproducible synthesis • Controlled release, pro-drug strategy	Efficacy in animal models and PK/PD studies on platform (Parrott et al., 2012)
DOPC conjugates	siRNA (EphA2, survivin), miRNA	• Entering clinical trials	(Vivas-Mejia et al., 2011), NCT01591356

Table 1. Overview of therapeutic delivery vehicles being developed by Alliance researchers, including information on tested cargo, unique features of the platforms, and maturity of platform

siRNA Therapeutics

Delivery of small interfering RNA (siRNA) for therapeutic applications is an area in which nanoparticle delivery can make a unique and valuable contribution. Although the RNA interference pathway is widely exploited in experimental cell biology and functional genomics studies, successfully using it *in vivo* for therapeutic applications has proved difficult. The most promising approach is the use of siRNA, but free siRNA can be immunogenic, prone to nuclease degradation in serum, and has a short circulation half-life even when not degraded, factors which greatly limit systemic delivery of free siRNA. In addition, accumulation in a tumor is not enough to produce a therapeutic effect, as the siRNA must also enter cells and escape the endosomal compartment in sufficient amounts to induce a therapeutically meaningful level of gene silencing and protein knockdown. Delivery of siRNA into cells initially proved so difficult that many major pharmaceutical companies, including Roche, Novartis, Pfizer, Abbott Labs and Merck, deprioritized the field in the midst of the financial crisis in 2010 and 2011 (Ledford, 2010) (Pollack, 2011). However, nanoparticle formulations of siRNA for cancer indications have since shown early success in Phase I clinical trials. Calando Pharmaceutical's CALAA-01, a polydextrin nanoparticle formulation developed by Alliance researcher Mark Davis of Caltech, and Alnylam Pharmaceutical's ALN-VSP have both reported positive safety and even preliminary efficacy data from their Phase I clinical trials (Davis et al., 2010, Tabernero et al., 2013).

Target	Indication	Developer
N-ras	Melanoma	Davis, Caltech
EGFR	Glioblastoma	Sharp, MIT
PARP (BRCAdef.), Myc, PKM2, Claudin-3, ERB-3	Ovarian	Sharp and Bhatia, MIT
Pgp, survivin, BCL2		Torchilin, Northeastern
BCL2	Breast	O'Halloran and Cryns, Northwestern
K-ras, PI3K, MEK, Myc	Lung	Huang, UNC
Eph A2, FAK	Ovarian	Lopez-Berestein, MD Anderson
EZZH2, TEM7	Ovarian	Sood, MD Anderson
Survivin	Breast	Mirkin, Thaxton, Northwestern
MDR-1, mrp-1, BCL2, Survivin	Multiple	Amiji, Northeastern
Survivin	Pancreatic	Yang, Emory

Table 2. Targets for silencing by RNA interference therapy under pre-clinical development with Alliance support

Nanoparticle formulations can improve siRNA pharmacokinetics and protect siRNA from serum nucleases while in transit to the tumor, increasing tumor exposure to the siRNA. Surface decoration with targeting ligands can facilitate transport of nanoparticles and siRNA cargo into the cell interior, while additional membrane penetration or endosomal disruption capabilities can be engineered into nanoparticles to enable siRNA escape into the cytoplasm. The Alliance supports a significant amount of research on siRNA delivery, ranging from proof of concept for innovative platforms based on structural RNA or DNA components to pre-clinical studies for mature technologies. Platforms being explored for siRNA delivery include Joseph DeSimone's PRINT PLGA particles, Lily Yang's polymer coated iron oxide nanoparticles,

Gabriel Lopez-Berestein's liposomes, and the silicon multistage vectors developed by Mauro Ferrari. Table 2 lists siRNA targets and indications being formulated into nanoparticle vehicles by Alliance investigators. This section introduces delivery vehicles that utilize nucleic acids as structural as well as functional components of nanoparticles for RNA interference therapy. The ability to covalently bind the siRNA to the nanoparticle while retaining function represents a major advance, as Alliance supported research by Mark Davis has shown non-covalently bound siRNA-polymer formulations are subject to disassembly in the kidney glomerular basement membrane, leading to rapid clearance from circulation (Zuckerman et al., 2012).

Peixuan Guo, PI of the Alliance Platform award at the University of Kentucky, is pioneering the synthesis of RNA nanoparticles for drug and gene delivery (Shu et al., 2011, Haque et al., 2012). RNA's chemical and tertiary structures can be controlled by exploiting both Watson-Crick and noncanonical base pairings to generate rigid, thermodynamically stable structural nanoparticle-like motifs (Guo, 2010). This stability prevents dissociation at low plasma concentration, and using chemically modified bases prevents RNase degradation, enabling systemic delivery of the RNA nanoparticles. The RNA nanoparticles are 10-50 nm in diameter and exhibit the extended circulation time characteristic of well-designed nanoparticles. But unlike typical nanoparticles, which show large batch-to-batch variation between syntheses due to the kinetically driven reaction conditions, RNA nanoparticles can be reproducibly synthesized with a known stoichiometry, controlled structures, and no *in vivo* aggregation. Guo's group has been developing stable nanoparticles made entirely of RNA and based on the structure of the bacteriophage phi29 packaging RNA (pRNA). Each pRNA contains a helical domain, a central domain containing right and left-hand loops and a three-way junction (3WJ) motif, shown in the top left of Figure 6. Interactions between these domains promote formation of pRNA nanoparticles with well-defined structures and stoichiometry, including simple nanoparticles formed by extending interlocking loops, higher order nanoparticles based on palindrome sequences and branched nanoparticles based on the 3WJ motif (Shu et al., 2013).

siRNA, targeting ligands, imaging agents, and small molecules can be integrated into the ends of the RNA oligonucleotides without inhibiting the folding of the RNA "nanoparticles" or compromising function of these components. Three RNA oligonucleotide sequences self-assemble at a stable three-way junction to form a trivalent RNA nanoparticle (3WJ-3pRNA) with three valencies for functional motifs (Shu et al., 2011), while four sequences form a tetravalent x-shaped motif (pRNA-X-4pRNA) (Haque et al., 2012), as shown in Figure 6. Western blot and qRT-PCR analysis showed knockdown of survivin gene products in cells exposed to 3WJ-pRNA incorporating an anti-survivin siRNA motif. Importantly, the group has established additive gene silencing from increasing numbers of functional siRNAs attached to the nanoparticles (81% with four siRNAs vs. 25% with one siRNA).

In vivo studies of the pRNA nanoparticles showed advantageous PK and biodistribution profiles compared to control 2'F modified siRNA alone. 3WJ-pRNA nanoparticles had a half-life of 6-12 hours, compared to less than one hour for the control. Whole body imaging of fluorescently labeled, folate targeted RNA nanoparticles showed localization at the folate expressing tumor and rapid clearance from the rest of the body, including lung, spleen and liver, as shown in Figure 7. This is atypical of nanoparticles following systemic injection, which usually accumulate in these organs, and is indicative of the high potential of pRNA nanoparticles for systemic drug delivery. Guo and his group have reported 14 different RNA nanoparticle structures and have integrated reporter molecules and small drugs along with siRNA into different RNA nanoparticles. The Alliance is supporting the preclinical development of these unique vehicles through Guo's Platform award and interactions with NCL. Guo will also test the efficacy of

siRNA treatment of ovarian cancer in an Alliance Challenge Project with collaborators from the Texas Center.

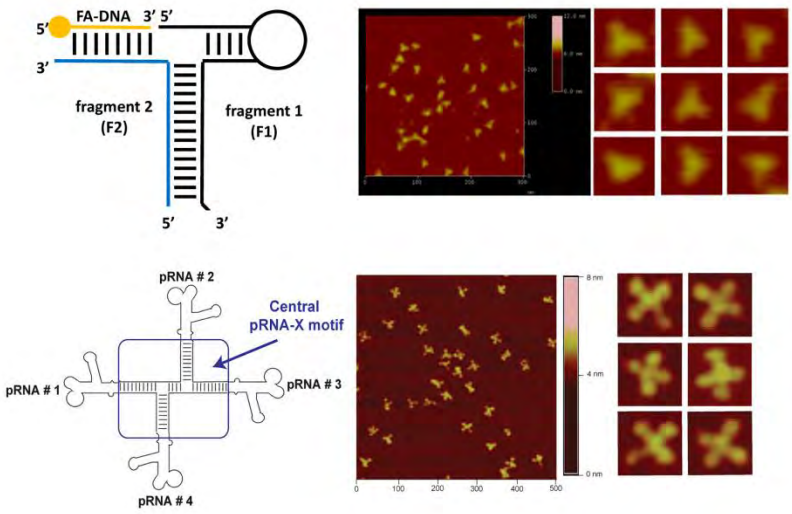


Figure 6. Trivalent and tetravalent RNA motifs. The upper panel shows a schematic of the trivalent RNA nanoparticle and the AFM image of 3WJ-pRNA-siSur-rZ-FA nanoparticles. The lower panel shows a schematic of the tetravalent RNA nanoparticle and the corresponding AFM image of the pRNA-X-4pRNA nanoparticles. Courtesy of Dr. Peixuan Guo. Adapted by permission from Macmillan Publishers Ltd on behalf of Cancer Research UK: Nature Nanotechnology (Shu et al., 2011), copyright 2011. Reprinted from Nano Today (Haque et al., 2012), copyright 2012 with permission from Elsevier.

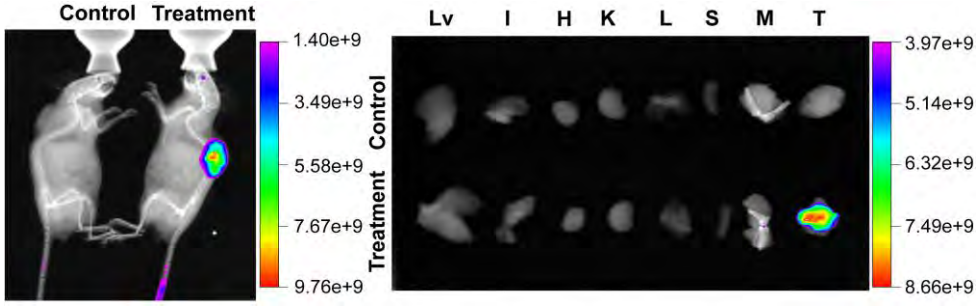


Figure 7. *In vivo* binding and entry of pRNA nanoparticles into cancer xenograft after systemic injection. Left panel: whole-body imaging reveals that pRNA nanoparticles containing folate ligands target to folate receptor positive tumor xenografts upon tail vein injection in nude mice. Treatment group was injected with pRNA nanoparticles containing folate and near infrared fluorescence marker Alexa Fluor 647. Control group was injected with PBS. Images shown are X-ray plus fluorescence imaging. Right panel: organ imaging showing pRNA nanoparticles target to folate receptor positive tumor after systemic injection (Lv, liver; K, kidney; H, heart; L, lung; S, spleen; I, intestine; M, muscle; and T, tumor). Scale bar indicates fluorescent intensity. Image courtesy of P. Guo.

Daniel Anderson of the MIT-Harvard Center collaborated with researchers from Alnylam Pharmaceuticals to develop DNA nanoparticles for efficient intracellular delivery of siRNA (Lee et al., 2012). Anderson and colleagues prepared DNA tetrahedrons through self-assembly of six single stranded DNA molecules with complementary overhangs at the 3' ends. Each edge of the tetrahedron is 30 base pairs long, leading to a theoretical edge length of 10 nm and a height of 8 nm. In the center of each edge is a nick where the 5' and 3' ends of successive DNA strands meet; siRNA chemically modified for greater resistance to serum nuclease can be attached at these locations during the initial synthesis reaction. As with Guo's RNA nanoparticles, the programmable nature of the synthesis leads to

a tightly controlled and reproducible size and structure, targeting ligand density and orientation, and siRNA loading (i.e., six siRNA per nanoparticle). Their control over ligand and siRNA density and orientation allowed them to test the effect of these parameters on gene silencing. They found that three folate ligands in close proximity were required for nanoparticles to effectively silence green fluorescent protein (GFP), even though nanoparticles with more widely space ligands were taken up by cells. The orientation of the ligands to the folate receptors may influence the intracellular trafficking or alternatively they may influence access of the endogenous processing enzymes to cleave the siRNAs from the nanoparticles. Biodistribution studies of the DNA nanoparticles pointed to high tumor accumulation, relatively rapid clearance from blood and low accumulation in lung, liver and spleen, while preliminary gene silencing experiments with anti-luciferase siRNA showed ~60% decrease in luminescence. These results point to the potential value of the DNA tetrahedrons as both a research tool and a therapeutic vehicle.

Chad Mirkin, PI of the Northwestern Center, is working with Amy Paller, a physician scientist, to develop his spherical nucleic acid gold nanoparticle conjugates (SNA-NCs) for use in siRNA therapy for skin diseases (Zheng et al., 2012). SNA-NCs are gold nanoparticles coated with highly oriented and covalently attached oligonucleotides that have been developed by Mirkin and his collaborators for a variety of biomedical applications, including *in vitro* diagnostic devices and cellular imaging. SNA-NCs can incorporate single or double stranded nucleic acids, with nucleic acid orientation with respect to the gold core determined by the nanoparticle shape. The density of the nucleic acid coating results in resistance to nuclease degradation, and the SNA-NCs are taken up by cell lines without additional transfection agents. Mirkin and Paller sought to exploit the transfection and permeability properties of SNA-NCs for topical delivery of siRNA, attaining efficient gene silencing while bypassing the side effects of systemic delivery. After establishing the safety of this approach by analysis of normal epidermal cells with nonsense siRNA, the group functionalized SNA-NCs with siRNA against EGFR and observed a 90% decrease in protein expression with as little as 0.01 nM of SNA-NCs, corresponding to 0.3 nM free siRNA, or 100 fold less siRNA than the positive control. Knockdown was persistent as well as efficient, with 50% knockdown after 96 hours. A 40% reduction in skin thickness compared to nonsense siRNA SNA-NC control showed translation of the silencing to phenotypic effect. Their results suggest this platform is a viable delivery system for siRNA treatment of skin lesions and tumors in humans. Mirkin is pursuing clinical translation through the startup company AuraSense Therapeutics.

Nanotechnology to Overcome Tumor Drug Resistance

Drug resistance in tumors is a major concern, with relapse of disease following emergence of resistant cells. The microenvironment of a tumor contributes to the development of multidrug resistant (MDR) cancer and determines a patient's response to treatment. Tumor microenvironment contributes to the development of MDR through several factors including formation of abnormal tumor vasculature, hypoxia, decreased pH, increased interstitial fluid pressure, and alterations in the expression of tumor suppressors and oncogenes. MDR cells often have increased DNA repair mechanisms, up-regulation of membrane transporter proteins by which drug molecules are removed from the cytoplasm, and a decreased apoptotic response (increased threshold for cell death). The diverse cellular mechanisms underlying MDR and tumor heterogeneity make combination therapy a requirement for effective treatment. Nanocarriers are well suited for simultaneous delivery of multiple agents with favorable PK. Endocytosis mediated uptake of nanocarriers can also be successful in diverting drug efflux through ABC-transporters by preferentially localizing agents in the peri-nuclear region of a cell, away from membrane localized efflux pumps (Wang et al., 2011).

Alliance Highlight – the Northeastern Platform and MDR

Over two rounds of Alliance support, Platform PI Mansoor Amiji at Northeastern University and his team have been exploring the relationship between hypoxia, MDR and glycolysis in cancer cells in order to develop a new animal model of MDR cancer (Milane et al., 2011a). The erratic supply of oxygen to cells within a tumor leads the cells to adapt an aerobic glycolysis pathway for generation of ATP, a transition known as the Warburg effect. To monitor and evaluate this transition, Amiji and his group first measured the expression of protein markers of hypoxia, glycolysis and MDR in breast and ovarian cancer cells. The researchers then chose a human breast cancer line (MDA-MB-231) and exposed the cells to either hypoxic conditions or to normal levels of oxygen (control condition) for five days before injecting the cells into the mammary fat pads of mice to develop orthotopic tumors. Once the tumors had grown to a predetermined volume they were removed and analyzed for markers of hypoxia, glycolysis and MDR. By analyzing these markers, the researchers were able to show that seeded cells preconditioned under a hypoxic environment had more MDR characteristics than those from the normoxic preconditioning, indicating that the method established a new orthotopic animal model of MDR breast cancer. There are currently few animal models that recapitulate resistance or its mechanisms, making this a valuable tool.

Amiji's group is using their mouse model to test the safety and efficacy of an EGFR-targeted polymer blend nanocarrier that treats MDR cancer with a combination of paclitaxel and lonidamine (Milane et al., 2011b). They prepared polycaprolactone nanoparticles incorporating a PLGA-PEG-peptide targeting unit and encapsulating paclitaxel and lonidamine. The nanoparticles were 120-160 nm and capable of high (65-70%) drug loading efficiency. Lonadamine is a hexokinase 2 inhibitor and has been shown to induce apoptosis in various MDR cancer cell lines. The nanoparticle formulation of the combination chemotherapy decreased tumor volume significantly more than co-administration of the free drugs and nanoparticle formulations of either drug alone. The decrease in tumor volume was sustained for 28 days post administration. Importantly, the treatment decreased the tumor density and changed the MDR phenotype of the orthotopic tumors.

Amiji's group also undertook PK and biodistribution studies of the combination therapy nanoparticle (Milane et al., 2011c). They looked at the plasma, tumor and vital organ distribution profiles and compared them to treatment with non-targeted nanoparticles as well as with drug solution alone. They also developed an isocratic high-pressure liquid chromatography method in order to quantify the amounts of both chemotherapeutics in the different plasma and tissue samples. Amiji's group found that their targeted treatment had a superior PK profile compared with the controls, with maximal tumor accumulation occurring 3 hours after administration. These results indicate that this targeted platform has potential to become a viable option for treating MDR cancer.

Amiji's work on MDR cancer demonstrates the broad scope of Alliance research, extending from basic studies of markers of MDR, to the creation of animal models that recapitulate these characteristics, to development and testing of nanoparticle therapeutics using these models. Amiji was one of the first extramural investigators to work with NCL and has been a vocal advocate of Alliance collaboration since then. He is currently working with William Zamboni of the UNC Center to understand drug release in his delivery system.

Targeted Drug Delivery

Site specific delivery or accumulation of nanoparticle therapeutics occurs through both passive and active targeting. Passive targeting strategies include optimizing nanoparticle size and shape to promote preferential deposition in tumors or diseased vasculature and exploitation of the EPR effect to enhance delivery to tumors. Passive targeting approaches generally increase accumulation at target sites but do not promote uptake of nanoparticles by cells, making them insufficient for delivery of agents that are activated within the cell nucleus or cytosol. EPR is not reliably strong in all tumors or patients, and animal models that accurately reflect the relevant vascular and lymphatic conditions in human tumors are rare, making EPR an unreliable method of tumor targeting. For these reasons, active targeting is considered an essential feature of next generation nanoparticle therapeutics. Active targeting of nanoparticles to tumor cells, microenvironment or vasculature, as well as directed delivery to intracellular compartments, can be attained through modification with small molecules, antibodies, affibodies, peptides or aptamers. This is an area of broadly shared interest across the Alliance (Goldberg et al., 2013), with activities ranging from development of new ligands to clinical translation of targeted nanoparticles for cancer therapy. Alliance researchers are also studying ligand-receptor interactions to elucidate the mechanisms of nanoparticle internalization and improve the efficiency of targeted delivery. This section reviews some of the new ligands and targeting approaches being developed within the Alliance.

Active targeting of nanoparticle vehicles is particularly important for delivery of drugs which must be internalized into cells to exert therapeutic effect, including siRNA, peptides and proteins. **Leaf Huang of the Carolina Center has developed a lipid-apolipoprotein nanoparticle to deliver one such protein, Cytochrome C (Kim et al., 2012).** Huang's group mixed a formulation of lipids with cytochrome C conjugated to a membrane permeable sequence peptide that enabled association of the non-lipophilic protein into the nanoparticle. The 20-30 nm nanoparticles were then modified with DSPE-PEG-Anisamide to enable site specific delivery to lung cancer tumors in a mouse xenograft study. Biodistribution studies of nanoparticles loaded with GFP showed preferential accumulation in tumors and low uptake by the liver. Cytochrome C nanoparticle retarded tumor growth, and immunohistochemical analysis showed activated Caspase-3 expression, consistent with apoptosis in the cells, evidence that the nanoparticles mediated delivery of the protein to the cytoplasm of tumor cells. Interestingly, delivery of nanoparticles mixed with unconjugated cytochrome C also showed tumor accumulation of the protein, although less than for conjugated protein, possibly due to charge interactions between the protein and nanoparticle. However, the unconjugated protein did not have an effect on tumor growth, suggesting that co-delivery is not sufficient to promote cellular uptake of the protein and that the peptide mediated incorporation into the lipid nanoparticles is necessary for therapeutic effectiveness.

Teri Odom, Director of the Nanoconstructs Core at the Northwestern Center, has developed gold nanostars coated with a nucleolin-specific DNA aptamer (AS1411) in which AS1411 acts as both targeting ligand and drug (Dam et al., 2012). Nucleolin is overexpressed in the cytoplasm and cell membrane of rapidly dividing cells, enabling active transport of nucleolin-targeted nanostars to the nucleus following binding of the nanostar to nucleolin receptors on the cell surface. Aptamer binding to nucleolin can also block its function and lead to cell death. TEM visualization of cells treated with AS1411-nanostars showed local deformations to the nuclear envelope in the proximity of the AS1411-nanostars, an effect not seen near control nanostars. Studies with free AS1411 at high concentration also showed nuclear deformation, although at higher concentrations of AS1411 than in the AS1411-nanostar experiments. This suggests the nanostars enhance AS1411 therapeutic activity by locally concentrating AS1411 near the nucleus. The group triggered release of AS1411 from nanostars near the nucleus with femtosecond laser pulses at the surface plasmon resonance frequency of the nanostars, increasing local aptamer concentration further. This led to increased deformations in the nucleus, which correlated with increased apoptosis and decreased viability. Studying the correlation between nanoparticle drug

interactions with the nucleus and increased therapeutic efficacy could provide insight into proper design of nuclear targeted therapy. Odom's work is also supported by the NIH Director's Pioneer Award program.

David Gorenstein, PI of the Texas Center, is developing next generation aptamers called X-aptamers that have enhanced nuclease resistance and expanded chemical functionality (He et al., 2012). Gorenstein's group began with a library of fully monothiophosphate backbone-substituted sequences (thioaptamers), to produce nuclease resistant aptamers against the target protein CD44-HABD. Small molecule ligands with high binding affinity against CD44-HABD were also screened, and ADDA (N-acetyl-2-3-dehydro-2-deoxyneuraminic acid) was chosen for chemical conjugation to the library. The binding affinities of ADDA conjugated and unconjugated X-aptamers were assayed, along with the binding constants of the binding motifs and stem-loop regions, which were extrapolated from secondary structure predictions for the X-aptamers. Gorenstein's group found that the addition of ADDA enhanced X-aptamer binding, with the strength of the effect dependent on the location of ADDA binding. They are currently investigating additional small molecules for conjugation, and the methodology can be applied to other target proteins with appropriate small molecules to create an inventory of X-aptamers with high selectivity.

Gorenstein is also continuing a collaboration with fellow Texas Center PI Mauro Ferrari on the development of liposomes targeted to the tumor vasculature using a thioaptamer targeted against E-selectin, which is selectively expressed in inflamed tumor vessels (Mann et al., 2011). By targeting the tumor vasculature, the group hopes to bypass difficulties in transporting liposomes from the circulation to interstitial tumor space for effective treatment. Surface modification with aptamers against E-selectin slightly increased the size of the liposomes from 110 nm to 120 nm and changed the surface charge from positive to negative. Fluorescent labeling studies of E-selectin targeted liposomes injected into mice bearing breast tumor xenografts showed preferential binding to E-selectin expressing endothelial cells. Additionally, accumulation of these liposomes in the tumor parenchyma was markedly increased after 48 hours, suggesting a possible benefit for tumor extravasation. PK studies of targeted and untargeted liposomes showed an increased area under the plasma drug concentration vs. time curve for the targeted liposomes, suggesting thioaptamer conjugation does not lead to increased immune activation and drug clearance or reduction of bioavailability associated with use of antibody targeting agents.

Molecular Imaging and Theranostics

The Alliance supports a significant body of work on nanotechnology enhanced imaging for cancer detection and monitoring. Much of this work is dedicated to synthesizing and testing new contrast agents, some for use with mature imaging technologies like MRI and others being developed in conjunction with instrumentation and protocols for other clinical imaging modalities like photoacoustic, near infrared fluorescence or Raman spectroscopy imaging. Targeted delivery of nanomaterials can be used to deliver contrast agents instead of, or in addition to, therapeutic agents. These imaging agents can preferentially accumulate at a tumor due to site specific recognition, or may even have their imaging properties activated by the tumor environment. Such molecular and functional imaging approaches are important areas of research across the Alliance, with multiple modalities being pursued at different award sites. These approaches are being used to enable diagnostics, therapeutic monitoring, theranostics and enhanced interventions through image guided surgery or radiotherapy.

New Imaging Agents

R00 PI Andrew Smith is developing bright and compact alloyed quantum dots with broadly tunable near-infrared absorption and fluorescence spectra through mercury cation exchange (Smith and Nie, 2011). This is a continuation of work Smith began as a graduate student under Shuming Nie at the Emory University Center during the first round of Alliance funding. Quantum dots (QDs) have many advantages over fluorescent dyes, including high quantum yield and an absence of photobleaching, allowing extended observation. Additional advantages of near infrared nanocrystal QDs over visible QDs including improved light tissue penetration at longer wavelength, lower background interference and reduced photochemical damage. Until recently, however, these QDs were limited in their medical applications due to their large size, broad emission spectra, low quantum yields, and poor photostability. Smith and Nie have been working on improving the fitness of QDs for medical applications and have developed QDs that are bright and compact with equalized particle size and tunable near infrared fluorescence emissions by alloying cadmium with mercury to modify the nanocrystal band gap structure and fluorescence emission. They are able to independently control the particle size and the degree of cation exchange, allowing broad tuning of the photoluminescence peak across a wavelength range from 500-1100 nm. Once capped with a multilayer shell of CdTe and $Cd_xZn_{1-x}S$, the QDs are better suited to biomedical applications, with quantum yields as high as 80% at room temperature. These mercury QDs are 2-3 fold smaller than previous near infrared QDs and are expected to show improved binding kinetics for both live cell imaging and *in vitro* diagnostic applications.

Over two rounds of Alliance support, Angela Belcher of the MIT-Harvard Center has been developing M13 phage templated nanomaterials as MRI and near infrared fluorescence imaging agents. Belcher uses the filamentous bacteriophage M13 as a scaffold for the attachment of nanoparticles, peptides and conjugated antibodies, as shown on the right side of Figure 8. In one example, a nanoparticle termed M13-SBP-MNP, monodisperse iron oxide nanoparticles are assembled along the phage by attachment to a material specific peptide motif engineered onto the phage coat, and the phage's distal end is engineered to display a peptide targeting SPARC (Secreted Protein, Acidic and Rich in Cysteines), a glycoprotein overexpressed in many cancers and associated with poor prognosis (Ghosh et al., 2012). The spatial separation between imaging and targeting units precludes functional interference between the two. The group confirmed enhanced T2 MRI relaxivity and SPARC targeting *in vitro* for the M13-SBP-MNP system. *In vivo* imaging studies of M13-SBP-MNP injected in high and low SPARC expressing prostate tumor mouse models showed T2 image enhancement only for tumors overexpressing SPARC.

The phage system is able to deliver larger numbers of nanoparticles to cells than observed when the magnetic nanoparticles were individually functionalized with equivalent amounts of targeting peptide, as shown in Figure 8. Delivery of individual nanoparticles is limited by the number and accessibility of cell surface receptors, while the M13-SBP-MNP system can deliver multiple nanoparticles per surface receptor. Due to the ease of genetically engineering the M13 coat to display peptides, the system is easily modified to attach different nanoparticles and display different targeting ligands. In another variation of the M13 system, single-walled carbon nanotubes (SWNTs) are attached to the M13 phage for use as near infrared fluorescence imaging agents (Yi et al., 2012). The distal end of the phage was conjugated to a SPARC-targeting peptide or biotinylated for conjugation to streptavidin attached antibodies, including an antibody against prostate specific membrane antigen (PSMA), commonly expressed on tumor vasculature. Both the SPARC and anti-PSMA M13 phage targeted tumors were imaged in a mouse model of prostate cancer, with the anti-PSMA system providing better contrast enhancement. Whether

this was due to different surface receptor expression or different cellular uptake for peptide or antibody mediated uptake is unclear and will be the subject of future studies.

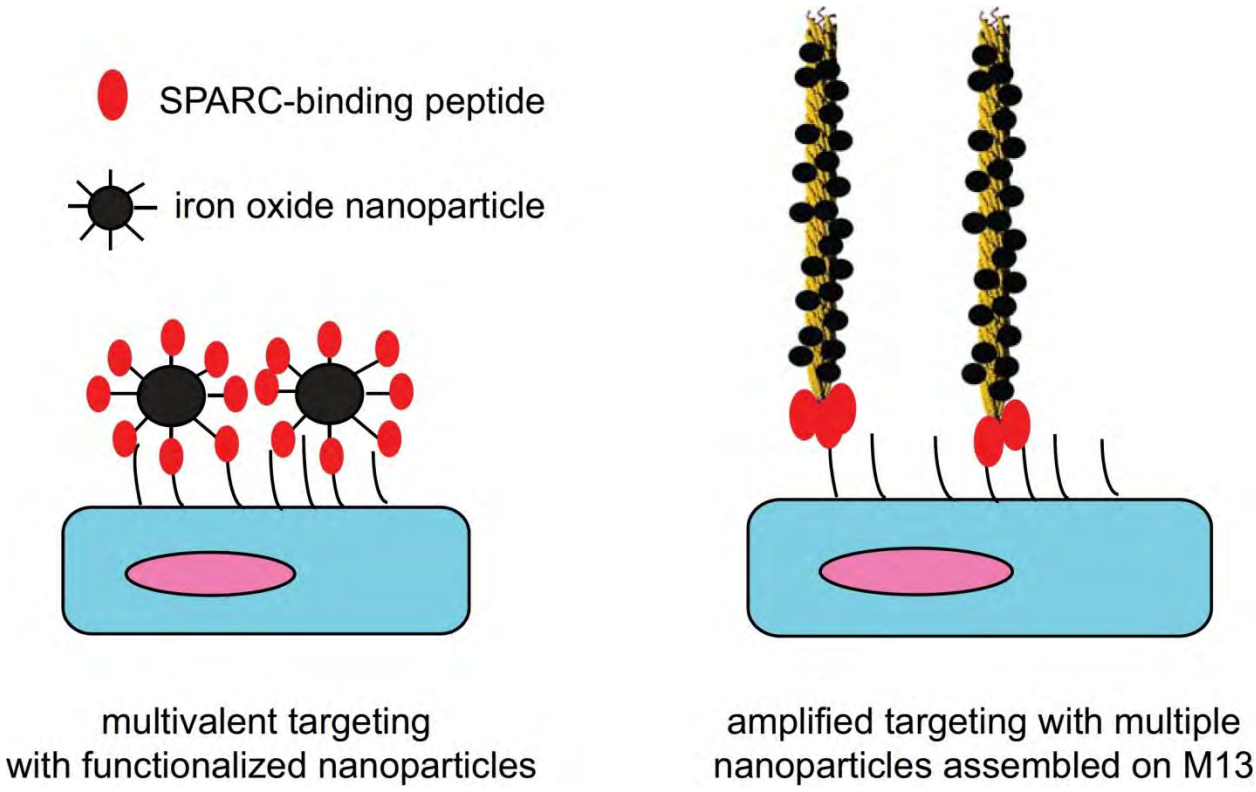


Figure 8. Left: schematic of SPARC-binding peptide ligand targeting of nanoparticles to targets via multivalent interactions. Right: M13 assembles multiple nanoparticles along its coat to deliver a higher cargo of nanoparticles per SPARC target than the ligand-functionalized nanoparticles. Image courtesy of A. Belcher.

Theranostics

The multi-functional potential of nanomaterials enables their use as combined diagnostic and therapeutic agents. The multi-functionality can be innate, as it is for gold nanoparticles that act as imaging agents through their light scattering properties and as therapeutic agents through light absorption mediated heating for thermal ablation of tissues. Multi-functionality can also be engineered by the combination of multiple materials into a single construct or the conjugation of biological or chemical species with imaging, detection, therapeutic and targeting functions to a nanoparticle. In both cases, targeted delivery can enhance localization and accumulation at a tumor. The unique multi-functional capability of nanomaterials suggests theranostics as an area in which nanotechnology cannot only improve on existing strategies for cancer care, but actually extend clinical capabilities beyond what would be possible with traditional materials and approaches. Over the past three years of Alliance support, theranostic nanoparticle platforms have matured considerably, advancing to *in vivo* efficacy, biodistribution and toxicity studies in preparation for Investigational New Drug (IND) filings with FDA.

Lily Yang and Hui Mao of Emory University have been developing theranostic platforms with funding from both Phase 1 and 2 of the Alliance. They are currently engaged in pre-clinical testing of a nanoparticle with an iron oxide nanoparticle (IONP) core for MRI contrast enhancement and a polymer coating that encapsulates chemotherapeutics or siRNAs (Cho et al., 2013, Lee et al.,

2013). Yang and Mao's efforts are focused on pancreatic cancer, a disease characterized by a dense, hypovascular fibroblast stroma. The stroma acts as a source of both intrinsic and extrinsic resistance to chemotherapy by releasing tumor promoting factors and stymieing transport of drugs to cancer cells. To confront the stroma, Yang and Mao conjugated their construct with an amino terminal fragment of a peptide targeting the internalizing urokinase plasminogen activator receptor (uPAR), which is overexpressed on both fibroblast and cancer cells in the tumor but not expressed by normal or inflamed pancreatic tissue. By delivering drugs to the fibroblast cells, the construct "chews through" the stroma and accesses cancerous cells in the tumor. The group conjugated gemcitabine to the polymer coating of the nanoparticle through a tetrapeptide linker that is a substrate for the lysosomal cystein protein cathepsin B; subsequent gemcitabine release is promoted by mild acidic conditions similar to those found in endosomes and lysosomes. Following uPAR mediated internalization of the construct, release of gemcitabine was observed only in the endosomes or lysosomes, suggesting deactivation of gemcitabine by enzymatic cleavage within the cytosol was prevented. Studies in an orthotopic human xenograft mouse model of pancreatic cancer showed increased tumor growth inhibition for targeted nanoparticles, compared to untargeted nanoparticle or free gemcitabine, and persistent IONP presence in residual tumors. This suggests the platform may enable monitoring of drug delivery and assessment of tumor drug resistance by MRI.

A modified version of the platform can be used for delivery of siRNA. In the place of gemcitabine, the polymer coating was conjugated to 10-20 double-stranded DNA nanocassettes containing a U6 promoter and a small hairpin RNA (shRNA) gene for *in vivo* siRNA expression. Constructs with both IONP and QD cores were synthesized. Inducing siRNA expression within the cell cleverly circumvents difficulties in siRNA delivery to the cytoplasm. The uPAR amino terminal fragment was retained as the targeting arm to promote tumor delivery and cellular uptake. The researchers first established gene inhibition with the platform by monitoring inhibition of Luciferase and then determined that delivery of multiple nanocassettes per nanoparticle increased the efficiency of gene expression knockdown. Knockdown was achieved *in vivo* following systemic delivery of the platform in a human xenograft model of breast cancer. In those studies, targeting increased accumulation in the tumor relative to liver and spleen as compared to untargeted nanoparticles. Studies on breast and pancreatic cancer cell lines extended the work to gene silencing of survivin and showed induced cell death in target cells. They were also able to increase sensitivity to gemcitabine following survivin knockdown, a significant finding since resistance to gemcitabine treatment is an important factor in poor treatment outcomes for pancreatic cancer.

In addition to incorporating multiple therapeutic strategies into their platform, Yang and Mao are also optimizing the diagnostic potential of their platform through ultra-short time echo MRI methods (Huang et al., 2012a). Superparamagnetic nanoparticles are typically used as negative contrast T_2 agents, which limits their sensitivity against background. At very short measurement times, however, the large longitudinal relaxivity of these particles can be exploited for T_1 weighted positive contrast imaging with relatively little influence from the T_2 signal. Mao and collaborators have been investigating MRI sequences for positive contrast imaging and combining the results with image analysis and sequence simulations to uncover relationships between image formation and nanoparticle core size and concentration (Zhang et al., 2011). Validated relationships would enable quantification of nanoparticle delivery to tumors. The platform has also been investigated for use in image guided-interventions, which was the subject of one project in the Emory Center in phase 1 of the Alliance and remains an area of active research interest for the group and their collaborators at Emory. Versions of the nanoparticle with a fluorescent tag were developed for dual mode MRI and near infrared fluorescent imaging and combined preoperative MRI and intraoperative fluorescent imaging of sentinel lymph nodes (Zhao et al., 2011, Zhou

et al., 2013). Mouse studies in both investigations revealed good localization of the nanoparticle to tumors and affected nodes and strong co-registration of the MRI and fluorescence images.

Yang and Mao's platform is truly multifunctional, incorporating cellular targeting, controlled intracellular drug release, enhanced therapeutic efficacy and diagnostic potential in a 70 nm diameter nanoparticle. Each component of the system has been carefully designed and tested, including the use of appropriate models that recapitulate the idiosyncratic molecular characteristics and microenvironment of pancreatic cancer. Designed for treatment of a highly lethal cancer for which there are no effective treatments, it is a strong test case for the clinical translation of a complex, sophisticated nanoparticle design. Yang and Mao are working closely with industrial partner Ocean Nanotech to prepare nanoparticles under GMP conditions for pre-IND studies. They have been successful in winning additional funding from NCI for their work, including R01 awards to investigate use of their theranostic platform in breast cancer and to develop stealth versions of the nanoparticles for image guided drug delivery and an NCI Small Business Innovation Research (SBIR) contract for Ocean Nanotech in support of preclinical studies They were recently awarded a supplement award from NCI to fund a collaboration with Haian Fu of the NCI supported Cancer Target Discovery and Development (CTD^2) Center at Emory. The platform will be used to deliver a peptide from Fu's lab to disrupt a crucial signaling network in pancreatic cancer cells.

Nanotechnology for Image-Guided Interventions

One of the most exciting applications for nanotechnology enabled imaging is in image-guided interventions. The tumor specificity of targeted nanomaterials can be used to delineate the margins of cancerous from healthy tissues, providing badly needed information for minimally complete removal of tumors. The diverse physical properties of nanomaterials and the ease of combining multiple materials into compound structures additionally enables design of agents that can be interrogated by different methods. This is particularly valuable for image guided interventions, in which different modalities can be used for pre- and intraoperative guidance. MRI and x-ray imaging provide high spatial resolution and tissue penetration but are prone to tissue drift issues that preclude real-time surgical guidance, while optical methods which cannot be used for deep tissue imaging or sizing of tumors are well-suited for such guidance. Co-registration of these complementary images is a serious challenge, and development of multi-modal imaging agents must be accompanied by design of instrumentation and imaging protocols to properly gather and collect image data.

Alliance Highlight – the Stanford Center and Interventional Imaging

Innovative design and careful characterization of nanoparticles for imaging in combination with the development of instrumentation and devices for diagnostic and interventional applications is a hallmark of the work of the Stanford Center. Led by Sam Gambhir, researchers in this Center collaborate across projects and work closely with the extensive facilities supported by the Nanocharacterization and Nanofabrication Core to perform in depth nanoparticle characterizations and associate nanoparticle properties with the results of *in vivo* studies. The Clinical Core and Project 4 associate results of imaging studies and investigations with *in vitro* diagnostic devices developed by Center researchers to develop highly informative diagnostics for cancer. Stanford leveraged the Phase 1 Alliance Center to garner additional support for their work with an award from the NCI Early Detection Research Network (EDRN) and a renewal of an *In Vivo* and Cellular Imaging Center (ICMIC), resulting in a highly resourced environment for translation of innovative technology for cancer diagnostics covering the pipeline from biomarker discovery to device testing.

Bryan Smith of Gambhir's lab established a research program on the use of real-time intravital microscopy imaging to quantitatively characterize the extravasational behavior of two different nanoparticles across three different murine models (Smith et al., 2012). Given that the dependence of tumor penetration on particle size is already well established, they were particularly interested in isolating the effect of particle geometry on extravasation, and they worked with high aspect ratio SWNTs and QDs of similar surface area, charge and PEG coating. For effective tumor delivery nanomedicine developers often rely on the EPR effect. However, Smith and Gambhir noted that the EPR effect is variable across models and inconsistent results are obtained for reasons that are poorly understood. They combined their intravital microscopy studies with detailed electron microscopy analysis of the nanoscale morphology of the vasculature to correlate their findings on nanoparticle extravasation with the size and shape of vascular pores, as shown in Figure 9. The ultimate goal is to enable observable features of the vasculature (e.g. flow rate or pressure) to inform the choice of appropriate nanoparticle properties for effective delivery to the tumor.

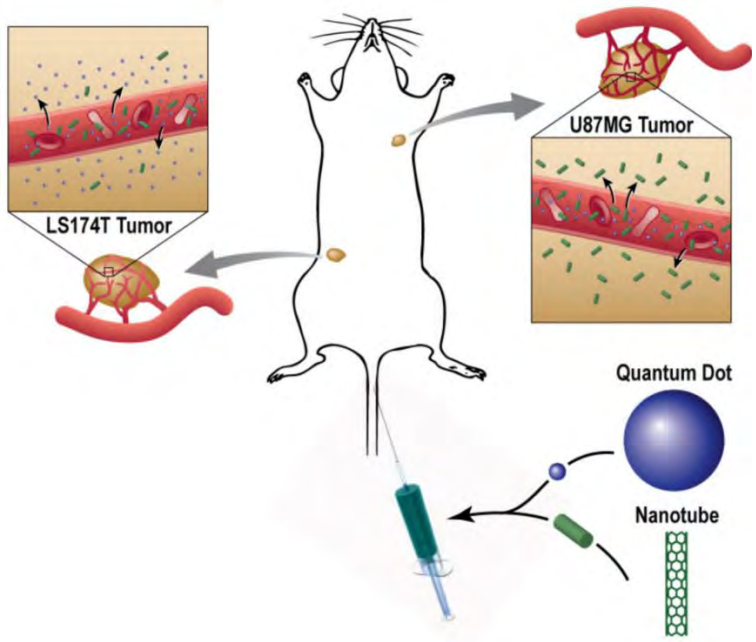


Figure 9. The extravasation of QDs and SWNTsS from the vasculature of murine tumor models. Overview depiction of nanoparticle extravasation. The schematic shows that QDs extravasate from LS174T tumors but not U87MG tumors, while SWNTs extravasate from U87MG tumors but only minimally from LS174T. Image courtesy of Stanford Center.

The group found that extravasational competence was highly dependent on nanoparticle geometry and tumor biology. QDs were much more successful than SWNTs in gaining entry to a human colon adenocarcinoma model tumor, but only SWNTs extravasated in a U87MG human glioblastoma model. Careful diffusion studies across membranes with controlled pore size suggested a slight increase in the interendothelial pore size in the colon cancer model may account for the QD extravasation there. The mystery of SWNT extravasation from the U87MG model may be solved by this model's fenestrated endothelium. That is, the existence of ~5.5 nm "holes" through which the SWNTs could diffuse out of the vasculature. One tumor type (SKOV3 ovarian cancer) did not appear amenable to EPR mediated delivery, with neither nanoparticle type entering the tumor interstitium. These results suggest that there is no "optimum" nanoparticle size or shape for tumor delivery, but rather that delivery to any tumor must be optimized based on its particular features. These results are consistent with findings from the Texas and UNC Centers in this area. Concerns about variation in EPR effect across tumor types and between

patients prompted the Alliance Office and TONIC consortium to organize a one day workshop on this topic in October 2012, to which Smith, a young researcher currently supported by an NCI K99/R00 award (external to the Alliance), was invited to participate.

Intraoperative Imaging

Gambhir and colleagues are also developing a triple-modality MRI-photoacoustic-Raman nanoparticle for accurate delineation of brain tumor margins both pre- and intraoperatively (Kircher et al., 2012). Completeness of resection is a major prognostic factor for patients with brain tumors, but brain tumors typically have indistinct margins and are often located very close to, or even invasive of, crucial brain features. Currently available tumor imaging techniques suffer from low spatial resolution, image drift, and background noise which prevent reliable discernment of tumor from healthy tissue. Gambhir and his group designed an imaging agent that would fulfill the requirements for successful brain tumor recognition – sufficient accumulation and retention within the tumor to allow observation over time; pre-operative, extracranial imaging capability to allow surgical planning; intraoperative imaging capability for real-time surgical guidance; deep tumor visualization; and sensitive and specific tumor margin detection.

To meet all these requirements in one agent, they combined three functionalities in one nanoparticle. A gold nanoparticle core with a Raman active layer enables high specificity, sensitivity and resolution imaging of tumor margins during and after surgery to confirm clear margins and also high resolution, 3D photoacoustic imaging of the tumor, while a surface gadolinium layer enhances MRI contrast for deep tumor visualization pre- and intraoperatively. Photoacoustic imaging is a new technology in which light excitation of an agent causes heat production and thermal expansion, which produces ultrasound waves that can be captured by an ultrasound transducer to create a 3D image. Sequestration and residence of the nanoparticles in the tumor is due to the EPR effect for the model used. The agent was studied in an orthotopic brain tumor mouse model, in which tumor bearing mice were injected with the triple modality agent and then successively imaged by the three modalities. Tumors were clearly visualized by all three modalities, and the images were co-registered. Enhanced image contrast was observed for all modalities over a 24 hour period, establishing persistence of the nanoparticles in tumors, in contrast to typical contrast agents which rapidly wash out of tumors. Comparisons of immunohistochemistry studies labeling tumor cells and microglia with Raman microscopy and SEM studies of nanoparticle uptake and Raman signal, showed that nanoparticles were located exclusively within the tumors and that Raman signal delineated the tumor margin successfully, as shown in Figure 10. They also tested the nanoparticles as intraoperative imaging agents, in which tumors in mice were resected 24 hours after injection of the nanoparticles. They initially resected tumors using visual inspection only, and then obtained high resolution Raman images to search for remaining tumor tissue. Raman images of what appeared to be completely resected tumors by visual inspection revealed small foci in the resection bed near the tumor-brain interface. Histological analysis revealed these to be finger-like extensions of the tumor into surrounding tissue, recognizable only through the Raman label. These results indicate MRI and photoacoustic imaging can be used to guide gross resection of tumors by providing information on the location and extension of a tumor within the brain, followed by sensitive intraoperative Raman imaging for careful excision along tumor borders.

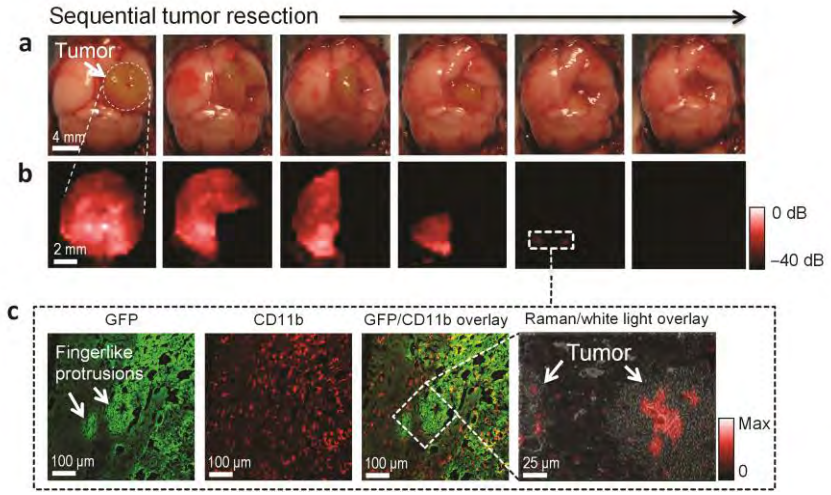


Figure 10. (a,b) Living tumor-bearing mice (*n* = 3) underwent craniotomy under general anesthesia. Quarters of the tumor were then sequentially removed (as illustrated in the photographs, a), and intraoperative Raman imaging was performed after each resection step (b) until the entire tumor had been removed, as assessed by visual inspection. After the gross removal of the tumor, several small foci of Raman signal were found in the resection bed (outlined by the dashed white square; some Raman images are smaller than the image frame). The Raman color scale is shown in red from −40 dB to 0 dB. (c) A subsequent histological analysis of sections from these foci showed an infiltrative pattern of the tumor in this location, forming finger-like protrusions extending into the surrounding brain tissue. As shown in the Raman microscopy image (right), a Raman signal was observed within these protrusions, indicating the selective presence of MPRs. The box is not drawn to scale. The Raman signal is shown in a linear red color scale. Reprinted by permission from Macmillan Publishers (Kircher et al., 2012).

Nanoparticle Enabled Raman Endoscopy

The Stanford group is also applying their nanoparticle imaging research to improving endoscopy, a technique that has dramatically enhanced a physician's ability to identify and diagnose many diseases. The ability to visualize suspicious tissue in parallel with clinical sampling has enabled earlier diagnosis of many types of cancer, most notably colorectal cancer. Even though colonoscopy has reduced colorectal cancer mortality by approximately 40%, it is estimated that 40-60% of flat or depressed lesions, which tend to be most malicious, are missed annually. Furthermore, the multiple biopsies associated with the procedure contribute to a 25 in 10,000 rate of adverse events, including infection, perforated bowel, and death. Surface-enhanced Raman scattering (SERS) is a technique that enables exquisitely sensitive detection of multiplexed targets by scattering laser light off gold nanoparticles layered with a Raman active layer over coated with a silica shell (Zavaleta et al., 2013).The gold surface enhances the inelastic scattering of photons to improve sensitivity, the Raman layer produces a unique spectrum from the scattered laser light, and the silica layer facilitates biocompatibility and the attachment of targeting moieties. The approach and materials are illustrated in Figure 11.

Gambhir's group has systematically been working to establish the utility and safety of these nanoparticles in humans. With support from Phase 1 of the Alliance, they worked with the Center's Nanofabrication Core to generate a suite of ten 120 nm nanoparticles, each with a different Raman active layer (Zavaleta et al., 2009). Using mice, the investigators established their ability to detect multiplexed Raman signals in an *in vivo* context, with linear extrapolation of the particles' concentrations from the detected Raman signatures. Gambhir and colleagues demonstrated the capacity of computer algorithms to accurately discriminate and quantify individual spectra through polynomial regression, deconvoluting the gross spectral output allowing linearly quantitative multiplex detection *in vivo*. This uniquely Raman multiplex-

ability from a single light source is expected to enable molecular imaging of interrogated tissues through specific targeting.

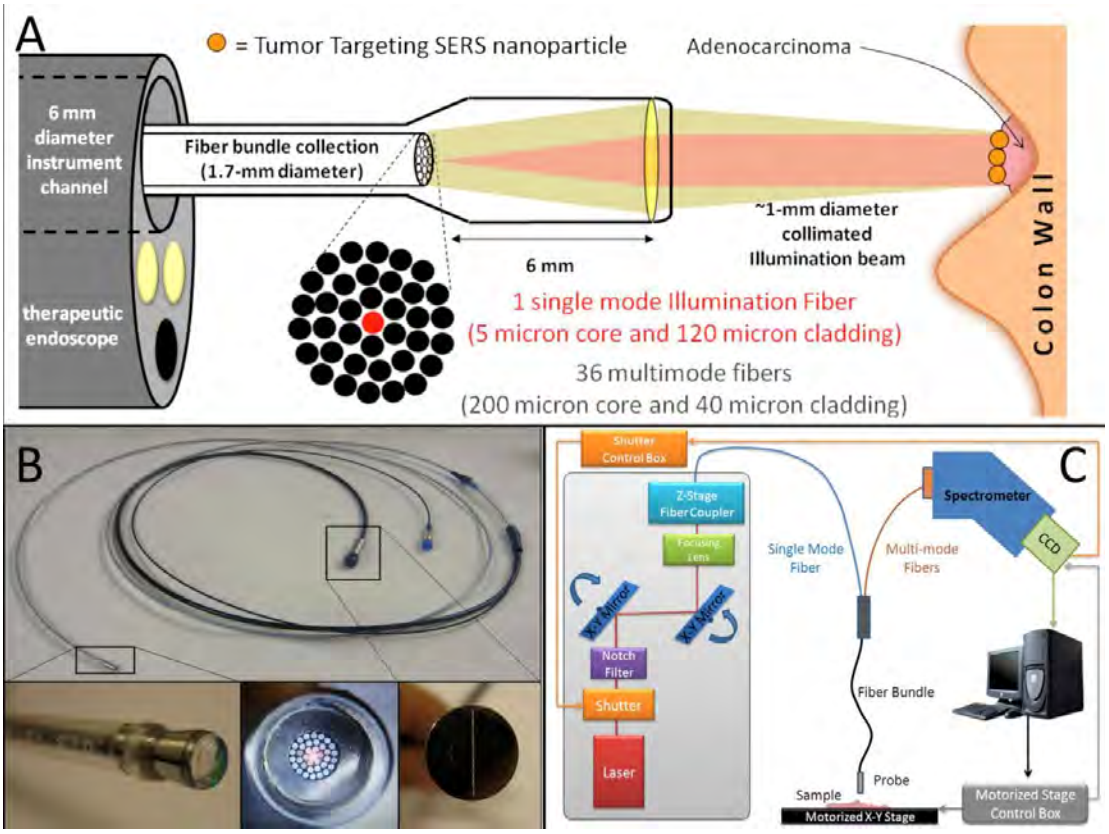


Figure 11. Raman endoscope design and setup. (A) Schematic of Raman endoscope designed to be inserted through the accessory channel of a clinical endoscope with a 6-mm instrument channel. The Raman endoscope is composed of a single-mode illumination fiber that is surrounded by a bundle of 36 multimode collection fibers, totaling a diameter of 1.8 mm. The excitation laser light is collimated by a lens to emit an illumination spot size of ~1.2 mm. (B) Photograph depicts the final fabricated Raman endoscope to be used for clinical studies. (Lower) Enlarged digital photograph of the endoscope head (Left), a magnified photograph of the fiber bundle (Center), and a magnified photograph of the back end of the device (Right) show a linear array of the 36 collection fibers that are specially aligned to fit into a spectrometer. (C) Schematic of the entire device setup starting with the 785-nm laser whose output is controlled by a shutter driven by a computer- driven shutter controller. The laser is then passed through a notch filter, which ensures a narrow 785-nm bandwidth, is guided through a series of mirrors, and is refocused to a single-mode fiber to illuminate a sample. The light collected by the multimode fibers is dispersed by wavelengths onto a CCD via a spectrometer. Figure from Proc Nat Acad Sci U S A, 110, E2288-97, 2013 (Zavaleta et al., 2013).

The nanoparticles still faced a challenge common to biomedical nanoparticle applications – systemically administered nanoparticles are prone to removal from circulation by the MPS. To bypass this, the Stanford group focused their efforts on delivery to the epithelially contained colonic system, with the hypothesis that nanoparticles delivered intrarectally would not extravasate to the circulation and would be excreted with normal feces. The group tested the preclinical fate, toxicity, and biodistribution of these particles (Zavaleta et al., 2011, Thakor et al., 2011). The results of these experiments were very encouraging. Biodistribution studies showed that while there was a preponderance of systemically delivered nanoparticles concentrated in the livers, kidneys and spleen, intrarectally treated animals predictably had very high levels of nanoparticles only in the large intestine. This diminished and was nearly undetectable after 24 hours, and no nanoparticle signal could be detected in any other tissue. Pathologic examination revealed that neither group of treated animals exhibited gross organ

abnormalities. The Stanford group is working with NCL on preclinical studies of these nanoparticles in preparation of an FDA submission.

In parallel to the preclinical testing of the nanoparticles, the Stanford group has been developing the medical instrumentation to detect these SERS particles endoscopically. The Raman endoscope has been designed with clinical utility in mind. Where other Raman devices require contact between the probe and tissue, the Stanford device's collimating beam enables working distances of 1-10 mm to accommodate tissue topology and user variability during examination. Furthermore, the two other Raman endoscopes previously published use laser power and interrogation times that exceed the maximal permissible exposure set by the American National Standards Institute. The instrument developed by the Stanford Center uses a laser one fifth the power and up to 30-fold less exposure time. Signal detection is achieved by a fiberoptic bundle that runs in the same 6 mm channel of the emission source, freeing other channels of the endoscope for other devices such as a nanoparticle sprayer, a biopsy device, or other tools to exploit other potential nanoparticle applications (Kircher et al., 2012). Using this instrument the Gambhir group demonstrated its ability to image the SERS nanoparticles using human tissue. With fresh biopsied pieces of human colon, the team readily detected ten different Raman signatures simultaneously and with co-localized multiplexing of a mixture of four particles. They also demonstrated linear sensitivity down to <1 fmol of target receptor, a 10-100 fold improvement over current fluorescent endoscopes. The endoscope has been tested in humans in IRB approved studies, discussed in more detail in Chapter 5. The long lasting collaboration between experts in electrical engineering and instrument design, nanomaterials synthesis and characterization and clinical application required for the invention and translation of this endoscopic system is uniquely enabled by the Center structure.

Development of imaging hardware based on nanotechnology

The basic design of the x-ray tube, the core of the most common type of medical scanner, has not changed significantly in the last century. A metal filament 'cathode' emits electrons when resistively heated to over 1000 °C and a metal target 'anode' emits x-rays when bombarded by the accelerated electrons. These systems typically have a slow response time, high power consumption, and relatively short lifetime, due to filament damage in the high operating temperature. Room temperature field emission of electrons is an attractive alternative mechanism, which produces stable electron beams at low power. The concept of cold-cathode x-ray tubes can be realized using carbon nanotubes (CNTs), which can produce high emission current, provided that high quality material with uniform properties is used. A schematic of such a source is shown in Figure 12. CNT field emission electron current can also be easily controlled by an external field to give instantaneous response time.

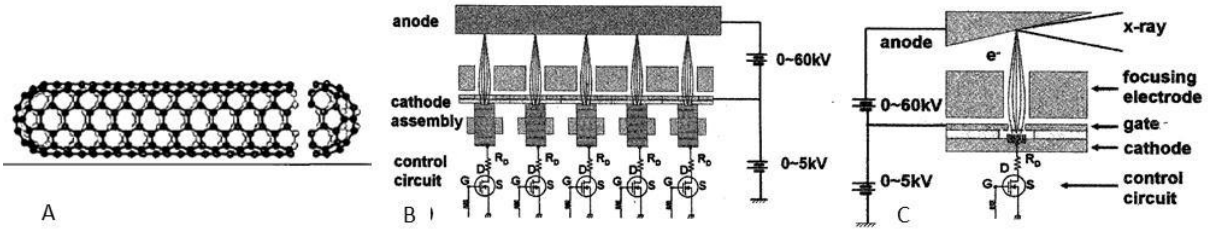


Figure 12. A - structure of single wall carbon nanotube. Typical dimensions are 1 - 50 nm in diameter and 1 - 10 mm in length, B - schematic of multi-beam X-ray source with a field emission cathode containing five emitting pixels, C - each emitting pixel is comprised of 1.5 mm diameter CNT film coated on a metal disk.

Otto Zhou of the Carolina Center and his group have developed technologies to fabricate macroscopic CNT cathodes that exhibit both high current and current density, long lifetime and high voltage stability (Yue et al., 2002, Zhang et al., 2005). They have demonstrated x-ray tubes with CNT-based cathodes that can generate x-ray flux comparable to that from conventional fixed-target x-ray tubes. The Alliance has supported Zhou's work over both rounds of funding.

Digital breast tomosynthesis for early detection of human breast cancer

Mammography is currently the most effective screening and diagnostic tool for early detection of breast cancer. However the current 2D-view mammography method lacks sensitivity and has a very high false positive rate, with 70-90% follow-up biopsies returning negative results. X-ray digital breast tomosynthesis (DBT) is a limited-angle computed tomography technique in which x-ray images at multiple angles are collected for a stationary compressed breast. The images are reconstructed into a 3D dataset, which can be viewed in thin slices with high in-plane resolution that do not suffer from tissue overlap confusion. The 3D images may improve detection of tumors in very dense breasts, which are difficult to image with current mammography techniques. The first commercial DBT scanner received FDA approval in early 2011. Several other DBT systems from different vendors are currently in clinical trials (Qian et al., 2012, Tucker et al., 2013).

All current commercial prototype DBT scanners use a regular full-field digital mammography system to generate a series of projection views from a limited angle range using a single x-ray source that moves along an arc above the compressed breast. However the long scanning time needed for source rotation leads to patient discomfort from breast compression, and motion blurring and system instability that limit spatial resolution. A stationary DBT scanner based on CNT multi-pixel field emission x-ray technology can overcome these limitations. Instead of mechanically moving a single x-ray tube to the multiple viewing angles, s-DBT employs a stationary x-ray source array, which generates x-ray beams from different viewing angles by electronically activating the individual sources (beams) prepositioned at the corresponding viewing angles without mechanically moving the x-ray tube, therefore eliminating the focal spot motion blurring (Yang et al., 2011a). The resulting increase in spatial resolution improves detection of microcalcifications, which are the basis for detection of 80-90% of ductal carcinoma *in situ*, which can be a direct precursor to invasive cancer. The data from the newly designed s-DBT system with CNT emitters shows higher contrast for micro-calcification as compared to images taken using commercial rotating gantry DBT system.

Zhou has been developing clinical instrumentation for DBT (Qian et al., 2012) and testing radiologist confidence in the resulting scans in a clinical trial, discussed in Chapter 4. He is also pursuing other avenues of research using his CNT X-ray source, including the possibility of applying the source to microbeam radiation therapy (MRT). MRT is a promising form of radiotherapy in which a single treatment of ultrahigh dose radiation (100s Gy) eradicates a tumor without functional damage to normal tissue. Despite its potential, MRT has not translated to clinical use in humans, due largely to a lack of accessible MRT irradiation devices, which currently can only be found in a handful of synchrotron light sources globally. Zhou's spin out companies, Xintek and XinRay, are commercializing next generation x-ray imaging systems based on his CNT emission technology. These start-ups are engaged in a commercial partnership with UNC and Hologic, a manufacturer of clinical x-ray systems.

In Vitro Diagnostics

Nanotechnology enabled *in vitro* diagnostics have been a focus area for the Alliance from its inception, due to their potential for high sensitivity and selectivity, their capability to perform simultaneous measurements of multiple targets and the existence of well-established techniques (e.g., lithography) that can be used for the manufacture of integrated, portable devices, enhancing the probability of commercial use as point-of-care devices. These devices have historically been designed for protein capture and detection, either to measure proteins as serum or tissue biomarkers, or to use proteins as tags to capture or label cells or vesicles. However, with the emergence of new biomarker classes, such as miRNA, the devices are readily modified for measurement of these molecules. Alliance projects extend across many facets of *in vitro* diagnostic development, including capture/probe design and synthesis, signal amplification and read out strategies, microfluidic sample handling, device fabrication and assay development by the use of these new devices for biomarker discovery.

Typically touted as higher performance alternatives to standard techniques such as ELISA, nano-enabled protein measurement devices have had striking recent success as tools for biomarker discovery. Alliance investigators have been using their devices to interrogate patient samples in the context of both clinical trials and practice, and to identify potential biomarker signatures of disease for diagnostic or therapeutic monitoring applications. The ease of use, integrated sample handling and high performance of their sensor platforms have enabled Alliance researchers to assay archived and fresh clinical samples to define and begin to validate biomarker panels for use with their platforms. Over the past three years, Alliance members have made significant progress in clinical translation of their devices, progress that has been accompanied by the delineation of clinically meaningful panels to assay with those devices.

Diagnostic Magnetic Resonance Devices

Ralph Weissleder and collaborator Hakho Lee of the MIT-Harvard Center have been developing nuclear magnetic resonance based diagnostic devices over both phases of the Alliance program, and their Diagnostic Magnetic Resonance (DMR) device is now in its third generation (Lee et al., 2008, Lee et al., 2009, Haun et al., 2011). The DMR-3 packages a miniature nuclear magnetic resonance sensor head (μ-NMR) with smartphone data readout and microfluidic sample handling into a device suitable for bedside use in the clinic. Its technical and clinical evolution is shown in Figure 13. The DMR-3 platform exploits changes in the transverse relaxation signal of water molecules in a magnetic field as a sensing mechanism for magnetic nanoparticle labeled analytes (e.g., cells, vesicles, proteins). Nanoparticles with optimal magnetic properties, an NMR head with high signal to noise ratio, a well-designed analytical protocol and a clever bio-orthogonal chemical conjugation scheme for attachment of the magnetic labels (Haun et al., 2010) result in a device with sufficient discrimination and sensitivity for clinical application. The design and testing of this device has involved expertise in biochemistry, nanoparticle synthesis, biomedical engineering and clinical practice, a range of disciplines that it is difficult to imagine could be easily brought together or efficiently collaborate outside of the center structure and associated centralized support and resources. The device is being investigated for a number of applications in cancer diagnostics, including identification of diagnostic protein expression signatures in tumor tissue, capture of tumor associated microvesicles, and detection and characterization of circulating tumor cells (CTCs). The device has been tested on human samples through half a dozen IRB approved protocols.

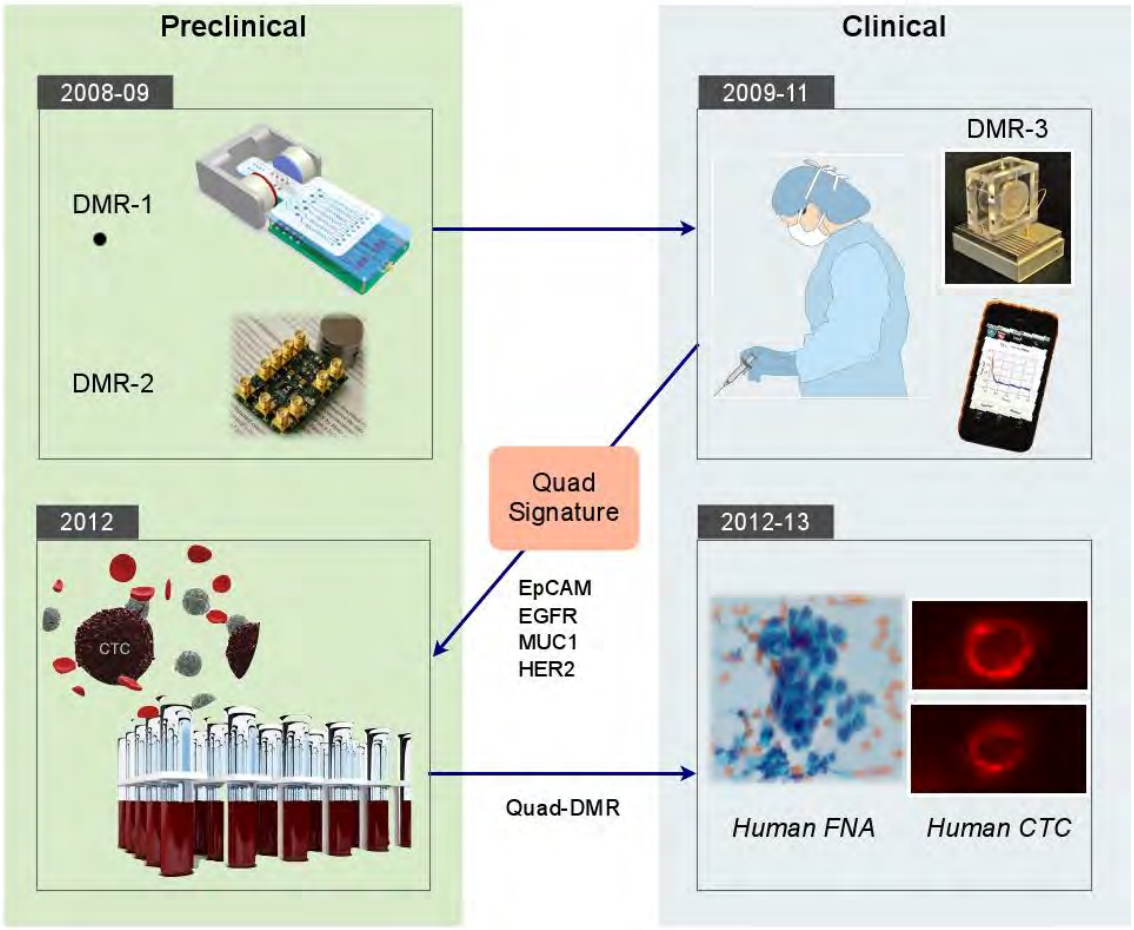


Figure 13. Evolution of the DMR device, including the first clinical configuration in the upper right hand, along with emerging applications. Image courtesy of R. Weissleder.

Evidence of the clinical value of the DMR device came from testing fine needle aspirates from 50 patients with suspected malignancies and validating the findings in an independent cohort of 20 patients (Lee et al., 2008, Lee et al., 2009, Haun et al., 2011). μ-NMR results were compared to current clinical gold standard results of traditional core biopsy samples from the patients to determine diagnostic accuracy. Eleven markers were measured in each sample, including nine cancer-related markers chosen based on current clinical practice or strong literature support for clinical utility, $CD45^{+}$ cell count and total cell density. There was considerable heterogeneity of marker expression across patients, and no single marker discriminated between benign and malignant growth. Even EpCAM, which is often used as a marker for CTCs, was not uniformly expressed and was not detectable in 20% of the cancers. Dual, triple and quadruple marker pairs were also analyzed for discriminatory power. The best performing panel, consisting of MUC-1, EGFR, HER2 and EpCAM, had 96% accuracy, and results were returned within 60 minutes. In comparison, clinical standard of care cytology and histology methods had 74% and 84% accuracy, respectively, and took from 1-4 and 1-8 days to return results.

The study interrogated marker expression heterogeneity within and across patients and found that different needle aspirates obtained along the same coaxial needle pass displayed high levels of marker variability, reaching 30% for extracellular markers and higher for intracellular markers. Variability is

greater still when samples from different tumor regions are considered. These findings have important implications for both molecular diagnostics and targeted therapeutics, and support the use of multiple over single marker detection. They also found that marker expression decreased significantly over time, with drastic changes occurring within one hour of sampling, suggesting that either rapid measurement or fixation of samples is necessary to maintain sensitivity in *in vitro* proteomic approaches.

Inspired by the results of this trial, the group investigated the best performing panel (i.e., MUC-1, EGFR, HER2 and EpCAM) for detection of CTCs from whole blood without prior purification in clinical samples (Ghazani et al., 2012). CTC results were compared to results using CellSearch, a clinically approved but not widely accepted method for CTC detection. Comparison of detection with single and quadruple marker labeling showed greater sensitivity for quadruple marker labeling, and the quadruple marker μ-NMR significantly outperformed CellSearch in the study of clinical samples. The study provides further preclinical validation of the quadruple marker set and established additional utility for the μ-NMR platform. The study also agrees with and supports current clinical methods of ovarian cancer detection, CellSearch and CA125 measurement, while substantially improving on their performance, opening the possibility of wider use of serum based detection and therapeutic monitoring for this disease. The study was extended to compare marker expression in CTCs and cells obtained by fine needle aspirates of bulk tumors (Ghazani et al., 2013). Only weak correlation was observed between the two sample types, throwing into question the use of CTCs as surrogates for tumors, although correlations between CTC/biopsy ratios and clinical trajectory suggest there may still be a role for CTC analysis in conjunction with biopsy. These studies again highlight both the clinical potential of the device and its ability to reveal previously unknown characteristics of cancer cells.

The μ-NMR platform has been adapted by the group for detection of microvesicles 10-100 times smaller than cells that are released into the blood by many tumors (Shao et al., 2012). These microvesicles are of particular potential value for glioblastoma multiforme (GBM) patients, who have large numbers of tumor associated microvesicles in peripheral blood, but for whom CTCs do not cross the blood brain barrier into circulation for detection. A quadruple panel of EGFR, EGFRvIII, PDPN and IDH1 R132H was found to discriminate GBM from host cell microvesicles with 90% accuracy. They were additionally able to use studies of microvesicles released from mouse and human GBM cell lines to show that microvesicle number and marker expression reveal differences in treatment. These differences could be used to define a treatment response index and further translated into a tumor progression index that could identify and predict treatment response, particularly for non-responders, in studies of human patients. The approach provides a rapid and clinically viable method of stratifying GBM patients by molecular characteristics, currently an outstanding clinical need.

The μ-NMR platform is versatile and scalable, being easily modified for measurement of different biomarker sets, and has the major advantage of compatibility with whole blood samples. It offers rapid and relatively inexpensive measurement of clinical samples. Continuing enhancements include work towards high-throughput capacity and on-board sample processing. Support for further development of the DMR device for detection of blood borne disease is provided by the National Heart, Lung and Blood Institute (NHLBI). Commercialization efforts through the spin out company T2 Biosciences are discussed in Chapter 4.

Tumor MicroRNA Profiling

Over the past decade, there has been increasing interest in using microRNAs (miRNAs) as diagnostic and prognostic cancer markers, based on findings that alterations in miRNA levels in cancerous compared to healthy cells correlate to disease state. miRNAs are short (~22 bases), single-stranded RNA

molecules that play a significant role in regulating gene expression, being implicated in regulation of more than 30% of protein coding genes. Although miRNAs are indicative of cell lineage and differentiation state, no single miRNA has been found to be clinically informative, and panels of miRNAs have instead been correlated to healthy or diseased states. This means selective detection of multiple miRNAs will be needed to establish miRNA profiles associated with a disease or stage. Additionally, miRNAs are found only in very low concentrations in both serum and tissue, making their unique identification and quantification technically challenging. Based on these considerations, clinically actionable miRNA based measurements will require high sensitivity, selectivity and multiplex capabilities. miRNAs are typically analyzed using standard high throughput molecular profiling techniques, such as microarray platforms and flow cytometry. Using these techniques typically entails a trade-off between throughput and specificity, and sample processing can consume large amounts of time and sample materials. Although miRNA studies so far have been highly suggestive of links between miRNA expression and disease progression, clinically meaningful miRNA profiles have not been established or validated. There is significant potential for nanotechnology based platforms to facilitate translation of miRNA diagnostics to the clinic, based on the exacting performance standards nanosensors are capable of meeting, particularly for sensitivity and on board sample processing techniques.

Work from Chad Mirkin and Amy Paller of the Northwestern Center on profiling miRNA levels in prostate tumors has led to the discovery of a signature potentially linked to progression that can be rapidly measured from clinical samples (Alhasan et al., 2012). The group adapted the scanometric DNA detection system invented and developed by Mirkin (Taton et al., 2000) for high multiplexity measurement of miRNA. The original system uses nucleic acid coated gold nanoparticles to label target oligonucleotides and is capable of single base pair specificity, a level of selectivity required to differentiate closely related miRNAs from one another. In the re-engineered system, christened the Scanometric miRNA (Scano-miR) Array, target miRNAs are enzymatically tagged with a universal single strand DNA linker and complimentarily bound to a high density miRNA array, leaving the ssDNA linkers free. Following a wash step, the array is exposed to the gold nanoparticle labels, which bind to the universal linker, enabling optical read-out of the array. The target miRNAs can be identified by the geographic addresses on the array to which they hybridize, each of which is associated with a specific sequence. The system was further engineered to improve sensitivity to the 1 fM level, to enable detection of typical serum miRNA levels, which are below 10 fM. The current standard fluorometric assays have a limit of detection of greater than 1 pM, preventing detection of up to 88% of low-abundance miRNA targets in a typical sample.

After establishing Scano-miR analytical performance for multiplex measurements using synthetic miRNAs and healthy donor serum samples, Mirkin and Paller applied the technique to analysis of clinical samples. They used the Scano-miR assay to study human prostate cancer biopsy tissue in an attempt to discriminate between stages of the disease. The Gleason system currently used to classify prostate cancer is based on examination and immunohistochemical staging of tissue sections by a pathologist, which can be highly subjective. Mirkin and Paller hypothesized that the morphological differences between high and low Gleason scored tissues could reflect differences in miRNA expression. They investigated the expression profiles of 706 miRNAs in RNA extracted from two tissue samples, one with a high Gleason score and one with a low Gleason score. One hundred and sixty-three miRNAs were found to be differentially expressed between the two samples, defined as at least a 1.5-fold difference in expression; 109 were more highly expressed and 54 had lower expression in the high Gleason tissue. Analysis of the gene targets of these miRNAs to identify prostate cancer implicated genes and their associated miRNAs (161 miRNAs obtained) revealed that the Scano-miR was able to detect potentially progression-linked miRNAs with 98.8% accuracy. A pilot functional analysis was also done, using gene

ontology network models, on a function-gene-miRNA network of 35 of the 163 miRNAs and 13 of their target genes that are associated with five cancer hallmarks. Network density near the 'cell differentiation' and 'regulation of apoptosis' gene ontology terms suggests the observed deregulated miRNAs are involved in regulation of tumorigenesis. These results are proof of principle that the Scano-miR can be used for miRNA expression profiling to identify new cancer biomarkers.

Integrating Nanotechnology and Cancer Biology

In addition to advancing cancer nanotechnology development and translation as discussed in the sections above, the Alliance supports a significant body of research focused on using nanotechnology tools to create new knowledge about cancer biology, including studies of the mechanisms underlying disease progression and therapeutic response. Alliance researchers are also expanding the application of nanotechnology approaches to emerging areas of interest in cancer research and clinical oncology, such as cancer genomics, metabolism and immunotherapy. Efforts in these areas have resulted in some of the most exciting research that the Alliance has supported over the past three years. This section will highlight some of the most forward thinking and promising work on integrating nanotechnology and biology being done by Alliance researchers.

The Cancer Genome Atlas (TCGA) and related projects are identifying gene alterations in various cancers and building large databases of information on gene copy number variations and genetic mutations. This information is the first step in determining the genetic alterations responsible for driving cancer initiation and progression. Follow-on efforts, like the Cancer Target Discovery and Development (CTD^2) project, are starting to analyze and translate the data generated by TCGA sequencing efforts into therapeutically actionable knowledge. Alliance researchers have begun to team up with investigators in these and other NCI programs, such as the Early Detection Research Network (EDRN), the Integrative Cancer Biology Program (ICBP), and the Physical Sciences in Oncology Centers (PSOC) to use tools from nanotechnology to test the validity of targets discovered and develop therapeutics against them.

Alliance Highlight – the Texas Center and Targeting miRNA Networks in Cancer

An effort to mine TCGA results for insight into potential targets for nanoparticle therapy was undertaken by researchers at the Texas Center and their colleagues at MD Anderson Cancer Center (Yang et al., 2013a). The Center brings together considerable expertise in clinical research in ovarian cancer, including precision medicine approaches, liposomal drug development experience, and extensive computational resources. This union of diverse specialties enables researchers to plan and execute ambitious plans to translate genomics findings into therapeutic candidates. Integrated genomic analyses of 459 cases of serous ovarian cancer from TCGA and 560 cases from independent cohorts led to the discovery of a miRNA-regulatory network defining a mesenchymal subtype associated with poor overall survival. 89% of the targets in the network were predicted to be under the regulation of eight key miRNAs, including miR-506, and functional studies determined that miR-506 increases E-cadherin expression, inhibits cell migration and invasion, and is correlated with improved prognosis. Based on these results, Texas PIs Gabriel Lopez-Berestein and Anil Sood tested the ability of a DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) liposome under development in the Center to deliver miR-506 to tumors to decrease invasiveness and improve outcomes.

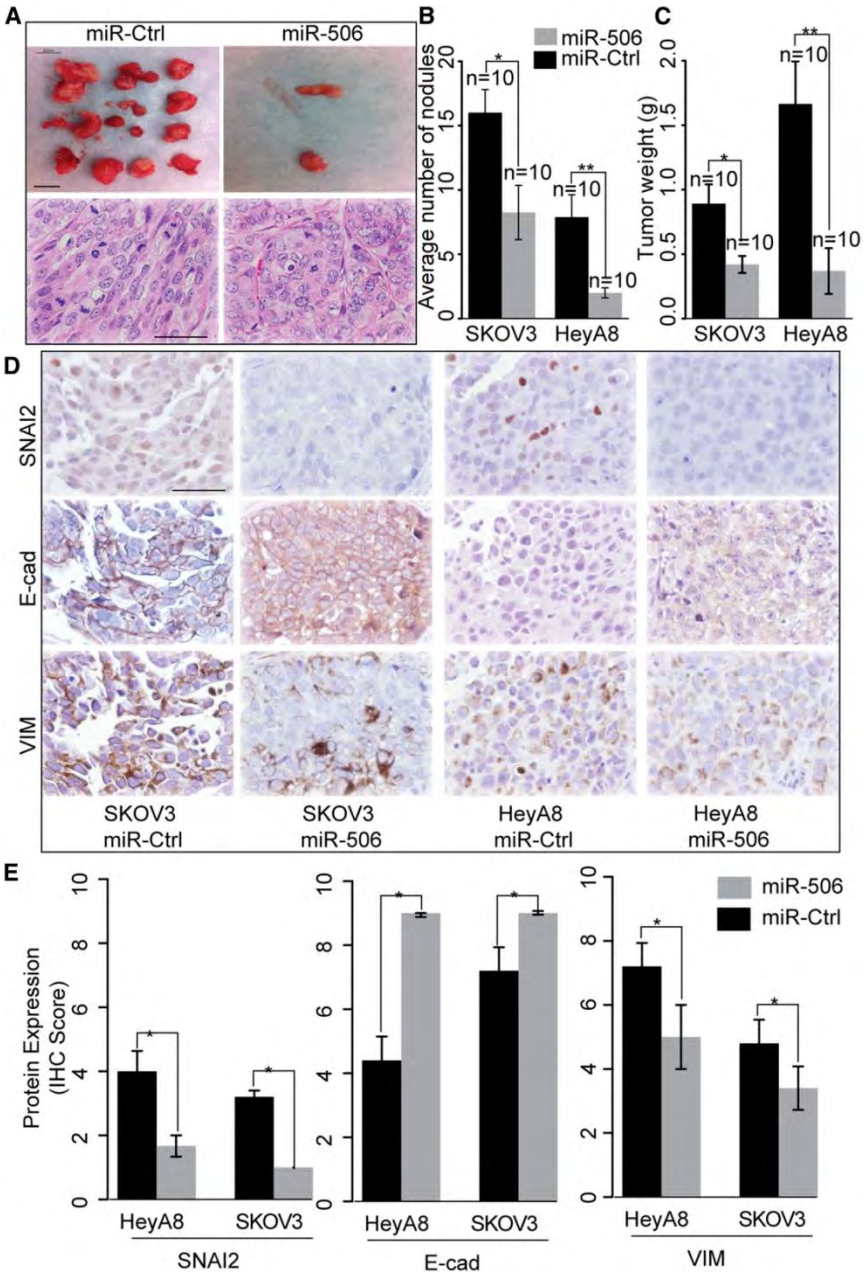


Figure 14. miR-506 inhibits tumor progression in the orthotopic mouse model of OvCa; (A) Representative images of tumor nodules and H&E staining of tumors in control miRNA- and miR-506-treated mice (HeyA8-ip1). Scale bar in the upper panel represents 1 cm. Scale bar in the lower panel represents 100 μm; (B and C) Quantification of tumor weights (B) and tumor nodules (C) in control and miR-506 treated mice (n = 10 for each group) that were injected intraperitoneally with HeyA8-ip1 (HeyA8) and SKOV3-ip1 (SKOV3) ovarian cancer cells, respectively. Error bars represent ± SD; (D) HeyA8-ip1 and SKOV3-ip1 tumor samples from control and miR-506 treated mice were stained for SNAI2, VIM and E-cad by immunohistochemistry. Scale bar represents 100 μm; (E) Quantification of SNAI2, VIM and E-cad protein expression. Error bars represent standard errors. ∗p < 0.05. Error bars represent ± SD. Reprinted by permission from Cancer Cell.

Invasiveness in ovarian cancer cells, and associated poor patient prognosis, is accompanied by a transformation from epithelial to mesenchymal features, known as the epithelial to mesenchymal transition (EMT). The molecular events leading to this mesenchymal subtype are not defined and poorly understood, but evidence has emerged that EMT is regulated by a miRNA network. TCGA mRNA data

had defined four subtypes of ovarian cancer, including a mesenchymal subtype, but these classifications were not prognostic. The Texas group integrated the TCGA mRNA data with miRNA, DNA copy number and DNA methylation data to discover clinically relevant subtypes. Their analysis separated samples into two clusters, one with a higher expression of miRNA-associated genes and associated with advanced stage disease and significantly shorter overall survival. The second cluster contained patients with consistent downregulation of miRNA-associated genes and improved survival. Pathological studies of the TCGA tissue showed samples that were sorted into the poor survival cluster lacked well-organized epithelial structures, resembling mesenchymal cells.

Based on these findings, the group identified miR-560 as a therapeutic target. miR-506 exhibited the greatest downregulation in their mesenchymal subtype and its expression was inversely correlated with the expression of 35 mesenchymal signature genes. They expanded their studies to protein level data, which was not collected by TCGA, using clinically annotated samples from Tianjin Cancer Hospital. Studies of miR-506 and the mesenchymal phenotype were consistent between these samples and TCGA data. From both the TCGA and protein data they could also determine that miR-506 expression was inversely correlated with *SNAI2* expression and positively correlated with E-cadherin protein expression. Again, high miR-506 expression was significantly correlated with longer overall survival. Having established *SNAI2* as a target for ovarian cancer treatment, and miR-506 as a potential therapeutic, the group established orthotopic mouse models and delivered miR-506 incorporated in DOPC nanoliposome (miR-506-DOPC). This resulted in the reduction in the number of tumor nodules and mass compared to delivery of control miRNA. Immunohistochemistry studies confirmed the effect of the miR-506 on *SNAI2* (suppression) and E-cadherin (upregulation) levels, as shown in Figure 14. Sood and Lopez-Berestein will continue their investigations of miRNA therapy with an NCI supplement to the Center award that will support collaboration with Michael White of the UT Southwest Medical Center CTD^2 Center on synthetic lethality approaches using key miRNA identified by White's research.

This DOPC liposome is a promising platform for drug delivery that Sood and Lopez-Berestein have been investigating for over ten years. The platform is also being investigated for RNAi therapy in a variety of contexts. It has been used to overcome taxane-resistance in an orthotopic mouse model of ovarian cancer using RNAi therapy against survivin, an inhibitor of apoptosis and a regulator of mitosis (Vivas-Mejia et al., 2011). The liposomes have also been loaded into Mauro Ferrari's multistage vector porous silicon particles to achieve sustained gene silencing of the EphA2 gene (Shen et al., 2013). High levels of the EphA2 protein are associated with robust tumor growth and can be used as indicators of tumor aggressiveness and poor patient survival. A clinical trial of the DOPC liposome loaded with siRNA against EphA2 sponsored by MD Anderson is slated to start in late 2013 (NCT01591356). The Texas group has been able to leverage the Center's resources to pursue multiple lines of research for this platform.

Validating Genetic Targets for Cancer Therapy

A large group of NCI supported researchers at the Broad Institute at MIT, including Alliance member Sangeeta Bhatia, are participating in an effort to study the essentiality of more than 11,000 genes in over 100 human cancer cell lines. Project Achilles systematically silences individual genes in cells to identify genes necessary for cancer cell survival. Cancer genome data has been integrated with this functional data to identify genes that are essential for survival and progression of cancer cells, and are also amplified or overexpressed in cancer cell lines and tumors, as identified by TCGA (Cheung et al., 2011). The revealed genetic information provides candidate targets for treatment, but the large number of candidates requires some method of prioritizing targets based on likelihood of clinical effectiveness. The Achilles team turned to nanotechnology to test target response to intervention. **Bhatia and her**

collaborators have developed an approach for *in vivo* validation studies of targets that uses a modular nanoparticle siRNA delivery platform to silence genes in tumor models and look for effects on tumor growth inhibition. This approach is ideal for gene targets that are considered "undruggable" by small molecule therapeutics.

Nanoparticle mediated delivery is a promising strategy for RNAi therapy, but access to deep tumor tissue is still a significant problem. High interstitial pressure, dysfunctional tumor vasculature and lymphatics and, in some cases, thick fibrotic stroma surrounding the tumor block access to deep tumor tissue. To overcome these difficulties, Bhatia's group engineered a modular tumor penetrating nanocomplex (TPN) vehicle consisting of a tandem peptide that combines a tumor penetrating unit, to bring the platform deep into the tumor parenchyma, with a membrane translocation unit for delivery of the siRNA into the cytosol (Ren et al., 2012). The tumor penetrating unit was developed by Erkki Ruoslahti with support from the first round of Alliance funding (Teesalu et al., 2009, Sugahara et al., 2010, Sugahara et al., 2009). It stimulates transvascular transport and enables passage of nanoparticles into tumor tissue (Teesalu et al., 2009). The peptides are tumor specific and are established vectors for delivery of small molecules, antibodies and nanoparticles out of the endosome and into the cytosol (Sugahara et al., 2010, Sugahara et al., 2009). The peptides maintain their functions when bound to free siRNA, forming stable nanocomplexes in water and phosphate-buffered saline (~200-400 nm in diameter). siRNA sequences can be plugged into the modular vehicle without interfering with the tandem peptide, allowing the TPN to be easily modified for multiple targets, an important characteristic for rapid screening and credentialing of targets. A vehicle containing a Transportan domain paired with a fixed cyclic LyP-1 domain was chosen as the lead candidate, due to its having the highest efficacy in delivering siRNA to cells, relative potency (>25% gene suppression at 25 nM siRNA) and known secondary structure.

With their delivery vehicle in place, the researchers used the Project Achilles data to choose their first target oncogene, *inhibitor of DNA binding 4* (*ID4*). *ID4* was chosen since it is overexpressed in a majority of primary ovarian cancers but not normal ovarian or fallopian tissue, and it is also associated with other cancers, including breast and glioblastoma multiforme. The oncogenic potential of *ID4* was tested *in vitro*, and three ovarian cancer cell lines were chosen for *in vivo* knockdown studies. The TPNs showed an extended (>12 hr) circulation half-life and siRNA release at endosomal pH, indicating this nanocomplex formulation would be appropriate for *in vivo* efficacy studies. Biodistribution studies of the TPNs in a xenograft mouse model of melanoma showed TPNs homing to tumors within 30 minutes, with a four-fold increase in tumor loading compared to untargeted control nanocomplexes. Histological studies showed the TPNs traveled into the interstitial tumor space significantly more than untargeted nanocomplexes.

The Project Achilles data were also used to identify another class of potential targets for cancer therapy. These are genes that are neither oncogenes nor implicated in driver mutations, but whose copy number loss in cancer cells is correlated with a greater vulnerability to additional gene suppression compared to suppression in healthy cells (Nijhawan et al., 2012). The copy number loss of these genes is typically a passenger mutation to the loss of a tumor suppressor gene. The researchers termed these genes CYCLOPS (for **c**opy number alterations **y**ielding **c**ancer **l**iabilities **o**wing to **p**artial **l**oss) genes. Studies in cell lines with and without loss of CYCLOPS genes confirmed the increased sensitivity to suppression, consistent with a hypothesis of the ability of cells to survive partial but not complete suppression of the gene. Many of the identified CYCLOPS genes are proteasome, spliceosome are ribosome components, essential cell machinery that can typically survive loss of one but not both alleles. The robustness of healthy cells against loss of one copy of these genes suggests that knockdown sufficient to inhibit cancer cell growth will not pose limiting toxicities to healthy cells. Additionally, passenger mutations significantly outnumber driver mutations and therefore offer a richer field of targets for potential treatment. The Bhatia

group tested the therapeutic potential of their results by suppressing the CYCLOPS gene *PMSC2*, part of the proteasome regulatory complex, in orthotopic tumor models of ovarian cancer using the TPN delivery vehicle and found a decrease in tumor burden in $PMSC2^{Loss}$ sensitive models.

Alliance Highlight – the NSBCC and Monitoring and Predicting Response to Cancer Therapy

Over two rounds of funding, the Alliance has supported efforts at the NanoSystems Biology Cancer Center (NSBCC) at Caltech/UCLA/Institute of Systems Biology (ISB) to develop devices and assays for cancer diagnosis. The Center brings together expertise in *in vitro* devices at Caltech with the world class PET imaging program at UCLA and cutting edge systems biology techniques at ISB. The Center's projects are highly integrated. When the NSBCC began, the technology mostly originated from Caltech, the biological content came from the ISB, and the translation to patients was at UCLA. Eight years of Alliance funding has blurred this boundary which, in turn, has accelerated innovation and translation. Caltech scientists now have labs in the UCLA medical school to support patient studies, biological content is now jointly developed across all three institutions (and their spin-off companies), and the core nanotechnologies of the center are now broadly developed and deployed across all three institutions. This highly integrated effort means that researchers can now move from concept to patient in less than a year. This approach enables NSBCC scientists and clinicians to uniquely combine discovery and clinical research.

This evolving story can be illustrated by following the development of a microfluidic/nanotech based set of devices, designed as a multiplex proteomics tools for tissue and blood analysis, developed by Center PI James Heath. During the first round of Alliance funding, Heath developed the DNA Encoded Antibody Library (DEAL) platform, a versatile array technology for integrating measurements nucleic acids, proteins and cells within a single assay platform (Bailey et al., 2007, Kwong et al., 2009). Shortly there-after, DEAL was expanded through the development of a microfluidics-directed, molecular patterning technique called barcoding that permits the construction of extremely high quality, but highly miniaturized DNA and antibody arrays (Fan et al., 2008). Several generations of development followed (Shin et al., 2010, Ahmad et al., 2011), each involving theoretical chemical physics, fundamental surface science, and micro- and nanoscale engineering. The resultant combination of molecular, micro- and nanotechnologies have provided a foundation two general classes of devices which provide enabling tools for both clinical oncology and basic cancer biology investigations. The first is the Integrated Blood Barcode Chip (IBBCs) (Fan et al., 2008, Wang et al., 2012, Qin et al., 2009), which permits large panels of blood protein biomarkers to be rapidly assayed from just a pinprick of blood.

The second platform is the Single Cell Barcode Chip (SCBC). The SCBC can be designed with between 300 and 10,000 individual 0.1 to 2 nanoliter volume microchambers, each of which contain a full copy of up to a 20-element antibody barcode array. These platforms permit highly multiplex assays of secreted, membrane, or cytoplasmic proteins from rare cells. The design of the basic SCBC device components required that the assays exhibit yield true quantitation (proteins are measured in copy numbers per cell), with a measurement error that is only 5%. This type of measurement flexibility, reproducibility and demanding performance standards permits wholly new types of measurements for clinical applications that require direct comparisons between patients, between tissue types, or between time points. The design details of an SCBC are dictated by the designated application. An overview of SCBC applications and associated parameters published so far is given in Table 3.

Heath and oncologist Antoni Ribas of UCLA have used the SCBC and the IBBC platforms to analyze patient samples and profile the immune response to adoptive cell transfer (ACT) cancer immunotherapy (Ma et al., 2013, Ma et al., 2011). The patients were enrolled in a clinical trial of ACT for melanoma

patients (NCT00910650), in which patients were treated with engineered $CD4^+$ and $CD8^+$ T cells. Following therapeutic transfusion, peripheral blood monocytes were analyzed using 10-parameter flow cytometry to permit the separation of highly defined T-Cell phenotypes. Those phenotypes were then analyzed for a panel of 20 functional (secreted) proteins that included cytokines, cytotoxic granules and chemokines. Each patient was analyzed at up to 10 points across a 3 month period, starting with administration of the engineered tumor-antigen-specific T cells. The measurements enabled Heath, Ribas, and their coworkers to make major observations that would not have been possible without the single cell proteomic analysis enabled by their technology platforms. They found that functional behavior of a given T cell phenotype was highly diverse, but could also be shown to be focused, at different time points, into basic biological functions, such as inflammatory, anti-tumor, immunorepressive, etc. Additionally, the T cells that secreted the largest numbers of different proteins also secreted the largest abundances (by far) of any given protein. This means that 10% of a given immune cell phenotype dominated the immune response of that phenotype, by an order of magnitude. This led to a new parameter, the polyfunctional strength index (pSI), to account for the functional behaviors of that dominant minority of cells. The kinetics of the pSI were found to provide a much stronger correlate of clinical response than the population kinetics of that same T Cell phenotype, thus establishing that importance of T Cell functional performance, rather than population abundance, in terms of influencing clinical outcome.

SCBC Parameters	Application
20 secreted proteins/cell **1000 microchambers / chip** **~10^3 copies /cell = detection limit**	Cell biology: stem cell functional capacity Clinical: adoptive T cell immunotherapy inflammatory bowel disease (Ma et al., 2013, Ma et al., 2011)
12 cytoplasmic, secreted, or membrane proteins/cell **350 microchambers/chip** **10^3 copies/cell**	Cell biology: hypoxia as a phase transition, PI3k signaling coordination in GBM cells Clinical: oncogenic signaling coordination in tumor cells (Shi et al., 2012, Shin et al., 2010, Wei et al., 2013)
8 – 16 cytoplasmic, secreted, or membrane proteins/cell **10,000 microchambers/chip** **10^2 copies/cell**	Cell biology: cell-cell distancedependent interaction functions; tissue assembly (Vermesh et al., 2011, Wang et al., 2012)

Table 3. Design parameters and applications for Single Cell Barcode Chip. Table adapted from James Heath.

The SCBC, as applied to such immune monitoring studies, has several advantages. First, it is readily integrated with flow cytometry to allow for analysis of highly defined immune cell phenotypes, or for the analysis of rare cell types. Second, it permits a very high degree of multiplexing (up to 50 proteins have been demonstrated in unpublished work). Third, the single cell resolution it provides allows analyses that can extract coordinated biological behaviors, or can identify those cells which dominate the immune response. Both types of information are lost in bulk assays. Fourth, the platform is comprised of only glass, plastic, and reagents – thus providing an extremely high value in terms of information returned per cost.

The SCBC is now being used in conjunction with numerous other clinical studies of ACT across the U.S., including work being done in collaboration with Steve Rosenberg of NCI's intramural program at the NIH Clinical Center as part of an NCI Provocative Questions award. SCBC analysis is also providing a foundational diagnostic for a major Stand Up to Cancer Immunotherapy Program (James Allison, PI) that

involves MD Anderson, MSKCC, UCLA, and 3 other comprehensive cancer centers. The most recent generations of this type of SCBC are now permitting the full panel of secreted proteins to be analyzed from each cell, followed by analysis of a panel of up to 100 specified transcripts from those same cells (unpublished). This capacity will allow for understanding the relationships between the gene regulatory networks and those highly functional immune cells that dominate the anti-tumor response in immunotherapy patients.

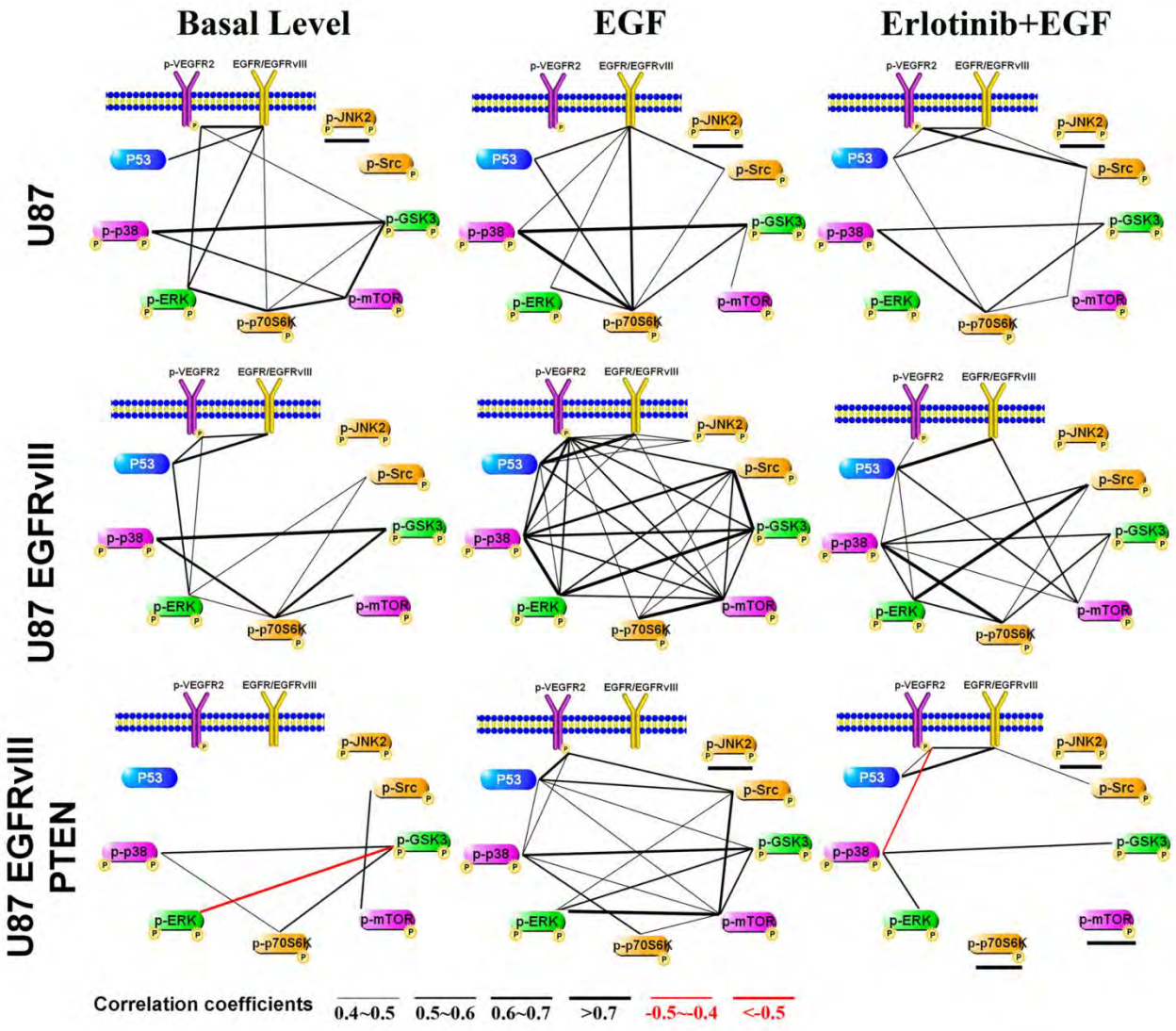


Figure 15. Protein correlation maps for U87 glioblastoma cells under different genetic and environmental perturbations. All indicated correlations pass a Bonferroni corrected p-value test (p = 0.05). Underlined proteins are below the detection limit. Figure reprinted from Proc Natl Acad Sci U S A. 2012 Jan 10;109(2):419-24. Copyright belongs to the authors.

A second set of SCBC applications have been directed at elucidating, in a wholly quantitative fashion, the phosphoprotein signaling pathways in single cancer cells. These SCBC platforms are designed for on-chip lysis of ~400 individual cells, followed by quantitative analysis of a panel of proteins and phosphoproteins that are released from those cells upon lysis. Heath, together with neuro-oncologist Tim Cloughesy and pathologist Paul Mischel are using the SCBC to assess protein-protein interactions in

glioblastoma multiforme cells and gain insight into cellular response to genetic and environmental perturbations. Such perturbations included various targeted molecular therapies, as well as environmental perturbations such as hypoxia (Shi et al., 2012, Wei et al., 2013). Figure 15 shows protein correlation maps elucidated using SCBC measurements of model GBM cell lines.

As the technology has improved, it has been extended towards the analysis of primary cancer cells separated from solid tumors. Most recently, these same researchers have been applying the SCBC to determine independent signaling pathways in tumor derived GBM cells. A unique aspect of these measurements is that, because of their multiplexed and wholly quantitative nature, they permit the introduction predictive analyses that are derived from the physico-chemical laws (Shin et al., 2011, Wei et al., 2013). This, in turn, has permitted the identification of independent signaling pathways within tumor cells, and the identification that those pathways can provide extremely facile resistance mechanisms to targeted inhibitors. Such analysis is also suggesting approaches for designing effective combinations of therapies that can successfully anticipate resistance. These researchers are currently working within NCI's High Content Data Integrated (HCDI) Working Group Biomarker Consortium to translate this approach into a clinical study.

Although the Center has established significant clinical utility for the SCBC, IBBC and related platforms, a significant obstacle to widespread adoption of protein based diagnostic devices is the reliance on expensive and fragile antibodies as capture and detection agents. Building on work supported during the first phase of Alliance funding (Agnew et al., 2009), Heath's group has created a series of peptide-based multi-ligand protein capture agents that provide drop-in replacements for monoclonal antibodies (Manetsch et al., 2004, Millward et al., 2011, Farrow et al., 2013, Pfeilsticker et al., 2013, Nag et al., 2013). The synthetic molecules, called Protein Catalyzed Capture (PCC) agents, are built using the technique of iterative *in situ* click chemistry, in which peptide libraries are sequentially screened against a target protein, or the target epitope of the target protein. This means that the protein, or protein epitope, actually provides the catalytic scaffold for assembling its own capture agent. To do so, it selects between millions of chemical reactions, resulting in an exquisitely selective process. The protein only couples those peptide library elements that can fit onto its surface in just the correct way.

A primary target for PCC agent development in the Heath lab has been Akt, with PCC agents developed against both Akt1 and Akt2. For Akt2, the agents were targeted at the S474 epitope, which is an 'undruggable epitope' (it provides no site for small molecule binding), but has a strong allosteric relationship to the functional activity of the protein. The PCC agents have not only served as monoclonal antibody replacements for standard assays such as ELISAs or Western blots, but they have also been shown to serve as very interesting drug candidates (Millward et al., 2011, Nag et al., 2013). The agents are being commercialized by Integrated Diagnostics (now InDi Molecular) by Heather Agnew, a former trainee in the Center who led the original PCC Agent development research. Research is being supported by the Gates Foundation and United States Department of Defense for applications in developing world diagnostics (PCC agents require no refrigeration chain, and so are readily used in harsh environments), as well as bioagent detection They are additionally being investigated for use as molecular imaging agents by Indi Molecular™. InDi Molecular, and its parent company Integrated Diagnostics, were both founded by Heath and NSBCC co-PI Leroy Hood, and are discussed in Chapter 4.

Bioresponsive and Bioactivatable Nanomaterials

The latest generation of nanomedicine platforms is ambitious in design, and the platforms increasingly dynamic in their interaction with the *in vivo* environment. In many cases, accumulation at a site isn't

sufficient to generate activity, and instead diagnostic or therapeutic activities are triggered by conditions at the disease site. In the most conceptually advanced platforms, the nanomaterials modulate the host physiology to enhance the platform's performance, moving past simple diagnostics or therapeutics into the realm of *in vivo* engineering of biological response.

Bioactivated nanomedicine will increase the demand for enzymatic imaging capabilities past even the current enthusiasm for the approach. Agents that provide detailed information about *in vivo* activity are in the early stages of development, but will hopefully be incorporated into diagnostic and therapeutic decision matrices sooner rather than later. **Jianghong Rao of the Stanford Center is designing strategies to interrogate enzymatic activity in the extracellular matrix and elucidate the cell-cell and cell-matrix interactions that drive tumor progression, invasion and metastasis (Xia et al., 2011).** Rao synthesized a fusion protein, CB-Luc, of the collagen binding protein CNA35 and the engineered mutant luciferase Luc8-535. Following tail vein injection of the CB-Luc into a mouse model, persistent, ubiquitous signal was observed, indicating binding to collagen throughout the animal. Having established that the probes could access the whole body and were stable *in vivo*, the group turned to tumor matrix specific protease activity. They chose matrix metalloproteinases 2 and 9 (MMP-2/9) as targets, since they are overexpressed in many tumors and process multiple molecular targets, including growth factors, which are implicated in tumor progression. To image MMP activity, they attached dye quenchers to an MMP substrate at the N-terminus of the Luc-835. Cleavage of the quencher by the MMP restores bioluminescence and indicates MMP activity. Significant differences in signal between tumor and normal tissue were observed in a xenograft mouse model, and this continuous MMP activation was observed for six days. Following normalization, differences in bioluminescent signal can be attributed to difference in enzymatic activity. This sort of functional imaging could enable prognostic and predictive screens that indicate tumor aggressiveness or potential responsiveness to therapy.

One of the most innovative new materials developed within the Alliance comes from the MIT-Harvard Center, where **PI Robert Langer and investigator Daniel Anderson have developed a nanoparticle system that can synthesize proteins in response to an external trigger (Schroeder et al., 2012).** They formed phospholipid vesicles around a minimal E. coli S30 extract, which acts as a source of energy, ions and T7 RNA polymerase, and a plasmid DNA template encoding a reporter protein, as shown in Figure 16. They first showed that the vesicles could produce GFP and that the GFP was located inside the vesicle, not at its membrane. They then incorporated a template encoding the enzyme Renilla luciferase. Particles were washed and lysed and luciferin added to the lysate, resulting in luminescence, indicative of successful luciferase production by the particles. By exploring the necessary nanoparticle conditions for protein production, they found a minimum size greater than 100 nm was required for the plasmid to fit into the nanoparticle without additional manipulation of the plasmid coil. However, they also found that the close proximity of the components promoted greater protein production in smaller (170 nm) rather than larger (400 nm) nanoparticles.

Having established optimized protein production, the group then sought to enable trigger-controlled production by conjugating a photo-cleavable protecting group to the DNA. They tested cleavage of the protecting group in microfluidic channels to test luciferase production under varying flow rates; production varied inversely with flow rate as illumination time and incident energy decreased at higher flows. They then tested activation of luciferase production in mice using local injection and irradiation of the nanoparticles, and achieved efficient luciferase production. The work has implications for the production of therapeutics *in vivo*, on demand, from inert precursors, enabling localized and highly controlled therapy.

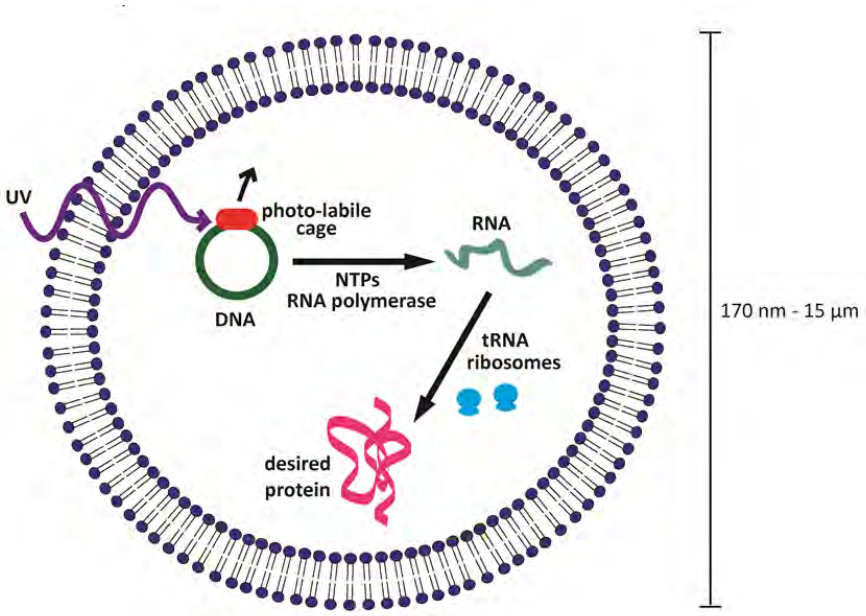


Figure 16. Protein producing particles. A schematic of an encapsulated *in vitro* transcription/translation nano- and microscale particulate system. DNA, tRNA, ribosomes, amino acids, ribonucleotide triphosphates (rNTPs), and ions were loaded into lipid vesicles. Image courtesy of D. Anderson.

Cooperative Nanoparticle Systems

Sangeeta Bhatia of the MIT-Harvard Center and her collaborators are developing a co-operatively acting system of nanoparticles that recruits the body's coagulation signaling networks to enhance drug and imaging agent delivery to tumors (von Maltzahn et al., 2011). The approach is conceptually similar to an earlier system developed with support from the first round of Alliance funding, in which tumor fenestration was increased by light mediated heating of gold nanorods, and the more fenestrated tumors showed increased loading of liposomal doxorubicin and chains of magnetic nanoparticles (magnetic nanoworms) suitable for use as MRI contrast enhancement agents (Park et al., 2010). In Bhatia's more recent and sophisticated approach, the coagulation cascade is triggered site specifically either physically or chemically, by "signaling" modules (nanoparticles or engineered proteins) that deliver a minor wound to the tumor tissue. Fibrin deposition and the coagulation cascade in tumor vessels was induced by either near infrared irradiation induced gold nanorod-mediated thermal damage of tumor tissue or activation of the extrinsic coagulation pathway by a tumor targeted tissue factor which induces coagulation when bound to receptors on the cell surface. Nanoworms were derivitized with a peptide substrate for the coagulation transglutaminase FXIII or with a fibrin-binding peptide, and mixtures of targeted and untargeted worms were systemically delivered to tumor bearing mice. Externally heated tumors (45 °C) showed increased accumulation of targeted nanoworms, along with greater extravasation and tumor permeation due to the heat mediated disruption of tumor vasculature. Similar results were observed for FXIII targeted liposomal doxorubicin, establishing that the receiving nanoparticles could home to nanoparticle triggered coagulation signals in tumors, as shown schematically in Figure 17.

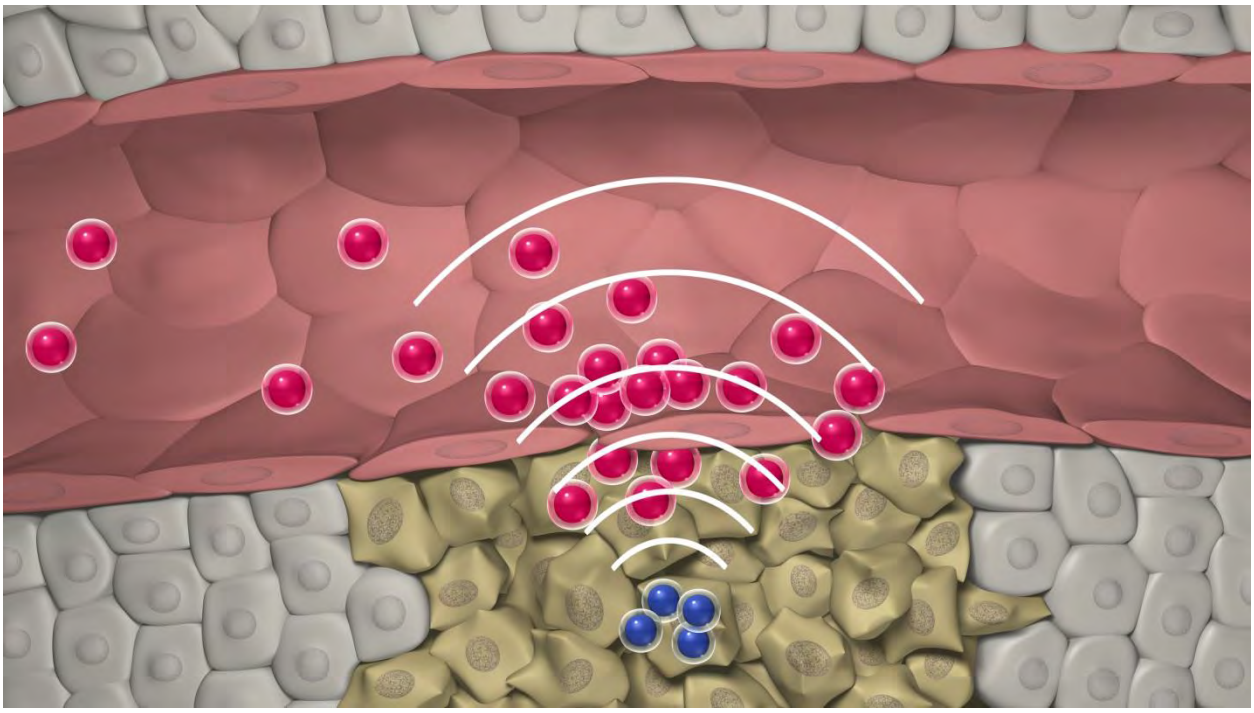


Figure 17. Schematic of communicating particles in vivo. Image courtesy Bhatia group.

Integrated tests of the signaling and receiving modules were done on mice bearing bilateral xenograft tumors. Gold nanorods were injected and allowed to clear from the mice before co-injection of distinctively fluorescently labeled FXIII and FXIII control nanoworms. Subsequent NIR irradiation of the whole right flank of the mice locally heated tumors where AuNRs accumulated. Fluorescent studies at 96 hours revealed a ten-fold increase in accumulation of FXIII nanoworms in irradiated tumors, compared to unirradiated tumors. When co-injected with FXIII nanoworms, targeted tissue factor also increased nanoworm accumulation by several fold. For both nanorod heating and the tissue factor treatment, the amplification of tumor targeting could be canceled by co-administration of the coagulation inhibitor heparin. A test of therapeutic enhancement using liposomal doxorubicin showed a 40 fold increase in tumor accumulation over plain liposomes and six-fold improvement over liposomes targeted to specific receptors. Importantly, it was also shown that the wound-induced doxorubicin accumulation produced improved tumor bulk reduction and survival than thermal ablation alone. Doxorubicin release in tumor tissues was also enhanced, due to improved permeability resulting from tumor heating.

Bhatia's group has devised another elegant system using mass-encoded peptides conjugated to nanoworms that act as synthetic biomarkers for noninvasive urinary monitoring (Kwong et al., 2013). The protease sensitive nanoworms passively accumulate in fenestrated tissues, such as tumors or inflammation sites, where the peptide substrates are cleaved by local protease activity. The released peptides are filtered from the body through the urine, where they are collected and analyzed by mass spectroscopy to identify aberrant protease activity at the target site. Bhatia's group was able to use these markers to monitor liver fibrosis and detect resolution or onset of cancer in mice without invasive biopsies. The approach should be readily generalized to multiple diseases and diagnostic settings.

Although the systems designed by Bhatia have obvious potential therapeutic and diagnostic applications, their greatest value is as proof of principle that cooperative systems of simple nanoparticles, synthetic peptides and proteins can transmit information *in vivo* by co-opting biological processes and acting as

artificial inputs and outputs to signaling pathways. The systems also exhibit innate signal amplification. Each signaling gold nanorod was capable of recruiting more than 100 FXIII nanoworms or over 35,000 doxorubicin molecules encapsulated within the FXIII liposomes, and each targeted tissue factor recruited more than ten FXIII nanoworms, with the advantage of not requiring an external trigger. Similarly, the synthetic biomarkers are activated by protease cleavage, enabling generation of large signals from repeated activity of the protease. Systems like this have the potential to marshal significant biological response to an activating signal that could itself be a quite small perturbation, enabling less invasive diagnostics and less toxic therapies. Given the wide diversity of biological cascades and available nanoparticles and synthetic proteins, it appears possible to engineer a very wide assortment of programmed responses for sensitive detection, diagnosis and treatment of disease.

Chapter 4 Translational Activities

Clinical translation of Alliance technology is an important goal for the program, and the Centers of Cancer Nanotechnology Excellence in particular are expected to aggressively pursue clinical application of the technologies they discover and develop. Each Center is expected to have advanced at least one of their projects to Investigational New Drug (IND) or Investigational Device Exemption (IDE) submission status to FDA by the end of the funding period. Alliance funding cannot be used to support clinical trials, but members are strongly encouraged to leverage Alliance funding and resources to garner additional support for clinical trials of their technologies. The Alliance seeks to assist its investigators in effectively testing and commercializing their products by providing access to industrial representatives, through forums and panels at Alliance sponsored meetings and through the Translation of Nanotechnology in Cancer (TONIC) consortium. The Alliance also supports translation through development and dissemination of standardized protocols and best practices, along with resources and information about nanomaterials characterization and device fabrication. In this chapter we will discuss the clinical translation and commercialization efforts of Alliance members, along with the program activities intended to support these efforts.

Clinical Trials

A number of Alliance affiliated institutions and companies are pursuing clinical trials of therapeutic nanoparticles and diagnostic approaches enabled by nanotechnology. Some of these trials are sponsored by companies that were started to commercialize technology supported by Alliance funding in the early stages of development, such as BIND Therapeutics. In other cases, trials of technology platforms are being pursued concurrent with further development and investigations performed with Alliance support. Examples of these concurrent developments Mark Davis and Cerulean Pharma's ongoing collaboration on the polymeric conjugation of camptothecin (Weiss et al., 2013, Han and Davis, 2013) and the continuing support by MD Anderson for DOPC RNA transfection vehicles developed by Gabriel Lopez-Berestein and Anil Sood (Vivas-Mejia et al., 2011, Liu et al., 2012). A complete list of clinical trials associated with the Alliance is given in Appendix C. This section provides highlights of Alliance research that has progressed to the stage of clinical trials or Institutional Review Board (IRB) approved clinical studies.

Therapeutics

BIND Therapeutics, a company based on the work of Alliance researchers Robert Langer and Omid Farokhzad at the MIT-Harvard Center, launched a Phase 1 clinical trial (NCT01300533) of its lead candidate, BIND-014, in 2011 and reported preliminary results in 2012 (Hrkach et al., 2012). BIND-014 is a PEGylated polymeric nanoparticle encapsulating docetaxel and targeted to prostate specific membrane antigen (PSMA), a receptor overexpressed on prostate cancer cells and on the vasculature of most solid tumors. BIND's delivery vehicles, marketed as the Accurin™ platform, are designed for extended circulation time due to a PEGylated surface; drug release is controlled by their polymer composition and targeted delivery to cells through surface ligands. BIND's proprietary particle screening process, which they call the Medicinal Nanoengineering® Platform, is an integral part of their development strategy. Combinatorial libraries of targeted nanoparticles are first prepared with systematically varied physical and chemical properties, such as particle size, surface PEG and ligand density and drug release profile. This library then undergoes an iterative *in vitro* and *in vivo* screening

process to optimize drug release, cell surface binding, PK/PD, biodistribution, and efficacy for a given indication. Optimization includes appropriately balancing physical properties for passive targeting of tissues and bio-chemical properties for effective cellular targeting, given that there are trade-offs between surface ligand density for improved cellular targeting and increased size and MPS recognition, which decreases tissue accumulation. The candidate Accurins are then manufactured using a well-defined and robust nanoemulsion process. Figure 18 shows a schematic of the optimization process for BIND-014, which was chosen from a starting library of over 100 different nanoparticle compositions.

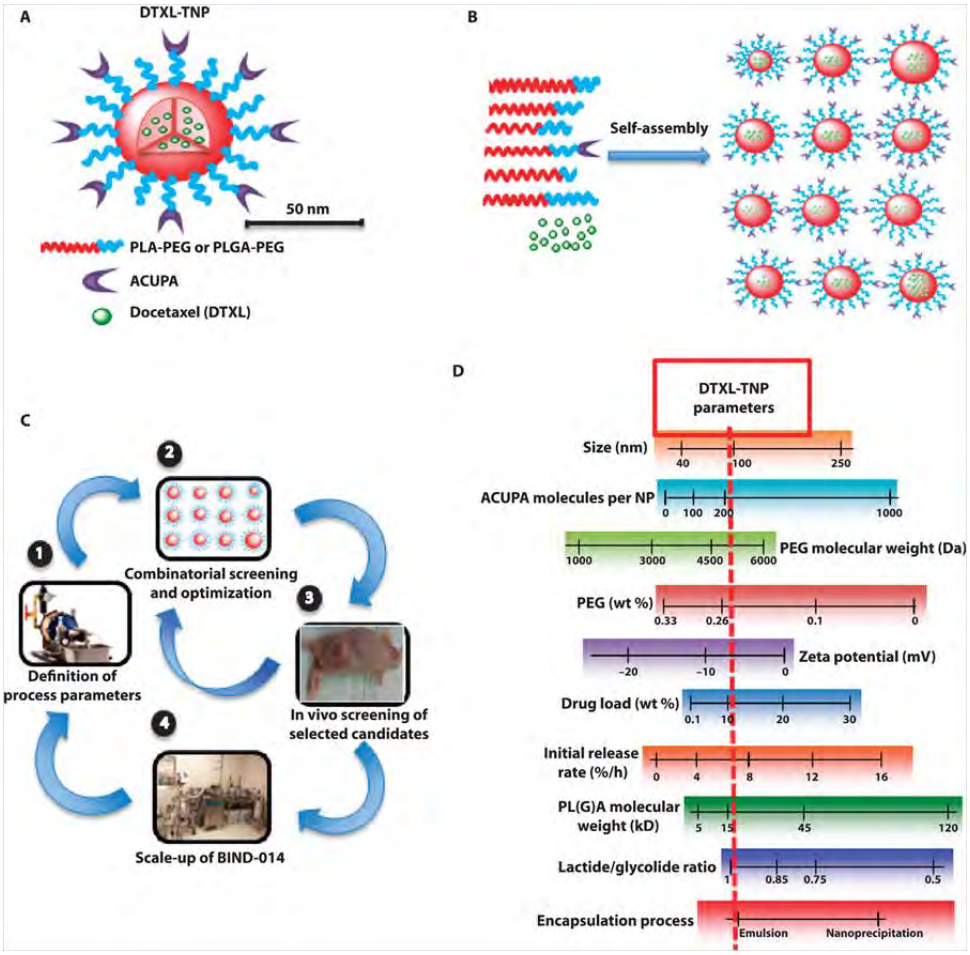


Figure 18. Combinatorial screening and optimization of DTXL-TNPs. (A) Schematic of DTXL-TNP, a PSMA-targeted polymeric nanoparticle (NP) composed of a hydrophobic poly-lactic acid (PLA) polymeric core encapsulating docetaxel (DTXL) and a hydrophilic PEG corona decorated with small molecule (ACUPA) targeting ligands. (B) Generation of a library of DTXL-TNPs prepared by self-assembly of particles from mixtures of DTXL, PLA, PLGA, PLA- or PLGA-PEG (with varying PLA, PLGA and PEG block lengths), and PLA-PEG-ACUPA. (C) Development and clinical translation of PSMA-targeted DTXL-TNPs. (1) A nanoemulsion process for efficiently encapsulating DTXL in NPs was developed. (2) Small-scale batches of DTXL-TNPs were prepared and evaluated with respect to drug load and encapsulation efficiency, particle size distribution and reproducibility, and *in vitro* release kinetics. (3) DTXL-TNPs with promising physicochemical properties were evaluated with respect to PK in rats, and tolerability, tumor accumulation, and efficacy in tumor-bearing mouse models. *In vivo* results informed additional formulation optimization and led to selection of DTXL-TNP composition and process. (4) The DTXL-TNP manufacturing process was scaled up and used to manufacture sterile clinical supplies under cGMPs. (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 (Hrkach et al., 2012).

The preliminary results of the Phase 1 trial were promising, with few adverse events recorded and positive responses to treatment observed in some patients with advanced disease at doses as low as 20% of the typical dose of free docetaxel used in the clinic. An important finding was the correlation between nanoparticle PK/PD and synthetic parameters, supporting the use of the Medicinal Nanoengineering Platform for rational design of Accurins for specific indications. There was agreement between the PK profiles in multiple animals and humans. This is particularly promising for future development of Accurins, as relevance of preclinical results to clinical studies has been a frequent and serious obstacle to the translation of nanomedicines to humans. The animal data also showed improved efficacy with receptor mediated uptake of BIND-014 compared to non-targeted nanoparticles, another reassuring finding for the BIND strategy of targeted delivery. A Phase 2 trial of BIND-014 for castration resistant prostate cancer began in 2013 (NCT01812746), joining an ongoing Phase 2 trial of BIND-014 for non-small cell lung cancer (NCT01792479). In April 2013, BIND announced a deal in which the Accurin platform will be developed for delivery of a proprietary kinase inhibitor developed by AstraZeneca. The companies will collaborate to complete IND enabling studies of the lead candidate Accurin, with exclusive rights belonging to AstraZeneca. BIND has also entered into a partnership with Amgen to develop a targeted kinase inhibitor nanomedicine and has a global collaboration agreement with Pfizer to develop cell and tissue targeted Accurins for delivery of small molecule drugs.

NCI and the Alliance program in particular have contributed to BIND's success with both funding and resources. Langer and Farokhzad's development of techniques to reproducibly formulate and precisely control the biophysical and chemical properties of polymer nanoparticles was supported by the Phase I Alliance Center (Gu et al., 2008). BIND has licensed patents on polymers for functional nanoparticles that grew out of research supported by the first round of Alliance funding (Gu, 2012a, Gu, 2012b, Radovic-Moreno, 2012) and continues to utilize research from the Phase II Alliance Center on the effects of ligands on nanoparticle self-assembly and targeting (Valencia et al., 2011). BIND collaborated with NCL on characterization of their platform on the road to their IND submission to the FDA. NCI's SBIR program also provided a number of awards to BIND during their start-up period.

Another nanoparticle formulation that has received continued support from the Alliance while transitioning to clinical trials is a nanoconjugate of camptothecin and a linear cyclodextrin polymer developed by Mark Davis (Schluep et al., 2009). Camptothecin is a potent topoisomerase inhibitor with strong anti-cancer properties, but it has very poor solubility and stability and significant adverse side effects. These liabilities prevented clinical development of camptothecin, although its weaker analogues irinotecan and topotecan are FDA approved chemotherapeutics. Conjugation of camptothecin to cyclodextrin increases solubility by as much as three orders of magnitude and also prevents spontaneous lactone ring opening under physiologic conditions, which is undesirable since this inactivates the drug. The conjugate spontaneously assembles into 30-40 nm nanoparticles with a mean plasma elimination half-life of 17-19 hours, compared to 1.3 hours for free camptothecin (Numbenjapon et al., 2009).

Cerulean Pharma has licensed the technology from Davis' laboratory and Calando Pharmaceuticals (original licensee) and is developing it under the trade name CRLX101. Results from Phase 1/2a (NCT00333502) testing of CRLX101 showed favorable PK and encouraging safety and efficacy (Weiss et al., 2013). Davis continues to use his Alliance funds to investigate nanoparticle formulations of camptothecin (Han and Davis, 2013) and to collaborate with Cerulean to elucidate the behavior of CRLX101. Importantly, they recently published correlative studies comparing human data from clinical investigations with results from multiple animal models (Eliasof et al., 2013). PK studies of CRLX101 in mice, rats, dogs and humans reveal linear scaling of the dose in milligrams of camptothecin per m^2 for all

species. Plasma concentrations of free drug released from CRLX101 and urinary excretion were also consistent across species. These findings, similar to what BIND found for its lead candidate, suggest that preclinical animal studies can be predictive of human response for nanoparticle delivery platforms, significantly increasing the likelihood of success in clinical trials for indications with strong preclinical data.

Cerulean announced in March 2013 that CRLX101 failed to meet its primary efficacy endpoint, overall survival benefit, in a randomized 2b (i.e., efficacy focused) study in patients with advanced non-small cell lung cancer (NCT01380769). However, tumor reductions that met Response Evaluation Criteria in Solid Tumors (RECIST) were observed. These criteria are a published and widely accepted set of rules defining when cancer patients improve ("respond"), stay the same ("stable") or worsen ("progress") while receiving treatments. Cerulean remains committed to CRLX101 and is partnering with cancer centers around the country on additional trials for multiple cancer indications and combination therapies (NCT01612546, NCT01625936, NCT01652079, NCT01803269). The company is also a member of TONIC, the academic-industrial consortium started by the Alliance Program Office.

Anil Sood and Gabriel Lopez-Berestein of the Texas Center have been working together for almost ten years to synthesize and test liposomal vehicles for gene therapy and have been continuing this work under the current Texas Center grant (Landen et al., 2005). The resulting I DOPC nanoparticle for delivery of anti-EphA2 siRNA will enter Phase 1 clinical testing in Fall 2013 (NCT01591356), sponsored by MD Anderson. Sood and Lopez-Berestein have also been investigating the DOPC formulation for transfection of other RNAs with Alliance funding. As discussed in Chapter 3, they have delivered miRNA mimics for validation and treatment based on targets identified by TCGA tumor analyses (Yang et al., 2013a). They are now extending this work as part of an Alliance sponsored collaboration with members of NCI's Cancer Target Discovery and Development (CTD^2) network. They are also performing pre-clinical studies of DOPC mediated delivery of anti-survivin siRNA for ovarian cancer therapy (Vivas-Mejia et al., 2011).

Other Alliance associated nanoparticle formulations for siRNA delivery continue to progress in clinical trials. In Phase 1 of the Alliance, the initial results of Calando Pharmaceuticals's Phase 1 trial of CALAA-01, an siRNA encapsulating PEG based polymer conjugate with a cyclodextrin pendant developed by Mark Davis, were published (Davis et al., 2010), demonstrating RNAi mediated gene inhibition due to systemic administration of siRNA. Alliance partner Alnylam Pharmaceuticals published the results of a first in human trial for RNAi therapy targeting VEGF and KSP in cancer patients earlier this year (Tabernero et al., 2013). Although the ALN-VSP platform originated outside the Alliance, Alnylam was founded by Alliance member Philip Sharp of the MIT-Harvard Center andcollaborates with Alliance researchers on the discovery of new platforms for siRNA delivery (Lee et al., 2012). Alnylam maintains clinical programs for RNAi therapy for a number of indications, including amyloidosis, respiratory syncytial virus and hypercholesterolemia.

Imaging

As discussed in Chapter 3, Otto Zhou has been developing methods for x-ray imaging and computed tomography using carbon nanotube (CNT) based x-ray instruments with support from the Alliance and through two companies spun out to commercialize his technology, Xintek and XinRay Systems (Lu et al., 2013, Keel et al., 2012). His focus in the Phase 2 UNC Center has been clinical translation of stationary digital breast tomosynthesis using a CNT source device. This has included retrofitting a commercially available Hologic Selenia digital breast tomosynthesis system (http://www.hologic.com/en/breast-imaging/digital-mammography/selenia/) with a CNT based emission head for the study, with engineering support from Hologic. The system has been in stable operation for

over two years and was used for an IRB approved comparative imaging specimen study in which readers compared images of clinical samples taken with standard 2D mammography and the retrofitted system. The study compared image quality with respect to malignancy and accuracy of lesion margin identification, and all readers were more accurate in diagnosing malignancy and reported greater confidence in margin identification with Zhou's system. These positive results led to initiation of a Phase 1 trial sponsored by the UNC Lineberger Comprehensive Cancer Center in collaboration with NCI (NCT01773850). The trial is expected to be completed in January 2015 following recruitment of ~100 patients. The goal of the study is to compare the confidence level of radiologists evaluating patients using the CNT based device compared to conventional mammography. The recruited patients will have known breast lesions and will receive conventional mammograms as part of their standard of care. A second system will be retrofitted for this study, again with support from Hologic and with a CNT x-ray source on loan from industrial partner XinRay.

Sam Gambhir and his colleagues at the Stanford Center have made significant process towards clinical deployment of a nanotechnology enhanced strategy for colonoscopy discussed in Chapter 3 (Zavaleta et al., 2013). Their coupled preclinical successes of establishing nanoparticle utility and low toxicity with instrument development have enabled Gambhir's group to initiate applications to conduct clinical trials on their system. A protocol to test the ability of the Raman endoscopy component to detect intrinsic Raman signal *in vivo* as potential background in future clinical studies received approval from the Stanford IRB (Stanford IRB-15766). The Raman endoscope is a flexible, 5 mm diameter fiber optic device for detection of Raman signal, which can be fitted into the accessory channel of a standard clinical endoscope. Three male patients undergoing routine colonoscopy consented to participate in the study. The endoscope could detect both illumination from the standard xenon-short arc lamp outfitted on the clinical endoscope and auto-fluorescence from tissue illuminated by the Raman laser. Independent variations between the two were used to compensate for background signal arising from the laser illumination. This demonstrated that the instrument is able to compensate for background fluorescence from its laser light and can give useable Raman spectra in a clinical setting. Figure 19 shows the configuration of the endoscope for the study, along with an image of the endoscope illuminating a spot on the colon wall of a human patient.

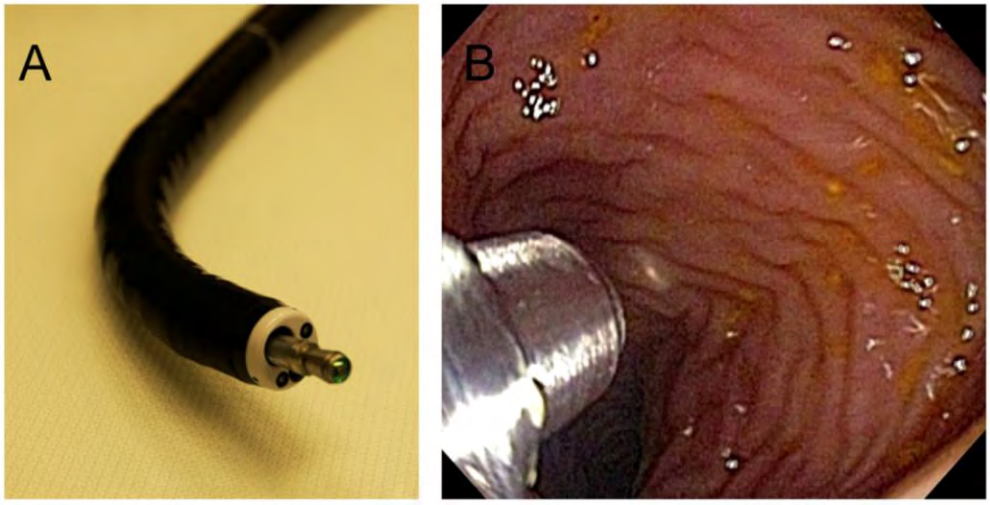


Figure 19. Clinical application and utility of the Raman endoscope in patients. (A) Raman endoscope inserted into the instrument channel of a conventional clinical endoscope. (B) Digital photograph taken from the white-light endoscopycomponent of the clinical endoscope portraying our Raman endoscope protruding from the instrument channel and illuminating a spot on the colon wall in a human patient. Figure from (Zavaleta et al., 2013).

Although the group could test the device component with IRB approval and guidance from FDA on acceptable parameters for laser performance, including power and intensity, the nanoparticle component of this work requires FDA approval before administration in humans. To this end, the Gambhir Lab has been working closely with NCL to address regulatory questions. Should the nanoparticle-enabled Raman endoscope system receive FDA approval, a successful clinical trial may pave the way toward commercialization and broader application of this promising preclinical instrument.

In Vitro Diagnostic Devices

The FDA approval process for *in vitro* diagnostic devices is significantly different than for therapeutics and imaging agents intended for *in vivo* use. Devices are stratified by risk classification, with regulatory controls for approval increasing as devices move from class I to Class III, with Class III devices subject to pre-market approval. However, US regulation allows devices to be used as laboratory developed tests (LDTs) regulated by the Centers for Medicare & Medicaid Services (CMS) through the Clinical Laboratory Improvement Amendments (CLIA) process, for which FDA approval is not required. In this case, the device cannot be marketed or shipped for clinical use but the approved laboratory can accept clinical samples for diagnostic testing. LDTs must be developed in the same laboratory where testing is performed to fall under this enforcement discretion. CLIA certification assures analytical validity of the test and operator performance, but not clinical validity or clinical utility, although companies can apply for and receive approval for reimbursement through CMS and insurance companies. These differences in the approval process lead to fewer registered clinical trials for *in vitro* diagnostic devices. They are more likely to be tested using existing clinical samples under IRB approved protocols for which patients have given consent for sample or specimen use. The pre-market approval (PMA) process for Class III *in vitro* devices is stringent, and it isn't uncommon for companies to release products under CLIA authority while preparing for the PMA submission.

A number of Alliance supported devices are being tested on clinical samples under IRB approved protocols, as in the studies by the Heath and Weissleder groups discussed in Chapter 3. Heath's microfluidic chip for single cell proteomic analysis is being used to characterize patient response in clinical trials of cancer immunotherapy, including one sponsored by the Jonsson Comprehensive Cancer Center at UCLA (NCT00910650) and others sponsored by NCI in support of Steven Rosenberg's work. Heath is also collaborating with Paul Mischel and Tim Cloughesy of UCLA to use a modified configuration of these chips to study protein signaling networks in glioblastoma tumor samples and identify targeted therapy combinations likely to work for individual patients. He recently undertook an Alliance Challenge project with surgeons at UCLA and investigators at Stanford to pursue this work further. The analysis will be done under UCLA IRB approval #10-000655, in collaboration with the UCLA Institute for Molecular Medicine.

Heath has previously developed the underlying DNA encoded antibody arrays as the basis for microfluidic chips for blood proteomics studies (Fan et al., 2008). These chips are being used to search for serum markers for glioblastoma or melanoma in patients and healthy volunteers and to characterize response to treatment by patients (Caltech IRBJH-228). Blood based diagnostics have been a focus area for the Center since its start in 2005, and Center partner Integrated Diagnostics (Indi) is sponsoring two clinical trials in this area. One is for the identification of serum and/or protein panels for lung cancer diagnosis in patients with lung nodules (NCT01752101), and the other is to determine the predictive values of a multiple protein panel based on observed prevalence of lung cancer in study participants (NCT01752114). The successful culmination of this work was recently published (Li et al., 2013) and the Xpresys lung cancer diagnostic product, sold by Indi, was launched in October 2013 at the CHESS

pulmonology meeting (http://www.indidx.com/xpresys_overview). The Xpresys diagnostic test is carried out in a CLIA certified laboratory that utilizes blood protein multiple reaction monitoring mass spectroscopy assay to classify lung nodules as benign or malignant. It is expected to reduce by 50% the numbers of unneeded operations that are carried out on patients with benign lesions (over half of all patients). Indi's long term goal is to migrate this and other assays to the chip and PCC agent technologies developed by the Caltech/UCLA/ISB Center, so as to make those diagnostics broadly available.

The DMR device developed by the MIT-Harvard Center is be used for a number of studies on clinical samples, all of them done under IRB approval. These studies cover bacterial phenotyping and exosome analysis for Tb antigens in addition to cancer biomarker studies. The rapid profiling of fine needle aspirates, exosome and CTC analysis discussed in Chapter 3 were all done under IRB approved protocols, and the MIT-Harvard team is currently conducting studies on CTC detection and analysis in gynecologic cancer patients and tumor biomarker analysis of blood and tissue from melanoma patients and people with suspicious lesions and suspected malignancy. Microvesicle analysis is being optimized through a study of healthy volunteers.

Shan Wang, PI of the Stanford Center, has fabricated a device using giant magnetoresistive (GMR) sensor technology that is standard in the computer hard drive industry. Working with this mature technology, Wang has developed a detection platform relying on GMR detection of biomolecules labeled with magnetic beads. These devices enable automated readout, mass production and development of a disposable test format, compatible with point-of-care diagnostic use (Gaster et al., 2011). Funding for development of the platform was also provided by the first phase of the Alliance (Osterfeld et al., 2008). The sensor technology is analogous to an ELISA immunoassay, with detection of the magnetic nanoparticle label replacing optical readout of a fluorescent label. The sensor is highly sensitive, the magnetic tag signal is not confounded by background or absorption from biological samples, and the ability to address multiple GMR elements simultaneously leads to multiplex detection capability. The integrated device was determined to have a limit of detection of 10 fM with a linear dynamic range greater than three orders of magnitude for a test biomarker (secretory leukocyte peptidase inhibitor, SPLI) with potential relevance to ovarian cancer. As part of the Stanford Center's focus on combined *in vitro* and *in vivo* diagnostics, the device is currently being tested in an IRB approved study "Detection of serum biomarkers for patients with a lung nodule undergoing FDG-PET imaging." The technology is also being explored for use in global health settings (Gaster et al., 2011) and is being commercialized by MagArray, Inc., which is making user-friendly instruments and biochips based on these sensors that are being marketed to selected customers including diagnostic and pharmaceutical companies.

Leveraged Funding

It was a stated expectation in the funding announcement that center funding would be used as a base to gather additional resources to extend applications and utility for center technology, for clinical testing and for translation to the clinic. The centers have been highly successful at leveraging their awards for these purposes, with external support from private, state and other federal sources totaling over $115M since the beginning of Phase 2 Alliance awards, as reported to the program office through progress reports, an amount exceeding the NCI budget over that period of phase 2 of the Alliance.

Many centers have received strong local and state support based on the NCI award. Researchers at the Caltech/UCLA Center received a $20M California Institute for Regenerative Medicine (CIRM) award, "Genetic Re-programming of Stem Cells to Fight Cancer," which will use the microfluidic chip

technologies developed in the Center to inform clinical development of new therapies. Brian Rutt of the Stanford Center received a $1.9M award from CIRM for “Development of single cell MRI technology using genetically-encoded iron-based reporters.” The Northwestern Center received $5M from the Illinois state government for capital equipment upon receiving the NCI award. They also received a $2.1M Lever award from the Chicago Biomedical Consortium to establish a new facility enabling center discoveries to be shared with Consortium-affiliated biology laboratories. This award could substantially broaden the impact of NCI supported research at the center. The University Cancer Research Fund at UNC’s Lineberger Comprehensive Cancer Center, which was established by the North Carolina state legislature, has invested $6M to create the Carolina Institute of Nanomedicine, directed by Carolina Center PI Joseph DeSimone. The fund has also made a direct commitment of $1M to fund pre-IND studies for the lead candidate to come out of the Center, which is comparing multiple nanotechnology platforms for drug delivery.

Alliance researchers have also raised significant funds from philanthropic sources. Sam Gambhir received a $10M award from the Ben and Catherine Ivy Foundation, “Development of a neuro-oncology imaging program using MRI-PET and molecular probes for improving the management of glioma,” based on his NCI supported work in molecular imaging of glioblastoma. Robert Langer received a $6M grant from Prostate Cancer Foundation to continue his work on targeted nanotherapeutics. Antoni Ribas of UCLA is part of the leadership team for a three year, $10M Stand Up To Cancer award, “Immunologic Checkpoint Blockade and Adoptive Cell Transfer in Cancer Therapy.” Jim Heath received a $1.7M award from the Bill & Melinda Gates Foundation Point-of Care Diagnostics Grand Challenges program award, “Protein capture agents with 40 °C shelf life for developing world POC diagnostics.”

Industrial Partnering

The Alliance model for translational research is that discoveries made in academic laboratories are handed off to for-profit partners for efficient development into research and clinical products. Alliance members have been eager to bring their technology to the clinic, forming over 75 start-up companies and partnerships with existing biotechnology firms (Chapman et al., 2012). A table of Alliance partners is given in Many of these start-ups are thriving and now offer products, research or consulting services to the academic and clinical communities. Others have attracted significant investment from large pharmaceutical companies and venture capital funds. Some examples of Alliance technology that has successfully transitioned to market, along with start-ups that have collaborations with large industrial partners, are discussed in this section.

Nvigen, Inc. (http://www.nvigen.com/) was started in 2011 by Aihua Fu, a post-doctoral researcher in the Gambhir lab, to commercialize multifunctional and biocompatible nanoparticles for sample separation, molecular imaging, *in vitro* diagnostic or therapeutic applications. The nanoparticles are based on research at Stanford and their partner, - the University of California, Berkeley, including studies of magneto-fluorescent particles for theranostic applications led by Fu while in the Gambhir lab (Fu et al., 2012). The company offers a variety of nanoparticles, including MagVigen™, magnetic nanoparticles for immunoprecipitation, molecular and cellular purification and identification; MaxVigen™, multifunctional nanoparticles for *in vivo* targeted delivery and imaging; MyQuVigen™, combination magnetic and quantum dot nanoparticles for imaging and manipulation; AuVigen™, biocompatible gold nanoparticles; and AngioGazer™, long circulating fluorescent nanoparticles for imaging angiogenesis. The company also offers custom nanoparticle design and synthesis for specific applications. Fu is one of a number of young Alliance members who have taken on leadership roles in commercializing the

products of their research, reflecting well on the implementation of a rapid technology turnaround strategy in the Alliance.

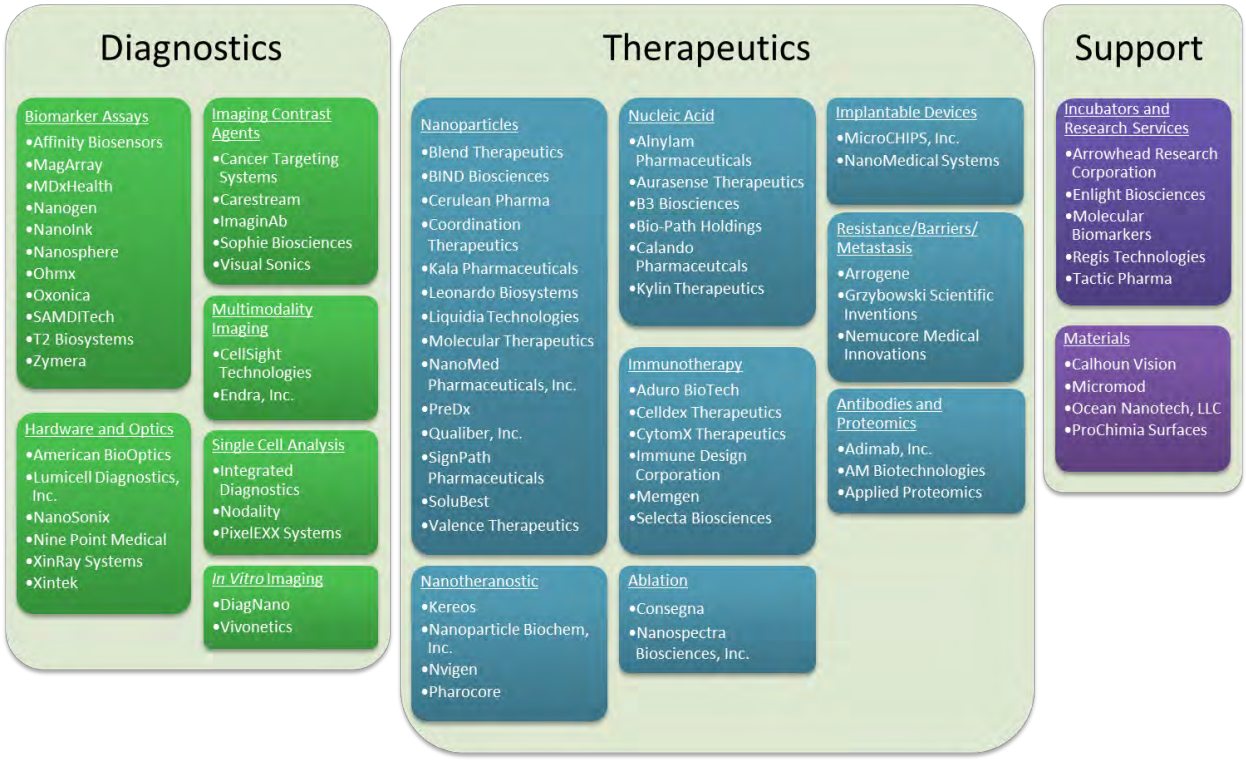


Figure 20. Alliance industrial partners organized by medical application and technology focus. Figure adapted from Chapman et al., 2012.

PI Chad Mirkin of the Northwestern Center founded AuraSense Therapeutics (http://www.aurasensetherapeutics.com/) with Center investigator C. Shad Thaxton in 2011 to pursue clinical applications of spherical nucleic acids (SNAs™), gold nanoparticles coated with highly oriented and covalently attached oligonucleotides. The SNAs have excellent potential for gene delivery applications, as the SNAs have demonstrated high (99%) transfection efficiency into a broad range of cell types without the need for additional transfection agents. The tight and dense nucleic acid binding prevents nuclease degradation *in vivo*, and low levels of immune response have been observed. Several clinical applications are under investigation, including intracellular imaging, topical siRNA delivery for skin disorders as discussed in Chapter 3, siRNA therapy for glioblastoma multiforme, and application in transplants and respiratory ailments. AuraSense, LLC licenses intellectual property from the Northwestern Center, providing a clear path to translation and commercialization for Center technology.

One especially promising SNA application is in NanoFlare™ genetic analysis tools. Nanoflares are SNas in which oligonucleotides on the nanoparticle surface act as capture sequences to which short, fluorophore-labeled DNA molecules, termed “flares,” are hybridized (Seferos et al., 2007, Prigodich et al., 2012). In this conformation the flares are quenched, but when the flares are displaced by target mRNA the fluorescence is recovered, signaling the presence of the target. Mirkin’s group has taken advantage of the polyvalency of the nanoparticle’s oligonucleotides to include multiple sequences on a single particle. By doing so, similar to real-time PCR, an internal control sequence can be included to account for mRNA-independent differences such as nanoparticle uptake rates. Further, multiplexed nanoparticles are

capable of quantifying mRNA levels, as seen in experiments where levels were modulated by siRNA. This technology is being commercialized through an agreement with EMD Millipore, which is selling SmartFlare™ Detection Probes for research use. Millipore offers a catalog of more than 360 SmartFlares™, along with develop-to-order (user-defined application) and custom (user-defined oligonucleotide sequence) design services. The probes are capable of multiplex imaging of RNA in live cells and are primarily used for visualizing siRNA knockdown effects in individual cells, although they are also useful for cell sorting applications.

AuraSense, LLC is also commercializing high density lipoprotein (HDL) nanoparticles developed by Shad Thaxton. Intended to elevate blood levels of HDL, "good cholesterol," for treatment of heart disease, the nanoparticles have also shown efficacy for treatment of B lymphoma (Yang et al., 2013b, Luthi et al., 2012). Uptake of the HDL-NPs changes cellular cholesterol flux by promoting efflux and limiting delivery of natural HDL, leading to relative cholesterol starvation. In *in vitro* tests, treatment of lymphoma cells with HDL-NPs decreased viability and increased apoptosis, an effect not observed with natural HDL treatment or treatment with free constituents of HDL-NPs, pointing to the necessity of the nanoparticle formulation for therapeutic effect. These results point to a new paradigm for lymphoma treatment based not on delivery of a cytotoxic drug, but on manipulation of cancer cell specific metabolism.

Sofie Biosciences (http://sofiebio.com/) was spun out of the Center at Caltech/UCLA/ISB in 2008 with a commitment to developing innovative molecular imaging technologies for positron emission tomography (PET). Sofie is organized on a hybrid model in which the university and faculty are investors in the company, aligning the interests of all stakeholders. Staff work closely with Center researchers to rapidly license and implement laboratory advances in instrumentation and probe synthesis in Sofie products. The company currently offers a number of products for PET imaging, including the GENISYS^4 small animal PET imaging system. Highly sensitive, the system is capable of imaging with 10-fold less than the typical dose of radioprobe, decreasing exposure for both animal and researcher. The compact design and turnkey operation allows operation in a research lab without specially trained personnel. Sofie also offers the ELIXYS radiosynthesizer, a single, versatile platform that can perform development studies on new probes and routine production of standard probes. Different probes can be synthesized on the one system by switching cassettes, kits and software protocols. Sofie licenses technology from and collaborates closely with Center member Mike Van Dam of UCLA on technology for fully automated microfluidic devices for microscale chemical synthesis (Ding et al., 2012, Keng et al., 2012). Van Dam's work with Sofie has received additional support from the Department of Energy's SBIR program.

The new molecular diagnostic ^{18}F-FAC (1-(2'-deoxy-2'-[18F]fluoroarabinofuranosyl) cytosine), a deoxycytidine analog and high-affinity substrate for deoxycytidine kinase (dCK), is available from Sofie for imaging activation of the immune system and for patient selection and drug dosing in select cancers, even as Center members continue to investigate potential applications for the probe. Center member Heather Christofk is investigating liposarcomas and has found high consumption of nucleosides, suggesting activation of the dCK dependent nucleoside salvage pathway in these cells (Braas et al., 2012). Sensitivity to treatment with the nucleoside analogue prodrug gemcitabine suggests that a subpopulation of patients (~10%) have tumors responsive to gemcitabine based on nucleoside salvage pathway activity that can be identified via PET. This should enable patient treatment stratification based on imaging results with ^{18}F-FAC-PET.In preclinical and clinical probe development, the company leverages the facilities at the Crump Molecular Imaging Center at UCLA to significantly reduce the cost and decrease the time required to prepare for an FDA IND submission. The Center has reported three INDs approved by the FDA for use in patient protocols at UCLA, [^{18}F]-D-FAC, [^{18}F]-L-FAC, [^{18}F]-L-FMAC

(2'-deoxy'2'-[^{18}F]fluoro-5-methyl-beta-L-arabinofuranosylcytosine). Subsequent to successful results in trials for imaging of the dCK salvage pathway in patients using these probes, these probes can be expected to join the Sofie product line. Sofie is also collaborating with Center investigators in an Alliance Challenge project to develop a whole body imaging assay for immune evasive tumor sites.

T2 Biosystems was founded by a group of MIT and Harvard faculty in 2006, including MIT-Harvard Center members Michael Cima, Robert Langer and Ralph Weissleder, to create medical devices and diagnostics based on magnetic resonance technology. The technology can perform analyte specific analyses when configured with magnetic nanoparticle probes, label free blood clotting or hemostasis assays. T2 Biosystems offers two instruments, the T2Dx® for diagnostic assays and the T2Stat® for hemostasis assays, for use in research settings only, although FDA clearance for clinical applications marketing is being sought. The company is also preparing rapid detection tests for blood borne infections – T2Candida®, T2Bacteria™, as well as T2Hemostat™, a test capable of monitoring coagulation and platelet function in patients following trauma or surgery or while receiving anti-platelet therapies. T2 is sponsoring clinical trials to assure uniform collection, handling, storage and transport of patient whole blood specimens and associated information to support validation of the T2Candida assay (NCT01525095) and to validate performance of the assay on the T2Dx instrument (NCT01919762). T2 Biosystems has licensed the diagnostic magnetic resonance technology for cancer diagnostics developed by Ralph Weissleder of the MIT-Harvard Center and will support clinical translation of the approach.

Investments in Alliance Companies

BIND Therapeutics was founded in 2007 by Robert Langer and Omid Farokhzad of the MIT-Harvard Center to provide an avenue to bring their polymeric nanoparticle therapeutics to clinical use. The company received initial financing from Flagship Ventures and Polaris Venture Partners, with funding from the NCI SBIR Development Center awarded soon after. Further support from existing and new investors came in 2010, when BIND secured $11M in Series C financing and $12.4M in Series C-1 financing. BIND's strong financing was a major reason for its success in rapidly establishing scaled-up GMP production and advancing its Accurin™ technology into clinical trials. Following initiation of the clinical trial for BIND-014, Bind received a $47.5M investment from a group led by RUSNANO, the Russian state corporation dedicated to promoting growth of a nanotechnology industry in Russia. The positive early reports from the trial led to agreements with Pfizer, AstraZeneca and Amgen to co-develop Accurins for delivery of proprietary compounds from these companies. These agreements have the potential to realize payments greater than $400M if all milestones are met by BIND, in addition to assuring BIND of resources for clinical testing and market access for the covered Accurins.

Liquidia Technologies was founded in 2008 by UNC Center PI Joseph DeSimone to commercialize his PRINT® nanoparticle system. Liquidia received early support from NCI's SBIR Development Center and NIST's Technology Innovation Program in 2007, before raising $20M in Series C financing in 2010. In 2011, the Bill & Melinda Gates Foundation made its first ever program-related investment in a private firm with a $10M investment in the company. The investment is intended to support development of safer and more effective vaccines and therapeutics. In 2012, Liquidia announced a partnership with GlaxoSmithKline (GSK) in which GSK acquired exclusive rights to research and develop certain vaccine and inhaled product candidates using the PRINT technology. The agreement could potentially result in payments of several hundred million dollars to Liquidia. Liquidia has already completed a Phase 1 clinical trial of its LIQ001 influenza vaccine candidate to evaluate its safety, tolerability and immune response when given in combination with the commercially available flu vaccine Fluzone (NCT01224262).

Integrated Diagnostics, now known as Indi, was founded in 2008 by James Heath and Leroy Hood of the Caltch/UCLA/ISB Center to develop technologies that incorporate systems biology methodologies to improve the accuracy and relevance of diagnostics. Indi exclusively licenses technology from the ISB and Caltech, much of it originating in the Alliance Center. Indi grew also from a partnership between the ISB and the University of Luxembourg, which is part of a $200M initiative by the Grand Duchy of Luxembourg to increase the pace of biomedical research, education and commercialization globally. Indi raised $30M in Series A financing in 2009, an additional $10M in 2010 and a final round of $10M of Series A financing in 2012. To expand its product line past the lung cancer diagnostics being developed by IndiDx, the diagnostics division of the company, Indi has recently launched IndiMolecular. This new division is dedicated to creating a new generation of PET probes based on Heath's protein catalyzed capture agents, originally intended solely for use in *in vitro* diagnostics as replacements for immunoassay technology but now recognized to have broader utility.

T2 Biosystems has been successful in raising investment capital as the company grows from a supplier of technician operated instruments for research use into a clinical instrument provider. The company received $5.5M in Series A financing in 2006 and $10.8M in Series B funding in 2008, with additional investors joining the later round. The company also received government support from the Department of Defense. An additional $15M in Series C funding followed in 2010, $23M in Series D financing in 2011 and $40M in Series E financing in 2013. This last round of financing is intended to further T2's clinical programs and support commercialization of its sepsis diagnostics as the company completes testing in support of FDA submissions for market clearance for clinical use of its instruments and assays.

TONIC – Translation Of Nanotechnology In Cancer Consortium

The tendency of Alliance researchers to seek commercialization of their technologies through establishing spin-off companies reflects an increasingly popular model for development of high technology products. While it is an attractive concept that capitalizes on the intellectual and creative aspects of academics, raising sufficient funds and meeting the demanding rigors of manufacturing in a small company environment poses several challenges and is often unsuccessful. Open innovation models and public–private partnerships are gaining popularity as means for small and large industry as well as academic researchers to unite with the same purpose of accelerating technology development or drug discovery as an alternative way of structuring pharmaceutical R&D. Such partnerships confront challenges on a scale and complexity not otherwise possible. Nanomedicines comprise a relatively young research area, with many fundamental issues that still need to be addressed adequately, such as toxicity, pharmacokinetics and biodistribution. These issues substantiate the argument to engage industry and academia in collaborations with the goal of accelerating drug discovery and development. Accordingly, the NCI has recently initiated a public–private industry partnership called the Translation Of Nanotechnology In Cancer (TONIC) consortium.

The main mission of TONIC is to create a consortium of the public, private, and academic sectors to accelerate the translation and development of nanotechnology solutions for the early detection, diagnosis, and treatment of cancer. This partnership model has several goals, including providing Alliance researchers insight into industry needs in technology platforms and drug targets, promoting collaborations between Alliance investigators and industry partners on pre-competitive and late-stage programs, providing TONIC members the opportunity to interact with regulatory authorities and NCL and serving as a sustained forum for exchange of ideas on nanotechnology. TONIC aims to provide venues for independent verification opportunities to ensure data reproducibility and robustness, ensure that

consortium project results are made available to the scientific community and promote the qualification, development, and regulatory acceptance of nanotechnologies in cancer.

It is expected that successful TONIC operation will create a discussion forum for opportunities in nanotechnology platform drug delivery, disease monitoring and imaging specifically in cancer, and possibly extend to other therapeutic indications if an opportunity arises. The group also seeks to develop a roadmap for the development of nanotechnology-based cancer products and a robust translational model to move promising nanotechnology strategies from academic research to the clinical environment. Members will also combine their expertise to evaluate the most promising technology candidates within existing R&D developments and generate case studies based on them. Together the group will seek to recognize and promote translational efforts at every stage of development through appropriate partnerships among industry, academia, government, and philanthropy. NCI Alliance investigators, and other interested parties, aware of the transformative potential of nanotechnology and eager to see their discoveries make a difference for patients, have eagerly embraced the TONIC initiative and dedicated their efforts to the development of products and technologies designed to take advantage of this potential.

Membership

TONIC consortium includes organizations which 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. The current membership of TONIC includes 16 industry members, three patient advocacy groups, NCL and FDA. The consortium has an advisory committee comprising key opinion leaders in the field, Dr. Robert Langer (MIT), Dr. Joseph DeSimone (UNC), Dr. Chad Mirkin (Northwestern University), Dr. Vladimir Torchilin (Northeastern University) and Dr. Larry Tamarkin (Cytimmune). Figure 21 shows the current non-government members of TONIC

Accomplishments to date

Since its inception in October 2011, TONIC has conducted several teleconferences and face to face meetings 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 interactions were instrumental in the identification of the Enhanced Permeability and Retention (EPR) effect as an important area for the group to focus on in order to achieve the maximum therapeutic effect with drugs using nanoparticle carriers, leading to the NCI hosted workshop on EPR in October 2012 discussed in Chapter 5. TONIC partnered with the Nanomedicine Alliance in Washington, DC to organize, conduct and present at a two-day symposium in Rockville, MD on 6-7 March 2013 on "Nanomedicines: Charting a Roadmap to Commercialization." This meeting was well attended by both academia and industry participants. Other meetings and presentations organized by TONIC to educate pharma and enhance awareness of the nanotechnology platform opportunities in developing cancer solutions have included a mini-symposium on "Nanotechnology Platform-Based Biomarker Assays" at the 2013 National Biotechnology Conference (May 20-22, 2013, in San Diego, California) and presentations at the 2013 Annual World Pharma Congress on "Development of Difficult-to-Deliver Drugs: Driving Innovation Through Effective Tools and Novel Drug Delivery Strategies" (June 4-6 2013, in Philadelphia, PA). TONIC also helped organize a Commercialization Panel at the 2013 Alliance Investigators' Meeting, intended to educate young investigators on industry practices. The hope is that a new generation of researchers will take best practices from industry into their labs for technology

development and begin seriously evaluating external opportunities and platforms, including due diligence and appropriate models for the partnering process.

Figure 21. TONIC corporate and foundation members.

Chapter 5 Alliance Network and Resources

The goal of all Alliance efforts is increased quality of cancer care through the use of nanotechnology. The Alliance was established as a network of awards, with the second phase of funding meant to create a cohort of participants engaged in nanotechnology development from discovery to clinical translation. Membership in the Network was intended to improve the efficiency of research and translation by giving access to the diverse and extensive suite of facilities, knowledge and expertise throughout the Alliance award sites. The hope was that the cost and time of development could be decreased by enabling Alliance researchers to leverage Network resources, and that the quality of research would be improved by regular meetings and interactions between investigators who are experts in their diverse fields. It was also hoped that the Alliance Network would over time increasingly act as a resource for non-Alliance members as well and that the Alliance researchers would become increasingly active with other NCI networks and programs.

Centers for Cancer Nanotechnology Excellence

The largest portion of Alliance extramural funds, ~70%, goes to the nine Centers for Cancer Nanotechnology Excellence located across the country. This reflects the large size of the individual Centers, which receive on average $2.3M total costs per year to support four or five research projects, three research support cores, and Alliance Challenge and Pilot projects. The Alliance funds Centers because the breadth and depth of collaboration necessary for successful translation of multidisciplinary research is difficult, if not impossible, to sustain in a single project. Although Centers are expensive to maintain, we believe they are more effective at supporting integrated research with a common focus than multiple independent projects linked externally. Each Center fills a unique position within the Alliance, defined by their clinical, scientific or organizational emphasis. The individual Centers were introduced in Chapter 1.

The centers are expected to be highly integrated and interactive. For some centers integration arises from shared scientific interest, as with magnetic hyperthermia therapy at Dartmouth, liposomal drug delivery at Northeastern and cutting edge nanomaterials at MIT-Harvard. In other centers key technology is shared across projects, like the multi-stage vectors and targeting ligands in the Texas Center and the microfluidic chips and PET imaging technology studied at the NSBCC at Caltech/UCLA/ISB. Disparate technologies can also be brought together by unified characterization and validation, as is happening at Stanford, Northwestern, Johns Hopkins and UNC.

The Center at UNC is illustrative of how strong leadership, efficient administration and thoughtful organization can support a cohesive research and translation program in cancer nanotechnology that is highly interactive and goal driven while still allowing individual investigators freedom to pursue their own scientific interests and technology development. The Center has benefited from Joseph DeSimone's dedicated leadership, substantial institutional commitment and strategic planning by UNC and the Lineberger Comprehensive Cancer Center. Based on the initial success of the Carolina Center for Cancer Nanotechnology Excellence in Phase 1 of the program, the investments by NCI have been leveraged to secure an additional $6M of state money from the University Cancer Research Fund at Lineberger to establish the Carolina Center for Nanomedicine, also led by DeSimone. Additionally, UNC has also leveraged its Alliance involvement through the recruitment of Platform PI Alexander Kabanov from the University of Nebraska Medical Center to direct the Center for Nanotechnology in Drug Delivery at the Eshelman School of Pharmacy.

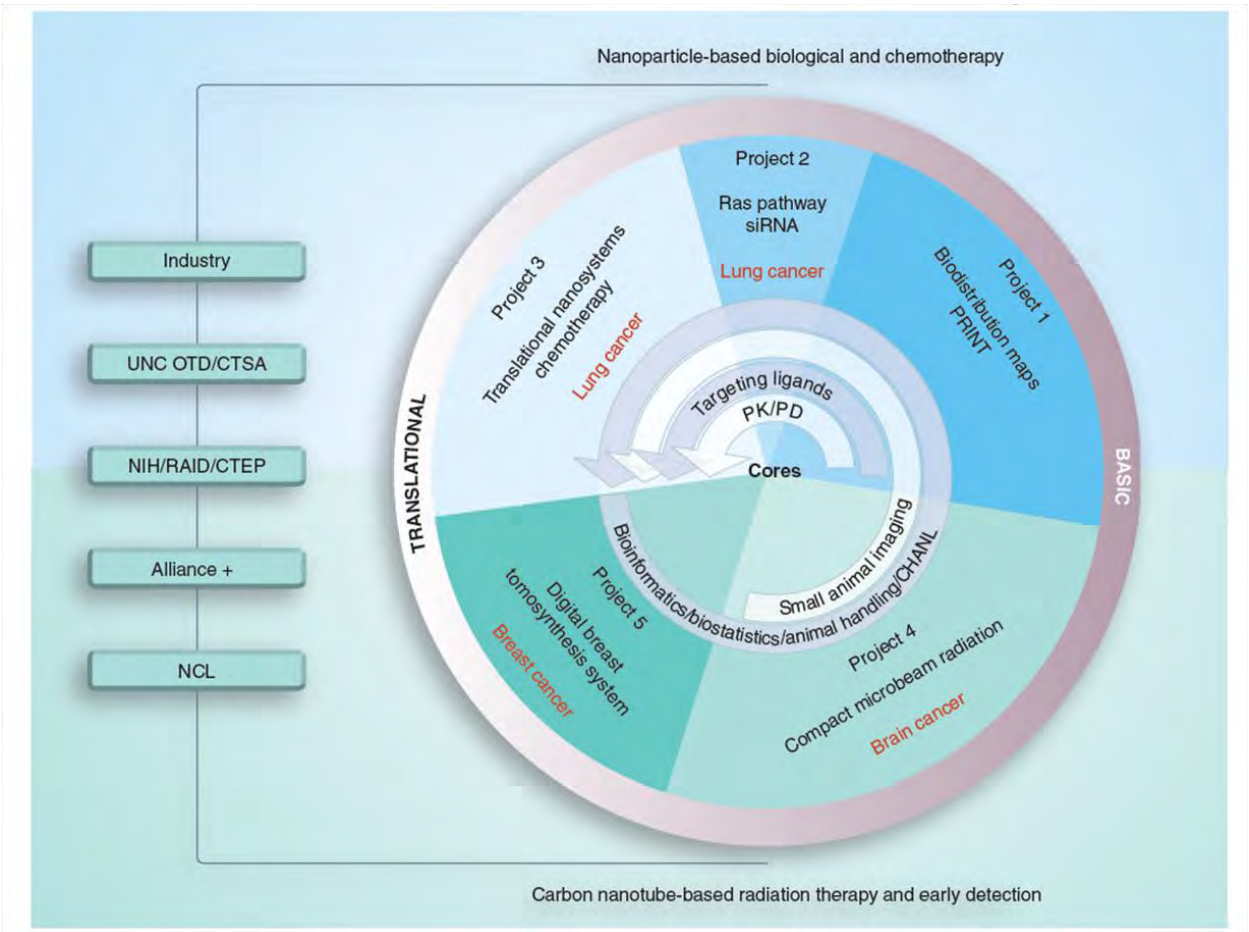


Figure 22. Model of interactions and programmatic strategy at the Carolina Center of Cancer Nanotechnology Excellence. Image courtesy of the Center.

A graphical representation of the UNC Center's structure and the ongoing interactions between center components and internal and external collaborators is given in Figure 22. Projects and cores in this Center are tightly integrated. Three projects collaborate with the targeting ligand core to design, produce, and test which types of targeting ligands (some of them being novel approaches such as multi-specificity ligands to more than one biomarker and heterodimeric ligands to signaling receptors) are most efficient for facilitating tumor cell uptake. The PIs of projects then incorporate the knowledge gained from the targeting core to build their respective particle types. Resulting nanoformulations are then tested *in vitro* for efficacy in the target tumor type. The Analytical and Pharmacokinetics (PK) Core conducts studies for each particle type to analyze tumor versus other tissue accumulation, particle clearance, half-life, etc. The shared development and characterization approach for these three projects enables direct comparison of platform performance and enables informed go/no go decisions similar to what is seen in the pharmaceutical industry. The Lineberger Cancer Center has seen the power of this approach and has dedicated $1M in funds to the top performing platform for preparative studies towards an FDA IND submission. Since joining the faculty of UNC, Alexander Kabanov has submitted his polyoxazoline micelle formulations for consideration in this nano-formulation "bake-off."

Lineberger also provides matching funds for the Alliance Challenge Project initiative, enabling an exceptionally high degree of Network collaboration at the Carolina Center, which participates in 13

Challenge projects. Center investigators are very active in Alliance working groups, with Russell Mumper acting as chair of the Bio-targeting Working Group during preparation of the perspective piece published early in 2013 (Goldberg et al., 2013) and William Zamboni acting as chair of the Animal Models Working Group and a regular contributor to Alliance workshops and publications (Zamboni et al., 2012, Prabhakar et al., 2013). Out of necessity, Russell Mumper has developed a reproducible strategy (94% success rate) to seed orthotopic non-small cell lung carcinoma xenografts into mice due to the inability to get published protocols from other groups to work well. He is now sharing this knowledge with other Alliance researchers working on lung cancer models.

Alliance Activities

TONIC EPR Workshop

The field of nanomedicine, is only now transitioning some of its developments from academic research to drug development and commercialization. To bolster this transition, the Program Office polled the industrial members of TONIC about what they believed were the most pressing issues in nanomedicine drug development. A consensus emerged that an incomplete understanding of the EPR effect, (which is believed to be responsible for nanoparticle deposition into tumors) in human tumors could become a major hurdle for cancer nanomedicine. The unique structural features of many solid tumors, including hypervasculature, defective vascular architecture, and impaired lymphatic drainage, are generally considered key factors in controlling nanoparticle delivery. However, the EPR effect has been measured mostly, if not exclusively, in implanted tumors in animals with limited data on EPR in metastatic lesions, making clinical relevance of the findings uncertain. Furthermore, tumor response alone is no longer considered a good endpoint for clinical trials, at least from the health authority point of view, as exemplified by FDA's withdrawal of approval for bevacizumab (Avastin) for patients with metastatic breast cancer, where impressive tumor responses did not correlate with improvement in overall survival. Limitations and challenges both in understanding tumor structural features and technology to probe them must be addressed and additional critical data generated before nanotechnology-based drug delivery approaches can be fully realized in clinical use in patients with cancer.

To begin a dialogue about nanomedicine and EPR, a one-day workshop was convened at the NIH on October 10, 2012, jointly organized by the Alliance and TONIC, to specifically address key issues related to understanding the EPR effect and its use to achieve the maximum therapeutic effect of drugs using nanoparticle carriers. Workshop attendees are shown in Figure 23. The main purpose of this meeting was to gain better understanding of the EPR characteristics affecting the use of nanoparticles in the clinic. Experimental evidence of EPR in animal models and humans, clinical relevance of EPR, gaps in knowledge, and ways to address these gaps were all discussed. The workshop was composed of eight talks by experts in the field, covering topics ranging from methods to investigate EPR in preclinical and clinical studies, such as diagnostic imaging, to the ramifications of EPR for enhanced drug uptake by different tumors and the predictability of preclinical and clinical outcomes. Following the workshop, a report was published in Cancer Research (Prabhakar et al., 2013). TONIC members also formed a nanodrug working group to work on clinical proposals/work ideas for effective imaging approaches to study EPR activity in patients. The working group has also recognized and is addressing the fundamental limitations and gaps in preclinical tumor models in recapitulating characteristics of solid tumors in patients. It is expected that data from the clinical trial and accompanying preclinical animal models will serve as seminal studies for the nanotechnology platform.

Figure 23. Participants in the Alliance hosted TONIC Workshop on EPR, NIH Campus October 2012.

Informatics

Biomedical nanotechnology as a whole is a field replete with multidisciplinarity. While enabling innovative discovery science is often a boon at the local level, this variety can hinder data transfer, even between collaborating groups. Progress in the field has been impeded by the lack of a knowledge-management infrastructure and associated standards to describe the complexity and heterogeneity of nanoparticles. Nanoparticle-based vehicles come in a wide variety of physical structures and chemical compositions, each aspect with an inherent distribution profile. This heterogeneity complicates characterization of nanoparticle effects on living organisms. Providing researchers with access to nanoparticle characterization methodologies and data, especially from *in vivo* experimentation, should expedite the use of nanoparticles in biomedicine. Informatics is an essential component of the nanotechnology data-sharing process, as it encompasses both terminology standardization and data management. This promotes interdisciplinary communication, allows data and protocol storage, and facilitates search, retrieval and modeling of data output.

To enable biomedical nanotechnology data sharing, the NCI Alliance supports two web portals, caNanoLab and the Nanomaterial Registry. The goal of these efforts is to provide resources where primary nanotechnology research data are no longer separate systems only affiliated with their originators, but standardized and shared across the scientific and clinical community. caNanoLab is a repository exclusively dedicated to cancer nanotechnology. It was designed as a resource with the potential to be interoperable with other nanotechnology, pre-clinical, clinical, and imaging resources. The Nanomaterial Registry has a broader mission to become a central tool for the various nanomaterial

stakeholder groups. The Registry has thus far collated data from biomedical, occupational health, manufacturing, regulatory, environmental, and standards-centric sources.

caNanoLab

caNanoLab is a web-based portal and data repository that allows researchers to submit and retrieve information on nanoparticles including their composition, function (e.g. therapeutic, targeting, diagnostic imaging), physical (e.g. size, molecular weight) and *in vitro* (e.g. cytotoxicity, immunotoxicity) experimental characterization, along with information on the protocols used for these characterizations and links to any related publications. While the majority of caNanoLab data has been entered through a contracted curation effort, web-based forms are available to facilitate data submission by data producers and users. Submitters can customize the visibility of their data to range from private, to organized collaboration groups, to fully public. caNanoLab can also be used for discovery purposes by searching the results of all the publicly available physical and *in vitro* characterizations as well as providing access to the associated publications. In addition to obtaining web-form-based query results, researchers are also able to download reports in a spreadsheet-based format.

The program office supports an ongoing data curation project to expand the dataset in the caNanoLab repository by applying standardized data elements and vocabularies to published nanoparticle data. The NCI instance of caNanoLab currently has 1,011 unique samples (including 17 different types of nanomaterial), 1,086 indexed publications, and 37 protocols in the public domain. Curation of data from publications requires significant effort and domain expertise, and it is almost always necessary to contact the authors for additional information. This is a very time consuming and inefficient process, as the papers are not written with interchangeable data storage in mind. Peer-reviewed journal articles often do not include sufficient information for a detailed understanding of the experiments described therein. This manual, expert-driven approach to information extraction has, however, led to the creation of a high quality and readily searchable repository of computable nanoparticle characterization data suitable to support the structure-activity relationship analysis that is a current focus of many nanotechnology informaticians.

Recently, members of program staff have expanded outreach efforts to publicize caNanoLab's upload feature using social media and journal comment pages. Greater relevance of the database is being achieved by encouraging journal editors to push caNanoLab as an outlet for detailed data sharing upon publication. Additionally, a polling strategy is being developed to gain insight into the profiles of caNanoLab users. This will better identify who is currently using the site and groups that should be targeted for future outreach efforts. Currently, the caNanoLab website averages 80-120 unique visitors per month.

caNanoLab software is also available for download and installation at local institutions. It is open source and the code is being deposited to the National Cancer Informatics Program (NCIP) channel in the GitHub code repository to support open, community led development. The goal is to encourage both end-users and developers to coalesce around caNanoLab to add new features and functionality. Going forward, the caNanoLab team aims to emphasize policies and resources that promote and incentivize standards-based data capture directly by the data producers. The team is also working together with the ISA data management community to extend the ISA Tools software suite to support the nanotechnology data extensions to ISA-TAB (ISA-TAB-Nano), an alternative standardized data architecture to caNanoLab (Thomas et al., 2013). The caNanoLab team will continue to promote data sharing in the nanotechnology community. In the future, the caNanoLab team will be participating in inter-agency collaborations through

the NCIP Nano Working Group towards the identification of information needed for obtaining regulatory approval on the use of nanotechnology in biomedicine.

The Nanomaterial Registry

The program office shares funding and management responsibilities of the Nanomaterial Registry with the National Institute of Biomedical Imaging and Bioengineering (NIBIB) and the National Institute of Environmental Health and Safety (NIEHS). The Registry has been developed and engineered under a contract to RTI International with broad stakeholder involvement from industry, academia and government. Registry partners are shown in Figure 24. In addition to quarterly reporting to the NIH leadership team, the Registry group also annually presents to its Advisory Board composed of leading scientists from National Labs, US regulatory agencies, and academia.

Figure 24. Government, industry and academic institutions involved in the Nanomaterial Registry project.

The goal of the Nanomaterial Registry is to become the definitive cross-disciplinary resource for nanoparticle characterization data for health, toxicity, and industrial concerns. It draws inputs from existing curated databases, including caNanoLab, and currently includes over 1,300 particle entries. Entries are populated on the web portal through curated data extraction using a Minimal Information About Nanomaterials (MIAN) characterization vocabulary architecture. MIANs capture the physico-chemical characteristics, biological interactions, and environmental interactions of the given particle. This homogenized vocabulary enables searches and comparisons based on MIAN similarity.

The curation process for the Nanomaterial Registry has advanced to include some automated data entry abilities. This has been facilitated by the development of a proprietary Registry Curation Tool that allows the curation team to easily update or retire a record as needed. The Tool also has several 'smart' features that pre-populate dropdown fields with relevant values based on data entered in previous fields. Collectively, these upgrades reduce the instances of free text entry, the time of curation, and content-

specific data entry errors. Furthermore, this has accelerated the pace of particle curation significantly. The average time to enter an individual data record and for it to undergo thorough quality control is 38 minutes/record.

The diversity of fields and the MIAN-enabled functionality on the Registry website has drawn broad international interest and use of the site. Using Google Analytics, which tracks visits to the site, has shown that the Registry repeatedly receives visits from dozens of different countries. Quarterly statistics show dramatic growth in site visits (1,852, an increase of 135% over the last reporting period), unique visitors (911, +85%), pages viewed per visit (4.61, +21%), and in time spent using the Registry per visit (7'26", +86%) over the past year. Interestingly the percentage of new visitors has declined slightly, possibly indicating a dedicated population of users. These high levels and increases in users are tied to multiple outreach efforts by the Registry staff. Over the past year representatives from the Registry group have presented posters and/or platform talks at nine national and international meetings. They have also participated in multiple regulatory standards ISO TC 229 Plenary Meetings and have produced a webinar for Greener Nano. Each of these outreach activities have tracked well with spikes in website visits and usage demonstrating the effectiveness of these efforts.

Now that the Nanomaterial Registry's site is up and running with expanding usership, the focus for the future is to build from this strong foundation. It is expected that over the next year the curation team will enter an additional 1,200 nanomaterials and reach a total of 3,000-4,000 new unique users. The Registry team will also work with the Purdue University Network for Computational Nanotechnology nanoHUB to integrate existing modeling tools to the MIAN-based database toward predictive applications of the growing body of characterization data.

National Nanotechnology Initiative Signature Initiatives

The program office participates in National Nanotechnology Initiative Signature Initiatives, intended to accelerate development in areas with the potential for significant advances to be made through close inter-agency collaborations. Advances in these areas are expected to serve the economic, security and environmental needs of the nation. Program staff played key roles in the National Knowledge Infrastructure and Nanotechnology for Sensors Initiatives. The Knowledge Infrastructure Initiative coordinates the federal community around collaborative modeling, a cyber-toolbox and data infrastructure. Through this initiative, program staff promotes wider acceptance and support for Alliance supported informatics efforts like caNanoLab and the Nanomaterials Registry. The Nanosensors Initiative brings together federal agencies with research, regulatory and defense missions to shorten the development time for inexpensive, portable devices to rapidly detect, identify and quantify biological and chemical substances for health and environmental monitoring. The initiative is also seeking to drive development of strategies for detection of nanomaterials in the environment and *in vivo*. Participation in this initiative has increased interaction between the program office and the FDA Center for Devices and Radiological Health, which has regulatory authority over nanotechnology based sensors for health and *in vitro* diagnostics.

Network Interactions

Working Groups

The Program office coordinates eight working groups in areas of scientific and practical importance to Alliance researchers:

- Biotargeting
- Genetic Therapy/RNA Interference
- *In Vitro* Diagnostics and Cancer Detection
- Imaging
- Nanoformulation and Nanosynthesis
- Animal Models
- Nanoparticle Biodistribution
- Communication and Integration

Each working group is chaired by one or two volunteer Alliance members. The chairs moderate the working group meetings and may consult with the Program office coordinator on the agenda and format. Levels of participation and output vary across the working groups, although most groups hold regular teleconferences to discuss topics of emerging and shared interest to the group. The most engaged groups, Biotargeting and Imaging, have prepared and published manuscripts reflecting the combined experience and insight of their members. Others, like the Animal Models and Nanoformulation and Nanosynthesis groups, are seeking consensus on appropriate models and minimum information standards for research and publications in the field, with the intention of sharing these standards across the Alliance. These efforts are also helping the program office to understand and define needs for research resources and data infrastructure. This input informs program participation in federal initiatives and cross-agency activities. Representatives from NCL also serve on the working groups when appropriate.

The Communication and Integration Working Group shares best practices in award management and functions as a medium through which the program office can communicate NCI policies to the investigators and award administrators and provide support in fulfilling reporting and administrative requirements. The group also gathers information and images for the quarterly Alliance newsletter, annual Bulletin, Alliance Calendar and image gallery. The cover image for the 2012 Bulletin, submitted by the Platform award at New Mexico, is shown in Figure 25.

One of the most active working groups is the Biotargeting Working Group, which jointly authored and published the perspective piece “Biotargeted nanomedicines: Six tenets before you begin,” in early 2013 (Goldberg et al., 2013). The piece addresses the biological and translational difficulties which must be navigated for successful market approval of targeted nanomedicines. One of the major biological challenges identified by the working group was choosing an effective membrane protein to target with an appropriate targeting moiety, the latter of which must be simultaneously selective and internalizing. An additional challenge is in developing a strategy for overcoming the multiple physiological barriers nanomedicines face in transit from circulation to the interstitial tumor space, to the plasma membrane and into the appropriate organelle. The perspective highlights the necessity to choose and design appropriate materials particularly with respect to surface properties and interaction with biological surfaces to maximize success. In keeping with the translational focus of the Alliance program, issues of manufacturing, including quality control and scale-up, regulatory review, and cost-effectiveness were addressed in the working group’s analysis.

The Imaging Working Group took on a similar task of reviewing the current status of nanoparticle-based cancer imaging and identifying the major challenges that have prevented successful clinical translation of numerous nanoparticle contrast agents (Chapman et al., 2013). This paper also addresses regulatory considerations, particularly important for imaging agents, which may face a higher regulatory scrutiny than therapeutics, due to the differing risk-benefit calculations for the two uses. The paper highlights opportunities in the area, while acknowledging the difficulties that existing nanoparticle contrast agents

like Feridex and Combidex have had in securing regulatory approval and market acceptance. The consensus of the group is that the greatest opportunity lies in the multifunctional capabilities of nanoparticle imaging, particularly for multi-modal imaging and the simultaneous collection of complementary images over space and time.

Figure 25. Image of protocell undergoing multivalent binding to and endocytosis into a cell. See Chapter 3. Image courtesy Mona Aragon, Carlee Ashley, Ph.D., and Jeffrey Brinker, Ph.D., New Mexico Platform

Annual Alliance Principal Investigators' Meeting

The Alliance hosts a yearly Principle Investigators' (PI) Meeting. The meetings are two and half days of primarily scientific talks given by investigators, some of whom are invited by the program office to give plenary presentations and some of whom are nominated by their award's PI(s). This allows for the fluid sharing of knowledge between the members of the Network as well as the opportunity for the researchers to meet with others in the Network face to face annually. Each year, the program office directs a theme to this meeting, to steer presentations and discussion towards areas of high priority to the program. The Kick-Off meeting, in November 2010, allowed PIs to provide summary presentations of the goals of their project(s) and members of the Network to meet each other. The second meeting, hosted in September 2011 by the MIT-Harvard and Northeastern Centers, introduced parallel discussion sessions dedicated to areas of cancer nanotechnology that were currently underserved by the research community. The third

meeting, held in November 2012 at the Texas Center included a panel of cancer survivors sharing their personal journeys through cancer treatment with the researchers. The Texas meeting also highlighted presentations from junior members of the Alliance and emphasized resource sharing across the Network, with a session dedicated to showcasing available resources. The 2013 PI meeting was held on the NIH main campus to provide an opportunity for NIH researchers and program staff to become better acquainted with the world of cancer nanotechnology and vice versa. The meetings typically host about 200 attendees, mostly Alliance investigators with an increasing presence from the pharmaceutical industry in the years since the inception of the TONIC consortium.

In addition to providing an outlet for sharing research results, the meetings are a unique setting for Alliance members to learn about and discuss other aspects of the field. There are typically tutorials for graduate students and postdoctoral fellows to learn more about specific areas of research at the host institution as well as information sessions about careers in the cancer nanotechnology field. There were plenary sessions organized around clinical applications of Alliance research during the 2011 and 2013 Meetings. NCL staff has led informational sessions and Lessons Learned Workshops to share the services offered by the NCL and the knowledge they have gained with the community. There have been informational sessions from FDA representatives about the regulatory process of approval and the current approach of the FDA towards nanotechnology based materials and drugs. The meetings have provided a venue for the working groups formed within the Alliance to meet in person and have presentations specific to their topics. The Annual Meetings have also served as opportunities for industry representatives to interact with Alliance investigators. The program office has organized panel discussions featuring industrial partners engaged in successful collaborations with Alliance members. The discussions have covered multiple facets of the commercialization process, from raising funds to establishing market viability. Since TONIC's inception, representatives of industry members have been invited to the Meetings to listen to presentations and meet individual researchers to discuss potential collaborations. A working lunch or dinner for TONIC members and invited Alliance investigators is also typically part of the Meeting agenda.

Each meeting also hosts the various guiding committees that steer the future course of the Alliance. First and foremost among these is the Coordination and Governance Committee (CGC) meeting. The CGC is composed of the NCI program office, the PIs of each Center, and rotating representation from the Platforms and Training Centers. At a given time, there are 15 investigators on the committee. The committee meets twice a year in person – at the PI meeting in the Autumn and at the AACR meeting in the Spring. The responsibilities of the CGC were set in the Alliance RFAs and include developing rules for the Alliance Challenge and Pilot Projects, determining rules related to information sharing, assessing opportunities for collaborations within the network as well as outside of the network, developing the format of and deciding priority areas for PI meetings, and assessing overall progress of the field and the program. The Alliance Clinical Committee is composed of leading physician scientists tasked with helping Alliance researchers identify goals and evaluate strategies, particularly clinical testing plans, for clinical translation of technologies under development within the program. The majority of members are funded program investigators. This committee is also active in shaping the translational agenda of the PI meeting and at times interacts with the TONIC Consortium. Committee members meet at the PI meeting and participate in ad hoc teleconferences. The membership of this committee is as follows:

- *Dr. Nahum Goldberg*, Professor of Radiology, Harvard Medical School
- *Dr. Steven K. Libutti*, Director, Center for Cancer Care, Vice-Chairman, Department of Surgery, Albert Einstein College of Medicine
- *Dr. Julia Y. Ljubimova*, Professor of Neurosurgery and Biomedical Sciences, Director of Nanomedicine Research Center, Cedars-Sinai Medical Center

- *Dr. Gabriel Lopez-Berestein*, Professor and Chief, Immunobiology and Drug Carriers Section in the Department of Bioimmunotherapy, M.D. Anderson Cancer Center
- *Dr. Steven Rosen*, Genevieve Teuton Professor of Medicine, Feinberg School of Medicine, Director of the Robert H. Lurie Comprehensive Cancer Center, Northwestern University, to become Provost/Chief Scientific Officer, City of Hope in March 2014
- *Dr. David Sidransky*, Professor, Otolaryngology—Head and Neck Surgery, Oncology, Pathology, Urology, and Cellular and Molecular Medicine, Johns Hopkins University.
- *Dr. Joel Tepper*, Professor of Radiation Oncology, University of North Carolina at Chapel Hill

The Alliance also had an Industrial Committee whose mission was to provide guidance on the commercialization of cancer nanotechnology. It was later determined by the program office that the Alliance researchers and their local intellectual property officers performed this task admirably and this committee was replaced by an Alliance Steering Committee in late 2012. The Alliance Steering Committee has a broader charge of providing oversight and perspective for all aspects (commercial, clinical and scientific) of strategic planning. The program office holds two to three teleconferences per year with the members of the Steering Committee, who are also encouraged to participate in the PI Meeting. The committee provides input on on-going program performance; strategies for clinical translation; collaborations within the network and between the network and foundations, pharmaceutical companies and the wider research community; strategies for educating/training the community in cancer nanotechnology; and future scientific direction and program composition from the stand-point of balancing basic and translational research. Additionally, this year committee members helped in developing the agenda for the Strategic Workshop in Cancer Nanotechnology in June 2013 and helped in securing a session on cancer nanotechnology at the upcoming American Society of Clinical Oncology (ASCO) meeting in 2014. The Committee members are:

- ***Dr. Neil Desai*** is Vice President of Strategic Platforms at Abraxis Bioscience, which is a wholly owned subsidiary of Celgene Corp. Prior to Celgene, Desai was with Abraxis Bioscience, where he invented a nanoparticle albumin bound (nabTM) drug delivery platform and was primarily responsible for the development of its nanotechnology drug, Abraxane, which was approved by FDA in 2005. Abraxane is currently used for the treatment of metastatic breast cancer, lung cancer, and pancreatic cancer.
- ***Dr. David Housman*** is a Ludwig Professor of Biology and a member of the Koch Institute for Integrative Cancer Research at MIT. His laboratory studies the genetic underpinnings of human disease and seeks to establish treatment strategies based on molecular targets for numerous diseases. He is interested in three major disease areas: trinucleotide repeat disorders particularly Huntington's disease (HD), cancer (glioblastoma and melanoma) and cardiovascular disease. Dr. Housman has co-founded a number of biotechnology companies and is a member of both the National Academy of Sciences and the Institute of Medicine.
- ***Dr. Larry Norton*** is Deputy Physician-in-Chief for Breast Cancer Programs and Medical Director at the Evelyn H. Lauder Breast Center of Memorial Sloan Kettering Cancer Center. His research has focused on anti-cancer medicines, particularly the application of mathematical methods to optimizing dose and schedule. He co-invented the Norton-Simon Model of cancer growth, and more recently the self-seeding concept of cancer metastasis and growth. He was a U.S. Presidential appointee to the National Cancer Advisory Board
- ***Dr. Roger Tsien*** is a Professor of Chemistry and Biochemistry at the University of California San Diego. He has been studying signaling inside individual living cells, in neuronal networks, and in tumors through the design and use of new molecules that detect or manipulate biochemical signals. Currently, his research is focused on using U-shaped peptides to image tumor margins and to provide real-time monitoring tools in surgery. Tsien received the Nobel Prize in Chemistry in 2008 for the discovery, expression and development of green fluorescent protein. He is a member of Institute of Medicine and National Academy of Sciences.

Alliance Challenge Projects

The Center and Platform awards contain restricted funds intended to pay for collaborative projects with other Alliance members. In some cases, the program office has approved use of these funds for projects with external partners, when a particularly valuable opportunity to expand the reach or value of Alliance technology is afforded. There have been three Rounds of Challenge Projects in Phase 2 of the program. The program has evolved over time, with increasing numbers of external partners being brought into the Network. Training Centers have participated in Challenge projects, although there is no requirement that they do so. The third round of projects included the option of using Challenge Project funds to scale up the production of GMP nanomaterials in order to begin a partnership with NCL. Dartmouth has chosen this route in Round Three. Figure 26 overlays the Challenge Project collaboration networks on the Alliance map.

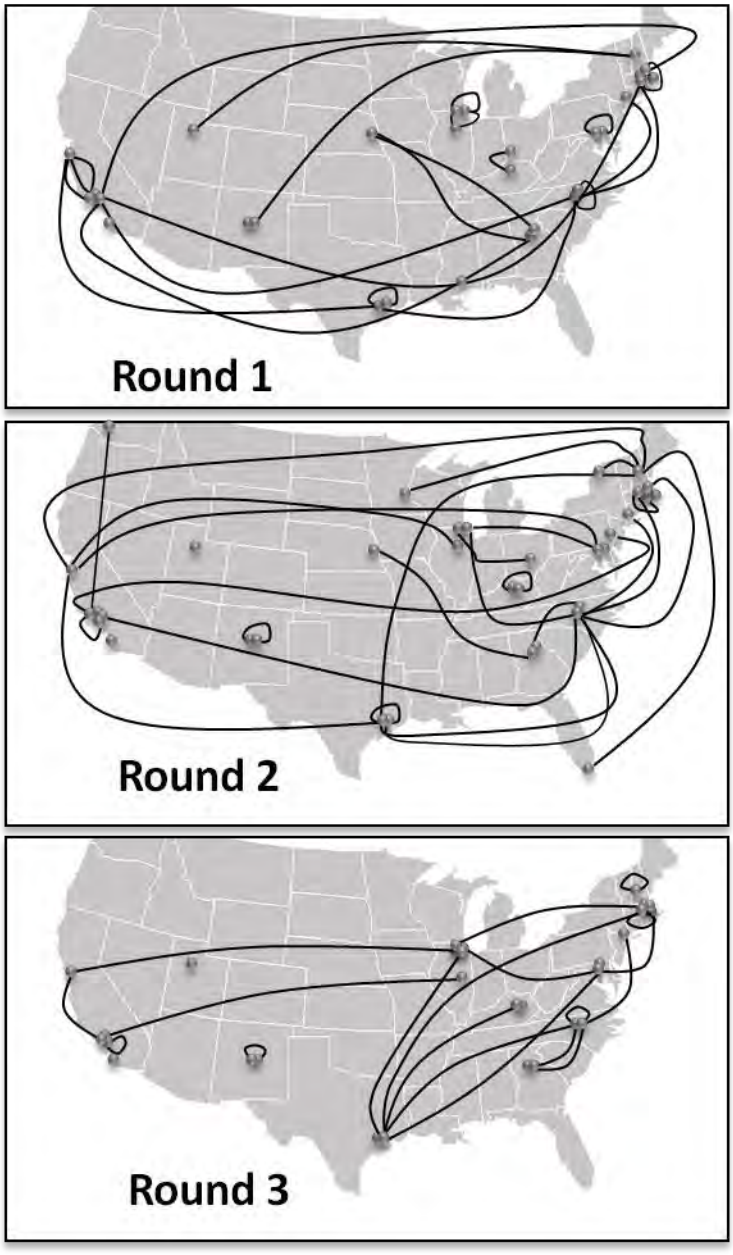


Figure 26. Maps of the Challenge Projects. Round 1 had 20 projects, Round 2 had 26 projects and Round 3 had 22 projects.

One example of a successful collaboration is the work detailed in a recent publication by Paula Hammond and Joseph Desimone from the MIT and UNC Centers' Challenge Project "PRINT and Spray: Building Functionalized Nanoparticles for Cancer," which details how technologies from each institute were combined to create built-to-order nanoparticles systems by spray coating PRINT particles to generate targeted nanoparticles (Morton et al., 2013). The PRINT technology has been at the center of many of UNC's efforts, and by incorporating the spray-assisted Layer-by-Layer deposition technology developed at MIT this collaboration was able to extend the attainable parameters of nanoparticle composition and surface functionality in a highly reproducible manner that neither program had been able to achieve previously.

The Challenge projects funded have been creative, and the initiative overall has been successful at promoting interactions and increasing technology and resource sharing across the Alliance. However, the initiative has met with uneven enthusiasm across the Alliance. Some sites, such as the UNC and Texas Centers, have embraced the

initiative and formed numerous collaborations, while others have been reluctant and have reported feeling compelled to seek collaborations of low intrinsic interest to them. UNC's strong interest may be related to the existence of external matching funds from the Lineberger Center for the projects. The initiative has not generated many publications, although this may be linked to the short time since the inception of the Challenge projects. There is also a relatively low level of discovery research within the Challenge Projects, compared to projects which focus on technology and resource sharing.

Challenge Projects are competitively decided upon within the institutions before they are submitted to the program office for approval and disbursement of funds, but the competition is for existing funds within the award. This lack of new funds may dampen enthusiasm for the initiative, along with a sense that the collaborations are compelled rather than arising naturally from the researchers. A competitive mechanism to award additional funding, perhaps in more targeted areas, may increase interest and drive innovative uses of Alliance technologies. The program office is investigating mechanisms to promote collaborations across the Network and with other NCI programs that may more effectively support higher risk research.

Interactions Across NCI

Office of Cancer Genomics

The Cancer Target Discovery and Development (CTD^2) Network (http://ocg.cancer.gov/programs/ctd2) in the NCI Office of Cancer Genomics promotes translation of genomic characterization data into cancer therapeutics. The program aims to extract therapeutic targets and diagnostic, prognostic and drug response markers from large genomic data sets like The Cancer Genome Atlas (TCGA), Therapeutically Applicable Research to Generate Effective Treatments (TARGET) and the Cancer Genomic Characterization Initiative (CGCI). A network of highly collaborative centers has been established for this purpose, and all data generated by the centers is released to the broader research community.

Collaborations between Alliance and CTD^2 researchers have emerged organically from existing relationships and at institutions hosting centers from both networks, as CTD^2 members seek technologies to validate and test their extracted targets. The joint efforts of Alliance member Sangeeta Bhatia and CTD^2 member William Hahn are discussed in some detail in the "Integrating Nanotechnology and Cancer Biology" section of Chapter 3.

Recognizing the potentially high impact of joint projects between the Alliance and CTD^2 programs, the two offices began to lay the groundwork to support collaborations between the two networks. Daniela Gerhard, Director of the Office of Cancer Genomics, regularly attends Alliance PI Meetings, and at the 2012 meeting in Houston she gave a presentation on datasets and resources available to Alliance researchers. She also attended the Alliance CGC Meeting to discuss ideas for collaborative efforts between the two programs. Alliance staff also attended the annual CTD^2 meeting in 2013, and office Director Piotr Grodzinski met with the CTD^2 steering committee to continue discussions on potential collaborations. Given the strong interest in collaboration by members of both networks, the Alliance office was able to obtain funds for administrative supplements to Alliance awards in fiscal year 2013 to support collaboration between Alliance and CTD^2 researchers. The goal of the supplement was to accelerate translation by funding the development of nanotechnology-based platforms for the *in vivo* delivery and validation of cancer therapeutics designed for these novel, functionally annotated genomic targets.

There was strong response to the supplement call, with ten applications received despite a period of less than one month between the supplement's announcement and due dates. A committee comprised of Alliance program staff and scientific experts from FNLCR reviewed the proposal and recommended three

awards. Most promising to note is that the three highly rated proposals pursue distinct therapy strategies, suggesting a broad applicability for nanoparticle therapeutics in this area. Sangeeta Bhatia and William Hahn will use nanoparticle delivery of siRNA to credential a potential target gene for ovarian cancer in *in vitro* and *in vivo* studies. Anil Sood and Gabriel Lopez-Berestein will collaborate with Michael White of the UT Southwestern Medical Center to determine the contribution of key miRNAs to tumor lethality and validate the efficacy of nanoparticle delivery of miRNA. Lily Yang and Hui Mao will team up with Haian Fu, also of Emory, to deliver a peptide antagonist to KRas and inhibit KRas-Raf interactions implicated in pancreatic cancer progression.

The program office noted that the enthusiasm for this supplement and the quality of applications seemed high compared to that found with the Alliance Challenge Project initiative. This suggests that competitive and focused calls for collaboration may be more effective at boosting collaborative discovery research than an obligation to use set-aside funds for projects in unspecified areas.

Division of Cancer Therapeutics and Diagnosis

The program office has collaborated with the Division of Cancer Therapeutics and Diagnosis (DCTD) in NCI to encourage the use of nanotechnology to improve delivery of promising chemotherapy candidates which encounter serious formulation problems, such as insolubility, poor PK, or instability. The Alliance sponsored a series of Requests for Proposals (RFP) soliciting contract offers under the heading “Highly Potent Nanotherapeutics” (solicitation numbers S10-038, S10-140, S11-108). Seven contracts were awarded in response to the RFPs, generally for early stage, proof-of-concept studies. For example, Aphios Corporation used its contract to perform *in vivo* studies of its Camposomes™ technology, a liposomal formulation of camptothecin, a highly potent but insoluble and unstable anti-cancer compound. Aphios continues to pursue development of Camposomes, building on the support for early animal studies from the contract. BIND Biosciences, an Alliance affiliated company that has since brought its lead candidate to clinical trials as discussed in Chapter 4, received funding for reformulation of bortezomib. A proeteasome inhibitor which has shown remarkable efficacy in treating refractory multiple myeloma, bortezomib is cleared very rapidly from circulation, limiting accumulation in solid tumors, a shortcoming that should be amenable to improvement through nanoformulation.

The program office has also been working with DCTD to develop additional mechanisms to support use of Alliance technology to reformulate failed drugs in the NCI inventory. The Deputy Director of DCTD, Joseph Tomaszewski, addressed the Spring 2012 meeting of the Alliance CGC to discuss agents and resources available from NExT/DCTD for reformulation. The Office is pursuing the possibility of preparing a contract or supplement solicitation through which Alliance researchers could use their vehicles to reformulate compounds designated as high interest by DCTD. These conversations are ongoing, and this is an area likely to remain of high priority to the Alliance.

SBIR

The program office works closely with the Small Business Innovation Research (SBIR) Office within NCI to provide support for small and start-up companies seeking to transfer their nanotechnology based platforms into the clinic or marketplace. Every year the Program Office participates in the preparation of the annual Omnibus NIH/CDC SBIR Contract Solicitation by developing one-time nanotechnology related topics, for example: Multifunctional Therapeutics and Theranostics Based on Nanotechnology (topic 285) Therapeutics and Theranostics Based on Nanotechnology (topic 300), “RNAi Cancer Therapeutics Using

Nanotechnology (topic 313).NCI SBIR staff regularly attend the Alliance PI Meeting and consult with Alliance Program staff on nanotechnology related SBIR applications and awards.

The program office also helped to prepare and served as Scientific Contact for the SBIR U43/U44 funding opportunity "Cancer Diagnostic and Therapeutic Agents Enabled by Nanotechnology" (PAR 10-286). This funding opportunity supports pre-clinical optimization and testing of nanotechnology applications for cancer indications. The end goal of SBIR financial support is to enable small companies to complete IND enabling studies for *in vivo* diagnostic and therapeutic agents. In addition, award recipients under PAR-10-286 are strongly encouraged to utilize NCL's technical services as well as regulatory knowledge. Alliance affiliated companies in general have done quite well in response to PAR 10-286, receiving two of the eight awards made in response to this call. Overall, ten companies associated with the Alliance received support from different SBIR initiatives. One Alliance affiliated NCI SBIR awardee, Nemucore Medical Innovations, is a clinical development start-up company originally supported through core funding from the Northeastern Center. Through PAR 10-286, the SBIR funding mechanism will support development of a targeted-delivery nanoparticle formulation of docetaxel. The company has garnered additional SBIR funding for image guided cancer interventions using an EGFR targeted nanoemulsion and has received an Academic-Industrial Partnership for Translation of *in vivo* Imaging Systems for Cancer Investigations R01 award. Nemucore regularly participates in Alliance meetings and industrial partnership activities, allowing them to further leverage the funding they receive from the Alliance to grow the company.

Nanotechnology Characterization Laboratory

The Nanotechnology Characterization Laboratory (NCL) is a formal interagency collaboration of NCI's Alliance for Nanotechnology in Cancer with the FDA and NIST. NCL was established in 2004 to facilitate preclinical characterization of nanomedicines and to accelerate the pace at which cancer-targeting nanomedicines get into clinical trials. Towards this goal, NCL has four main objectives:

- Characterize nanoparticles using standardized methods;
- Conduct structure activity relationships (SAR) studies to identify and characterize critical parameters related to nanomaterial ADME/Tox;
- Facilitate data supporting regulatory review of nanotech constructs;
- Engage in educational and knowledge sharing efforts.

NCL since its inception in 2004 has accumulated a significant knowledgebase about nanomedicines properties which is being shared with the scientific community through workshops, publications and access to databases.

Nanoparticle Characterization via Standardized Methods

The NCL has standardized more than 40 *in vitro* assays for nanomaterial characterization. These assays have been validated for a variety of nanomaterial types and undergo continual revision to ensure they meet FDA regulatory requirements. New assays are added every year, and are made freely available to the public via the NCL website (http://ncl.cancer.gov/working_assay-cascade.asp). Many of these assays have also been compiled into a recent methods book, *Characterization of nanoparticles intended for drug delivery* (McNeil, 2011). Figure 27 shows the breakdown by type of nanomaterials characterized by NCL.

The NCL's three-tiered Assay Cascade includes physicochemical, *in vitro*, and *in vivo* characterization. The NCL's physicochemical characterization of nanomaterials goes well beyond basic measurements of

size and surface charge. NCL's routine characterization also includes batch-to-batch consistency evaluation, measurement of drug loading, confirmation of targeting ligand conjugation, quantitation of surface ligands, and nanoformulation stability assessment. *In vitro* analysis includes sterility and endotoxin quantification, something many researchers often overlook, and analysis of hematological compatibility and immune cell functions using human whole blood. NCL's *in vivo* capabilities include toxicology, immunotoxicology, drug metabolism, pharmacokinetics, efficacy and imaging studies.

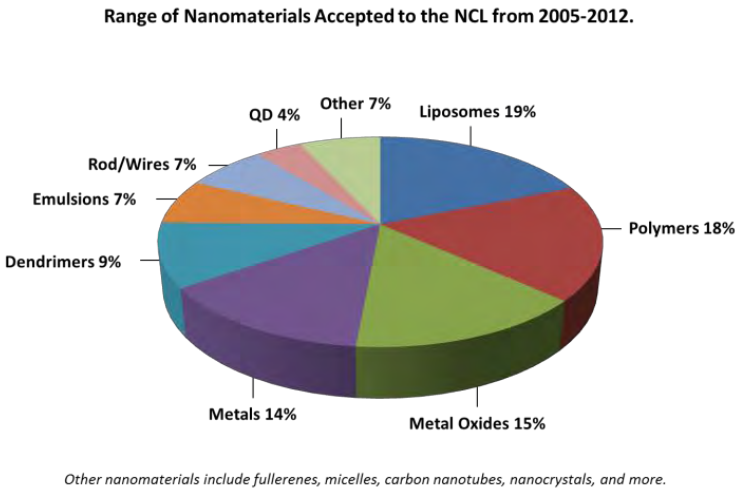
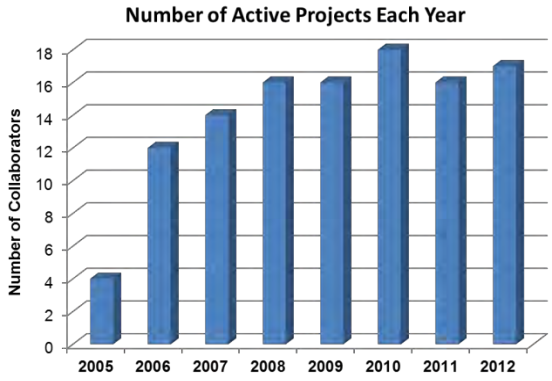


Figure 27. Types of nanomaterials characterized by NCL from 2007-2012. Image courtesy of NCL.

Since the NCL began accepting applications in 2005, it has characterized more than 300 different nanoparticles from nearly 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. NCL collaborations over time are shown in Figure 28. NCL works with investigators from all backgrounds, including academia, small biotech companies, large pharmaceutical companies, and independent investigators.

The NCL also routinely works with standards organizations such as ASTM International and ISO. Three NCL assays have been adapted as standards: ASTM protocol E2524-08, Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles; ASTM protocol E2525-08, Standard Test Method for Evaluation of the Effect of Nanoparticulate Materials on the Formation of Mouse Granulocyte-Macrophage Colonies; and ASTM protocol E2526-08, Standard Test Method for Evaluation of Cytotoxicity of Nanoparticulate Materials in Porcine Kidney Cells and Human Hepatocarcinoma Cells. In addition to developing and reviewing protocol standards for ASTM and ISO, the NCL also worked closely with NIST to develop the first ever nanoscale reference standards: RM8011, RM8012, and RM8013 for 10-, 30-, and 60-nm diameter gold nanoparticles, respectively.



	2007	2008	2009	2010	2011	2012
Nanoparticles Submitted (unique formulations)	37	40	34	48	27	32
Total Submitted Samples (multiple batches)	90	83	44	77	103	89
MTAs/CAs/CRADAs	11	15	19	12	14	7
Client Reports (confidential reports)	4	6	8	9	10	4
Publications (public documents)	5	10	8	10	32	12
Animal Studies	7	17	22	18	20	14

Figure 28. Left, number of active NCL projects by year, 2005-2012. Right, NCL nanomaterials submissions, transfer agreements, reports, publications, and animal studies, 2007-2012. Image courtesy of NCL.

Identification of Parameters Related to Nanomaterial ADME/Tox

In addition to the characterization projects performed for NCL's nanomaterial submitters, NCL also performs independent research projects and SAR studies directed at understanding the relationship between a nanoparticle's structure and its induced biological responses. NCL publishes the results of these studies to inform the nanotechnology 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.

In particular, NCL has conducted research into autophagy and lysosomal dysfunction as emerging mechanisms of nanomaterial toxicity and therapeutic efficacy. These studies have resulted in numerous publications over the years, culminating most recently in a review of the involvement of autophagy and lysosomal perturbation in nanomaterial toxicity (Stern et al., 2012). As a testament to the novelty and relevance of this work, this review was the highest accessed article published in 2012 in Particle and Fibre Toxicology, the oldest journal in existence dedicated to the study of nanoscale particle toxicology. Additionally, a research article evaluating autophagy dysfunction as a therapeutic mechanism underlying ceramide nanoliposome and vinblastine combination therapy was published in collaboration with The Pennsylvania State University Hershey Medical School (Adiseshaiah et al., 2013).

Another significant NCL SAR study has been aimed at mapping the physicochemical properties (e.g. size, charge, etc.) of dendrimers to their tendency to cause platelet aggregation and induce procoagulant activity in leukocytes. This work has been ongoing for several years now and has resulted in two publications to date (Dobrovolskaia et al., 2012a, Dobrovolskaia et al., 2012b).

NCL has also worked on numerous other studies. For example, NCL has developed and characterized several animal models for evaluating nanomaterial effects on the immune system, to include tests for T-dependent antigen response (TDAR), local lymph node assay (LLNA), local lymph node proliferation (LLNP), and adjuvanticity. NCL has also recently published a manuscript on the *in vitro-in vivo* correlations of immunotoxicity assays in an effort to assist researchers in finding predictable screening methods to detect nanoparticle immunotoxicities early in development, thereby enabling the engineering of a nanoparticle's physicochemical properties to decrease/eliminate their immunotoxicity (Dobrovolskaia and McNeil, 2013).

Facilitate Regulatory Review of Nanotechnology Constructs

NCL works closely with the FDA to ensure that NCL's activities are in line with the current regulatory requirements and interfaces with the FDA on a number of levels. Senior FDA personnel participate in NCL's Scientific Oversight Committee to review NCL-generated data to help ensure that NCL's assays capture important aspects of characterization relevant toregulatory submissions. NCL also interacts with FDA policymakers on national-level committees such as those sponsored by the National Nanotechnology Initiative. FDA and NCL also co-sponsor workshops, seminars, and meetings for standards development and characterization of nanomaterials.

Of NCL's nearly 100 collaborations, six NCL collaborators have obtained IND or IDE approvals from FDA. CytImmune Sciences and ProNAi Therapeutics are both in Phase II clinical trials. CytImmune's CYT-6091 therapy is being used for the treatment of solid tumors, and ProNAi's PNT2258 is being used to treat non-Hodgkin's lymphoma and other cancers. BIND Therapeutics' targeted nanotherapeutic BIND-014 has completed Phase I and is approved to begin Phase II clinical trials for prostate cancer. Azaya Therapeutics has completed its Phase I clinical trial with ATI-1123, for the treatment of solid tumors. Nanospectra Biosciences' AuroLase technology for the treatment of head and neck cancers is currently in Phase I clinical trials. PDS Biotechnology received IND approval in 2013 for PDS0101, a treatment for human papiloma virus and cervical cancers, and will begin a Phase I trial soon. NCL linked clinical trials are included in Appendix C.

NCL staff also actively collaborates and communicates with FDA scientists and reviewers to address specific scientific challenges in nanomedicine. NCL has worked with four FDA departments and completed six projects on a variety of nanomaterial concerns. NCL has worked with the Center of Device and Radiological Health studying the penetration of nanoscale titanium dioxide particles in rodent, pig and human skin, and studied the effects of sterilization procedures (mimicking those used in the sterilization of medical devices) on silver colloids. In collaboration with the Center for Drug Evaluation and Research, NCL has performed other dermal penetration studies of nanomaterials in sunscreens and cosmetics, as well as compared endotoxin levels in experimental nanomaterial products to levels in FDA-approved products. NCL has a longstanding collaboration with the Center for Food Safety and Applied Nutrition, recently completing a characterization and purification project of dendrimers for another dermal penetration study. Finally, NCL and the National Center for Toxicological Research have a collaboration to study immunological reactions to nanomaterials in non-human primates.

Educational and Knowledge Sharing Efforts

The NCL has published nearly 100 peer-reviewed manuscripts, top-level review articles, and book chapters. The NCL has also edited two books, *Characterization of Nanoparticles Intended for Drug Delivery* (McNeil, 2011) and *Immunological Properties of Engineered Nanomaterials* (Dobrovolskaia and McNeil, 2010). In 2011, NCL developed a "Lessons Learned Workshop". This was an intensive 2-day workshop designed for all levels of nanotechnology researchers that highlighted many of the common shortcomings and mistakes NCL had seen from its nearly 100 collaborations. The series provided detailed talks on many different aspects of preclinical nanomedicine characterization, breakout sessions on selected focus topics of interest, and half a day devoted to case studies describing the various nanomaterial deficiencies encountered during NCL's three-tiered Assay Cascade characterization process. The "Lessons Learned" workshop has been very well received and NCL has since put on six more workshops, including invited presentations to the FDA, the University of North Carolina-Chapel Hill,

Northeastern University, The Methodist Hospital Research Institute in Houston, and the Clinical Nanomedicine conference in Basel, Switzerland.

Chapter Six Training

While the Alliance currently supports an impressive roster of established biomedical nanotechnology scientists and clinicians, NCI recognizes that a pressing priority is to assure continuing success of the field through education, training, and outreach to succeeding generations of researchers in cancer nanotechnology. This is accomplished through informing and engaging the general public in the field of cancer nanotechnology as well as through support for training students and postdoctoral fellows in the field. The multidisciplinary research driven by the Alliance provides a unique opportunity to develop and provide innovative educational and outreach programs. The Cancer Nanotechnology Training Centers, funded through the R25 training mechanism, form the foundation of the Alliance's training and outreach, although the research Centers have an education, training and outreach mandate as well.

The main objective of the Training Centers is to educate and train early career researchers from diverse fields in the use of nanotechnology-based approaches, to advance understanding of cancer biology and to create new methods and tools for the prevention, diagnosis and treatment of cancer. The program of multidisciplinary research education is primarily focused on mentored laboratory-based training through participation in dedicated research projects along with a secondary focus on courses, seminars, and other forms of research education. The Training Centers have each also developed a complementary outreach component of educational materials for the general public and cancer patient community.

The research Centers have been tasked with training cancer nanotechnology scientists and performing community outreach as well. They are expected to establish efficient training and career development opportunities for young, new, and/or established investigators. This integrative training may include graduate programs, fellowships, certifications, courses, and internal seminar series to develop multidisciplinary trainees that can tackle cancer-related problems with physical sciences and engineering approaches. The Centers have budgetary restrictions requiring at least 2.5% of their budget to be dedicated toward education, training, and/or outreach efforts. Additionally, at least 3% of their budget is reserved to fund innovative pilot projects that primarily were internally awarded to young faculty and in some cases to postdoctoral research associates.

The second round of Alliance funding also supports seven K99/R00 Pathway to Independence Awards in Cancer Nanotechnology. The K99/R00 is a two phase award, in which up to two years of support are provided for mentored postdoctoral research and career development activities, followed by up to three years of support for the investigator to develop an independent research program. The intention of the award is to allow promising young investigators to broaden their training and acquire skills beyond what was gathered during their graduate and early postdoctoral training. This aspect of the K99/R00 award is particularly valuable for young researchers in multidisciplinary fields like cancer nanotechnology. For example, the K99 phase was used for training in animal model development and experimentation by four of the Alliance K99/R00 investigators with engineering backgrounds. Transition to the R00 phase is not guaranteed and requires appointment to an independent, tenure-track position at a research university, in addition to meeting K99 research and career development goals. All of the Alliance K99/R00 awardees succeeded in their postdoctoral positions and have transitioned into their first faculty positions for the R00 portion of their award. The Alliance K99/R00 awards are listed in Table 4.

Awardee	Postdoctoral (K99) Institution, Department	Faculty (R00) Institution, Department
Mingnan Chen	Duke University Center for Biologically Inspired Materials and Material Systems	University of Utah Pharmaceutics and Pharmaceutical Chemistry
Andrew Goodwin	University of California San Diego NanoEngineering	University of Colorado at Boulder Chemical and Biological Engineering
Aaron Mohs	Emory University Biomedical Engineering	Wake Forest University Health Sciences Biomedical Engineering
Prakash Rai	Massachusetts General Hospital Center for Engineering in Medicine	University of Massachusetts Lowell Chemical Engineering
Ravi N. Singh	Wake Forest University Health Sciences Cancer Biology	Wake Forest University Health Sciences Cancer Biology
Andrew Smith	Emory University Biomedical Engineering	University of Illinois Urbana-Champaign Bioengineering
Jin Xie	NIH National Institute of Biomedical Imaging and Bioengineering	University of Georgia Chemistry

Table 4. Alliance K99/R00 awardees.

Outreach

Community and public outreach about cancer nanotechnology is a systemic function of the Alliance Network. The Research and Training Centers have mandates and dedicated funds for this effort, and many of the Platforms are also pursuing efforts that involve the development and organization of educational and outreach programs, as well as the creation and distribution of educational materials for children, college students, technical professionals, teachers, and the general public. Many Alliance awards organize and host regular seminars throughout the year featuring invited speakers, which includes other Alliance members. Alliance PIs are encouraged to be actively involved in the planning and coordination of local seminar series and to recommend speakers who are experts in cancer nanotechnology research. These venues provide unique opportunities to initiate collaborations. Lectures may also be organized as Continuing Medical Education (CME) courses for the medical community. They also often choose to host seminars which are directed toward non-research audiences, such as meetings of the National Science Teachers Association, Institutional Board of Trustees, or lecture series targeted to the general public. These types of public outreach efforts generate a wider understanding of the potential impact of cancer nanotechnology on clinical practice. In addition to seminars, members of the Alliance have also organized symposia. Symposia often span 1-2 days and generally include a number of thematic presentations by leaders in the field for members of the research community.

Outreach to the General Public

Outreach to the general public, including programs for K-12 educators and students, has been very successful. Many of the universities choose to do community outreach by hosting special events for the

public around the topic of cancer nanotechnology. Northwestern University's Center hosts an "All Scouts Nano Day" which is targeted to both Boy Scout and Girl Scout troops. In March 2012, the "All Scouts Nano Day" had over 100 participants. They also host "Nanotechnology Town Hall" meetings to introduce nanotech and its potential applications to the lay audience. Other Centers within the Alliance reach out to the community by providing open access to symposia and seminars and by preparing educational materials for the public. The monthly bionanotechnology seminar series sponsored by the Stanford University Center is recorded and made freely available on the web (http://mips.stanford.edu/events/nanobiotech_seminar.html).

One nationwide nanotechnology outreach program that many of the Alliance members participate in is the National Science Foundation's Nanoscale Informal Science Education Network (NISE-Net) "Nano Days." Nano Days is NISE-Net's annual celebration of nanoscale science, technology, and engineering. NISE-Net encourages community-based educational organizations, such as museums, research institutions, universities, and libraries, to focus their efforts on bringing nanotechnology to the public during one week each spring. Many Alliance members participate in Nano Days either by developing their own outreach activities or by participating in activities organized in their area. NISE-Net provides purchasable hands-on activities, access to downloadable media, as well as science and education professionals that can help support institutions as they organize their events. More information can be found at the NISE-Net website: http://www.nisenet.org/nanodays.

The University of Kentucky's Training Center hosted a free one day mini-symposium, "Nanobiomotors: Structures, Mechanisms and Clinical Implications," which was open to the public and featured talks from a number of invited speakers from universities in the Kentucky and Illinois area. The Training Center also participates in the annual "Engineer's Day," where students in grades 1-12 and their parents learn what an engineer does by participating in contests, winning prizes, watching experiments and demonstrations, and talking to engineers about their jobs. Trainees staffed a booth and explained the enhanced permeability and retention (EPR) effect and how nanotechnology can be utilized for cancer therapy. The Training Center is also involved with the "Markey Cancer Research Day" which is an annual, daylong event showcasing cancer research at the University of Kentucky. Each trainee had at least one poster presentation at this event and their trainees often compete in the "best poster" competition.

The Training Center at the University of Illinois Urbana-Champaign also participates in a campus wide outreach effort, the Engineering Open House, in which over 200 visitors and elementary school students were introduced to cancer nanotechnology through visual displays and quiz show-style games. Concepts covered included the applications of gold nanoparticle based sensors and red to blue color shifts due to gold nanoparticle aggregation. Trainees also visited a local museum to teach elementary students about nanotechnology and engaged the wider Urbana-Champaign community during weekly "Science at the Market" at the local farmers market.

The University of California San Diego's Training Center has been pursuing internet and television outreach in addition to traditional campus-based activities. They maintain a Cancer Researchers in Nanotechnology Website (http://kummel.ucsd.edu/crin/home.html) which contains program information and participant research from their training program so that the public can learn more about their program. They also engage local TV news programming to educate the public about research in cancer nanotechnology. Clinical faculty and students in the program present their research in videos for these news clips, which are also made available on the website. An example can be seen at http://www.10news.com/news/25700294/detail.html.

Alliance researchers at The University of New Mexico are also engaged in digital outreach. Platform PI C. Jeffrey Brinker worked with the New Mexico Public Broadcast Station to produce a piece on nanotechnology for their monthly Connect program (http://portal.knme.org/video/2272153669/). The MIT-Harvard Center produced a flash mob re-enactment of nanoparticle entry into a cell (https://www.youtube.com/watch?v=jis2mXXY90Y). The New Mexico Training Center has hosted a number of student and faculty symposia, and also conducts periodic "The Art of Systems Biology and Nanoscience Days for Kids and Evenings for Grownups" events. These events deliver the artistic flair inherently wedded between scientist creativity and nanoscale imagery. Information on the most recent event can be found here: http://www.sfcomplex.org/2011/02/the-art-of-systems-biology/.

Educational Outreach to Undergraduates, Graduate Students and Medical Professionals

Consistent with the vision of the caNanoPlan for cancer nanotechnology training, many members of the Alliance Network have been incorporating biology and medicine into their nanoscience undergraduate programs as well as adding a focus on nanobiology or nanomedicine to their graduate programs in nanoscience. There has also been an increased focus on recruiting biological and medical researchers into nanotechnology research, in addition to the more typical addition of medical applications to engineering and nanotechnology research programs.

Northwestern University's Center has three educational outreach programs that educate undergraduates and medical professionals in the area of nanotechnology. They have a program supporting research experience for undergraduates, a medical student fellowship in nanotechnology, and a "Nano Boot Camp" for clinicians. Additionally they have developed nanotechnology courses in survey, cellular, and animal studies. These courses are open to medical faculty, graduate students, technicians, and others with an average of 14 students per course. A graduate level Certificate in Nanotechnology program is available and a web-based program is under development. In addition, trainees in the Center that take the needed courses can receive the Masters of Science in Clinical Investigation degree.

The Training Center at Boston University regularly hosts workshops targeted to different audiences to promote research in cancer nanotechnology. Examples include the annual "An Introduction to Cancer Care for Engineers and Physical Scientists," which covered surgical oncology, chemotherapy and biologics, radiation oncology and clinical information about specific tumor types, and a "Nanoparticle Synthesis and Characterization" workshop that introduced fundamental concepts in materials science such as optical properties of noble metal nanoparticles and their biomedical applications.

Each summer the University of Illinois Urbana-Champaign hosts a two week long Bionanotechnology Summer Institute dedicated to emerging areas in biomedical research. In 2011 the institute focused on cancer nanotechnology and in 2012 on biosensing and bioactuation. Participants included postdocs, faculty, and undergraduate and graduate students from around the world, who were introduced to the basics of cancer biology and cell mechanics, and trained at the intersection of biology and engineering. The institutes are intended to foster networking with other researchers. A number of other Alliance members also host summer research programs. The University of Kentucky supports mentored undergraduate researchers as a part of the University's summer undergraduate research program in the Colleges of Pharmacy and Engineering, a program jointly supported by the NSF's Research Experience for Undergraduates (REU) program. Researchers in the Alliance awards at Johns Hopkins University also participate in the REU program.

The UCSD Training Center holds an annual trans-Alliance NanoCancer Junior Investigator Conference. This two day conference invites trainees from across the Alliance to present and discuss their data to each other and a diverse panel of local faculty including Nobel Laureate Roger Tsien.

Training

The Training Centers' focus on comprehensive training program development has led to innovative strategies for training the next generation of nanotechnology cancer researchers. All six programs have developed strong training plans for their funded students and postdoctoral fellows and implemented coursework, seminar series and training networks that have value to students at the host institution beyond those supported directly by the awards. These programs have also made significant progress towards integrating curricula and research training in nanotechnology and cancer biology.

In addition to bringing students into nanotechnology research laboratories, they have also worked to improve the classroom training in this area to develop them into truly multidisciplinary researchers. The John Hopkins University received approval in 2012 from the university and the State of Maryland to offer a certificate program in Nanobiotechnology. All students that complete the Alliance supported Training Center program gain this recognition in addition to their Ph.D. UCSD offers two courses in nanoengineering for graduate students, and the University of Kentucky has developed two courses on nanotechnology. "Bionanotechnology: Interfaces and Devices" introduces the broad impact of small-scale technologies on the biological and medical fields and "Characterization of Nanoparticles for Medical Applications" is a multidisciplinary course covering nanomaterials/nanoparticle applications in medicine. At the University of Illinois Urbana Champaign the Introductory Course, "BioNanotechnology and Nanomedicine: Applications in Cancer and Mechanobiology," was successfully offered in Fall 2012 and will continue to be offered each Fall semester.

In addition to course development, many of the Training Centers have also chosen novel approaches to the overall structure of their training programs. An example is the format of the Training Center at UCSD. The program is made up of two tracks, one for trainees from the medical sciences and one for those from the physical sciences, to insure cross-training. Students enter the track based on their previous background, and are then cross trained in courses in the other area to ensure a well rounded cancer nanotechnology education. To continue this approach each trainee has two mentors, a basic scientist and a clinician scientist to promote translational research. Each trainee is required to have a translational medicine project as well as didactic training in nanomedicine technology commercialization. The UCSD program also includes training in entrepreneurship from the UCSD Rady School of Management. The Kentucky Training Center similarly requires all trainees to have both a research and clinical mentor. The clinical mentor provides training in standard clinical techniques such as administration of chemo- and radiotherapy and advises on the clinical utility and relevance of trainee research.

A novel approach to understanding training outcomes can be found at Boston University. The Boston University Training Center has developed an innovative method to measure the impact of its training paradigm. By applying social network analyses to trainee-trainee and trainee-mentor relationships, they are gathering data to provide insight into what it means to be collaborative and multidisciplinary in a research setting. These data, when applied to eventual trainee outcomes, are expected to provide unique insight into best practices in training and interpersonal professional relationships.

Training programs in the Alliance also focus on reducing disparities in cancer research and training. The UCSD Training Center recruits and retains a sizable number of trainees from under-represented groups,

including women in physical sciences and engineering. They currently have two participants who are being supported by Diversity Supplements from the NCI Center to Reduce Cancer Health Disparities. Diversity supplements have also been used by the Northwestern Center and Cedars-Sinai Platform to increase recruitment and retention of under-represented groups in cancer nanotechnology research. Administrative support for the Johns Hopkins University awards is used to maintain a diversity recruitment coordinator, an investment that has enabled them to recruit not only current students but to prepare a pool of potential future trainees.

Transitioning Trainees

A number of Alliance supported trainees have moved on to independent research and faculty positions in cancer nanotechnology. Some examples are given below.

Heather Agnew, who completed her doctoral work in Jim Heath's lab at the California Institute of Technology, is now employed at one of their spin out companies, Integrated Diagnostics, and is also an Adjunct Assistant Professor in the Molecular and Medical Pharmacology Department at UCLA. While in the Heath lab she was lead author of the first paper on protein catalyzed capture agents, which can identify, bind to and remove protein. Dr. Agnew first identified the chemical components at the core of these protein capture agents, which might someday replace natural antibodies in the healing process. Her work with Integrated Diagnostics will commercialize this technology. In 2010 Dr.Agnew won the Lemelson-MIT Caltech Student Prize. The Lemelson Foundation awards several prizes yearly to inventors in United States. She also received the Penn State Alumni Achievement Award, awarded to outstanding alumni under 35 years age, from the PSU Alumni Association in 2012.

Two Alliance postdoctoral fellows were on Forbes' 2012 "30 Under 30 – Science and Healthcare" list, http://www.forbes.com/special-report/2012/30-under-30/30-under-30_science.html. The first, Pedro Valencia, from the Robert Langer group in the MIT-Harvard Center, is currently a Consultant at The Boston Consulting Group. Valencia's work determined how to more quickly synthesize nanoparticles while improving their efficacy and reducing their toxicity. This work has resulted in the formation of a start-up company, Blend Therapeutics. The second was Adam de la Zerda from the Ghambir group in the Stanford Center. de la Zerda had a background in electrical engineering when he joined the group and is now a structural biologist who has developed a technique for imaging tumor cells. Since graduating from Stanford de la Zerda has completed a postdoctoral fellowship at UC Berkeley, and is now an Assistant Professor of Structural Biology in the School of Medicine at Stanford University.

Moritz F. Kircher worked during his postdoctoral training with both the Center for Molecular Imaging Research at Massachusetts General Hospital/Harvard Medical School with Ralph Weissleder and at the Molecular Imaging Program at Stanford with Sam Gambhir. Dr. Kircher is now faculty at the Memorial Sloan Kettering Cancer Center where he is a physician-scientist.

Jered Haun completed a post-doctoral position in the laboratory of Ralph Weissleder and joined the faculty of the Department of Biomedical Engineering at the Henry Samueli School of Engineering at the University of California, Irvine as an assistant professor. While in Weissleder's lab, Haun developed a bio-orthogonal chemistry for magnetic nanoparticle functionalization and worked on molecular detection applications using Weissleder's Diagnostic Magnetic Resonance device,

Jin Wang, a post-doctoral associate in the laboratory of Joseph DeSimone at UNC, has joined the faculty of the Baylor College of Medicine as a Cancer Prevention Research Institute of Texas (CPRIT) Scholar in Cancer Research. Wang will be an assistant professor in the Department of Pharmacology, a member of the Dan L. Duncan Cancer Center and an adjunct faculty member in the Department of Bioengineering at

Rice University. Wang's research interests lie in RNAi therapy and targeted drug delivery using nanoparticles.

Samantha Meenach was a postdoctoral fellow at the University of Kentucky under Drs. Kimberly W. Anderson and Dr. J. Zach Hilt, where she developed aerosol dispersions of phospholipid nanoparticles for inhalation delivery of chemotherapeutics to lung cancer patients. She is now an Assistant Professor of Chemical Engineering at the University of Rhode Island.

Daniel Scott also completed his training at Kentucky, where he formulated analogues of the anti-cancer drug mithramycin into self-assembled and cross-linked micelles for controlled drug delivery. He is now a Visiting Assistant Professor at Centre College in Danville, Kentucky.

Steven Millward was a graduate student and postdoctoral fellow in the Heath lab at the California Institute of Technology, where he also worked on the protein catalyzed capture agents. In Fall 2011 he started as Assistant Professor of Experimental Diagnostic Imaging at the University of Texas MD Anderson Cancer Center.

Cristina Zavaleta, a Postdoctoral Fellow in the Gambhir group at Stanford during the first phase of Alliance funding is now an Instructor in the Diagnostic Radiology program at Stanford.

Conclusion

The summary of Alliance research given here represents only a portion of the research funded by the Alliance program and the related translational efforts that have reached publication and clinical trial/commercialization stage. We selected contributions which, in our opinion, are most relevant from the perspective of lasting impact on cancer research and care in the near future. The technologies presented here are the culmination of efforts that have spanned the past decade.

We expect that further progress in the field will be moving along two parallel tracks. The first track will be associated with on-going translation to the clinical environment and the second with the development of new tools and techniques in the research arena. For example, small molecule drugs in nanoparticle-based formulations currently undergoing clinical trials will be joined by other modes of therapy with more emphasis being put on siRNAs. Active targeting, when appropriate, will be used more frequently. Imaging based on nanotechnology will evolve towards greater use of bio-activatable probes for assessing the tumor microenvironment. Imaging will be also be increasingly used in intra-operative imaging to guide real time surgery using single and multi-modality imaging nanoconstructs. In parallel, efforts on the development of novel nanoconstructs and studies towards basic understanding of delivery mechanisms and interactions of nanomaterials with biological systems will be continued.

The research community pursuing cancer nanotechnology is expected to continue relying on multi-disciplinary environments of chemists, physicists, and engineers driving innovation in nanotechnology devices and tools and biologists and clinicians defining compelling areas of applications and clinical needs. It is hoped that involvement of clinicians in the early stage of research and technology development will increase.

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Acronym List

3WJ	Three Way Junction
ABS	Asymmetric Bifunctional Silyl Ether
ACT	Adoptive Cell Transfer
ADME	Absorption, Digestions, Metabolism, Excretion
Alliance	NCI Alliance for Nanotechnology in Cancer
caNanoPlan	Cancer Nanotechnology Plan
CAPR	Center for Advancing Preclinical Research
Center	Center of Cancer Nanotechnology Excellence
CGC	Coordination and Governance Committee
CGCI	Cancer Genomic Characterization Initiative
CIRM	California Institute for Regenerative Medicine
CLIA	Clinical Laboratory Improvement Amendments
CME	Continuing Medical Education
CMS	Center for Medicare & Medicaid Services
CNT	Carbon nanotube
CTC	Circulating Tumor Cell
CTD^2	Cancer Target Discovery and Development
CTL	Cytotoxic T Lymphocyte
DBT	Digital Breast Tomosynthesis
dCK	Deoxycytidine Kinase
DCTD	Division of Cancer Therapeutics and Diagnostics
DEAL	DNA Encoded Antibody Library
DMR	Diagnostic Magnetic Resonance
DOPC	Dioleoyl phosphatidylcholine
DSPE	1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine
EDRN	Early Detection Research Network
EMT	Epithelial to Mesenchymal Transition
EPR	Enhanced Permeability and Retention
^{18}F-FAC	1-(2'-deoxy-2'-[18F]fluoroarabinofuranosyl) cytosine

FACS	Fluorescence Activated Cell Sorting
FAES	Foundation for the Advanced Education in the Sciences
FDA	Food and Drug Administration
FNLCR	Frederick National Laboratory for Cancer Research
GBM	Glioblastoma Multiforme
GFP	Green Fluorescent Protein
GMP	Good Manufacturing Practices
HDL	High Density Lipoprotein
ICMIC	*In Vivo* Cellular and Molecular Imaging Center
IDE	Investigational Device Exemption
IGERT	Integrative Graduate Education Research Traineeship
IND	Investigational New Drug
IONP	Iron Oxide Nanoparticles
IRB	Institutional Review Board
ISB	Institute for Systems Biology
K99/R00	Pathway to Independence Award
LDT	Laboratory Developed Test
LLNA	Local Lymph Node Assay
LLNP	Local Lymph Nnode Proliferation
M-CNTC	Midwest Cancer Nanotechnology Training Center
MDR	Multiple Drug Resistant
miRNA	MicroRNA
MPS	Mononuclear Phagocyte System
MRT	Microbeam Radiation Therapy
NCI	National Cancer Institute
NCIP	National Cancer Informatics Program
NCL	Nanotechnology Characterization Laboratory
NHLBI	National Heart, Lung and Blood Institute
NIBIB	National Institute of Biomedical Imaging and Bioengineering
NIEHS	National Institute of Environmental Health and Safety
NIH	National Institutes of Health

NIST	National Institute of Standards and Technology
NMOF	Nanoscale Metal Organic Framework
NSBCC	NanoSystems Biology Cancer Center
NSF	National Science Foundation
PD	Pharmacodynamics
PEG	Polyethylene Glycol
PET	Positron Emission Tomography
PI	Principal Investigator
PK	Pharmacokinetics
Platform	Cancer Nanotechnology Platform Partnership
PLGA	Poly(lactic-co-glycolic acid)
PMA	Pre-market approval
PRINT	Particle Replication in Non-Wetting Templates
pRNA	Packaging RNA
PSMA	Prostate Specific Membrane Antigen
QD	Quantum Dot
RECIST	Response Evaluation Criteria in Solid Tumors
REU	Research Experience for Undergraduates
RFA	Request for Application
RFP	Request for Proposal
RNAi	RNA Interference
SAR	Structure Activity Relationship
SBIR	Small Business Innovation Research
SCBC	Single Cell Barcode Chip
SERS	Surface Enhanced Raman Spectroscopy
shRNA	Small Hairpin RNA
siRNA	Small Interfering RNA
SNA	Spherical Nucleic Acid
SNA-NC	Spherical Nucleic Acid-Nanocrystal
SWNT	Single Walled NanoTube
TARGET	Therapeutically Applicable Research to Generate Effective Treatments

TCGA	The Cancer Genome Atlas
TDAR	T-dependent Antigen Response
TONIC	Translational of Nanotechnology in Cancer
TPN	Tumor Penetrating Nanocomplex
Training Center	Cancer Nanotechnology Training Center
UCLA	University of California Los Angeles
UCSD	University of California San Diego
UIUC	University of Illinois Urbana-Champaign
UNC	University of North Carolina
UNM	University of New Mexico
uPAR	urokinase plasminogen activator receptor

Appendix A

Alliance awards

Centers of Cancer Nanotechnology Excellence

Nanosystems Biology Cancer Center 2 (NSBCC) (Jim Heath, Ph.D., Leroy Hood, M.D., Ph.D. and Michael Phelps, Ph.D.)

Scientific Focus: Develop and validate tools for early detection, diagnosis and therapy of melanoma and glioblastoma through *in vitro* diagnostics, *in vivo* molecular imaging and targeted therapies, including adoptive T cell immunotherapies and siRNA delivery.

Unique contribution to the Network: This Center tightly integrates basic and clinical research to develop assays for gauging therapeutic performance that are not possible using any other method. They have a well-developed pipeline for clinical translation including close collaboration with and licensing to industrial partners.

Dartmouth Center of Cancer Nanotechnology Excellence (Ian Baker, Ph.D., and Keith Paulsen, Ph.D.)

Scientific Focus: Develop and use targeted magnetic iron/iron oxide nanoparticles which can be excited by alternating magnetic fields to induce localized hyperthermia in breast and ovarian cancer cells.

Unique contribution to the Network: This Center is exploring the potential clinical value of magnetic nanoparticle mediated hypothermia by optimizing nanoparticle and instrument design. The Center also supports development of new affibody targeting agents alongside studies on cell surface receptor density and nanoparticle trafficking *in vivo* for improved nanoparticle delivery.

Center for Cancer Nanotechnology Excellence at Johns Hopkins (Peter Searson, Ph.D. and Martin Pomper, M.D., Ph.D.)

Scientific Focus: Develop and integrate nanotechnology-based *in vitro* assays, targeted chemotherapy and immunotherapy for diagnosis, therapy and post-therapy monitoring of lung and pancreatic cancer.

Unique contribution to the Network: Project and core efforts focus on developing nanoparticles for deployment as sample processing and detection elements in *in vitro* devices and as imaging and therapeutic agents. They have strong efforts in materials science and a unique approach to the treatment of lung cancer using mucus penetrating particles. This Center is co-located with an Alliance Training Center.

MIT-Harvard Center of Cancer Nanotechnology Excellence (Robert Langer, Sc.D., and Ralph Weissleder, M.D., Ph.D.)

Scientific Focus: Develop and translate to the clinic a diversified portfolio of nanoscale devices for targeted drug and siRNA delivery, diagnostics, non-invasive imaging and molecular sensing for better diagnosis and treatment of melanoma, prostate and colon cancer.

Unique contribution to the Network: This Center is at the leading edge of engineering innovative nanomaterials and novel research concepts. Contributions include synthesis of tumor penetrating

nanocomplexes, targeted drug delivery vehicles currently in clinical trials, bioresponsive nanomaterials and magnetic nanoparticle based *in vitro* and *in vivo* sensors.

Center for Translational Cancer Nanomedicine at Northeastern University (Vladimir Torchilin, Ph.D., D.Sc. and Nahum Goldberg, M.D.)

Scientific Focus: Develop and characterize nanomedicines using extensive *in vitro* and *in vivo* testing and imaging capabilities, with a particular focus on lung, ovarian and pancreatic cancer. The Center pursues targeted delivery of multiple therapeutics using nanoformulations.

Unique contribution to the Network: This Center has created a pipeline approach to liposomal and micellar formulations based on outstanding expertise in nanoformulation. The Center also studies in detail approaches to overcome multidrug resistance using nanoparticle delivery. A core facility operated by their industrial partner, Nemucore Medical Innovations, is dedicated to good manufacturing practices (GMP) production and offers its services to researchers outside of the Center.

Nanomaterials for Cancer Diagnostics and Therapeutics at Northwestern University (Chad Mirkin, Ph.D. and Steven T. Rosen, M.D.)

Scientific Focus: Develop novel nanoscale technologies for the detection of circulating cancer stem cells and develop model matrices to clarify cancer biology processes. These technologies are being investigated for melanoma, glioblastoma and prostate cancer diagnosis and treatment.

Unique contribution to the Network: This Center focuses on highly innovative “nanoflares” based on spherical nucleic acid nanoparticles invented by the PI. Clinical applications being investigated range from mRNA profiling in tissues and cells to the delivery of siRNA and miRNA therapeutics.

Center for Cancer Nanotechnology Excellence and Translation at Stanford University (Sanjiv Sam Gambhir, M.D., Ph.D. and Shan Wang, Ph.D.)

Scientific Focus: Design and implement novel *in vitro* diagnostic devices and verify their performance using *in vivo* imaging to monitor lung cancer therapy and for earlier detection of ovarian and colon cancers.

Unique contribution to the Network: This Center integrates advances in biomarker discovery with nanoparticles for molecular imaging and *in vitro* diagnostic devices. Applications include nanoparticle based endoscopy, image guided surgery, multi-modal imaging, a device for blood based proteomics and tools for genomic and physical characterization of single cells.

Texas Center for Cancer Nanomedicine (David G. Gorenstein, Ph.D., Mauro Ferrari, Ph.D., Anil Sood, M.D., G. Lopez-Berestein, M.D. and Jennifer L. West, Ph.D.)

Scientific Focus: Develop and apply a diverse array of nanoplatforms for new therapeutics, develop methodologies for reliable monitoring of therapeutic efficacy, investigate early detection approaches using biological fluids and pursue advances in imaging and cancer-prevention protocols for ovarian and pancreatic cancers.

Unique contribution to the Network: This Center systematically integrates cancer biology and nanotechnology, developing animal models and therapeutic targets using data from The Cancer Genome Atlas (TCGA) and related efforts. The Center has extensive expertise in rational design of targeting ligands and nanoparticles for controlled release.

Carolina Center of Cancer Nanotechnology Excellence (Joseph DeSimone, Ph.D. and Joel Tepper, M.D.)

Scientific Focus: Develop innovative and significant core technologies, including PRINT® (Particle Replication on Non-Wetting Templates) nanoparticles and carbon-nanotube-based x-ray sources for cancer therapy and early detection of lung, brain and breast cancer.

Unique contribution to the Network: This Center possesses unique chemistry methods for synthesizing nanoparticles and utilizes a thorough and systemized approach to the preclinical characterization of their nanoparticles and the study of nanoparticle-host interactions. This approach is applied to the multiple nanoformulations developed by the Center's investigators, enabling direct comparison of performance across platforms.

Cancer Nanotechnology Platform Partnerships

Combinatorial-designed Nano-platforms to Overcome Tumor Resistance, Northeastern University (Mansoor Amiji, Ph.D., and Zhen-feng Duan, M.D., Ph.D.)

Goal: To design libraries of nano-assemblies for encapsulation and targeted delivery of siRNA and small molecule anticancer drugs in order to suppress multidrug resistance.

High-Capacity Nanocarriers for Cancer Therapeutics, University of North Carolina at Chapel Hill (Alexander Kabanov, Ph.D, D.Sc.)

Goal: To develop polymeric micelle carriers as effective drug delivery systems to overcome the limitations of low water solubility and improve the safety and bioavailability of anticancer drugs.

Magnetoresistive Sensor Platform for Parallel Cancer Marker Detection, University of Utah (Marc Porter, Ph.D., and Sean J. Mulvihill, M.D.)

Goal: The creation of a platform that not only detects the presence and/or change in the levels of large numbers of markers in sera, but also can handle samples of preciously limited volume and meet the demand for high-sample throughput.

Nanobioconjugate Based on Polymalic Acid for Brain Tumor Treatment, Cedars-Sinai Medical Center (Julia Ljubimova, M.D., Ph.D.)

Goal: To develop a nanoplatform based on polymalic acid that can cross the blood–brain barrier and the blood–brain tumor barrier to deliver anticancer drugs into the tumor cells directly. By systemic administration of this nanoplatform, anticancer drugs will inhibit the synthesis of several tumor specific targets, such as tumor vascular protein laminin 411, which plays a significant role in glioma growth, invasion, and metastasis.

Nanoscale Metal-Organic Frameworks for Imaging and Therapy of Pancreatic Cancer, University of Chicago (Wenbin Lin, Ph.D., and Jen Jen Yeh, M.D.)

Goal: To develop a new class of hybrid nanomaterials, namely, NMOFs (nanoscale metal-organic frameworks), for early detection and more effective therapy of pancreatic ductal adenocarcinoma, and thus provide new nanotechnology management strategies for cancer patients.

Peptide-Directed Protocells and Virus-like Particles: New Nanoparticle Platforms for Targeted Cellular Delivery of Multicomponent Cargo, University of New Mexico (Cheryl Willman, M.D., and C. Jeffrey Brinker, Ph.D.)

Goal: To develop generic, universal nanoparticle platforms tailored to target, identify, and treat arbitrary, select, and often minute populations of cancer cells in high-risk Acute Lymphoblastic Leukemia patients.

Preclinical Platform for Theranostic Nanoparticles in Pancreatic Cancer, Rice University (Naomi Halas, Ph.D., D.Sc., Amit Joshi, Ph.D., and Sunil Krishnan, M.D.)

Goal: To accelerate the preclinical testing of multifunctional hybrid nanoparticles as multimodal molecular imaging agents and targeted therapeutic agents for the diagnosis and treatment of pancreatic cancer.

RNA Nanotechnology in Cancer Therapy, University of Kentucky (Peixuan Guo, Ph.D., and John Rossi, Ph.D.)

Goal: To fabricate RNA nanoparticles to incorporate therapeutic siRNA, aptamers, and ribosomes to accomplish targeted delivery for lung, ovarian, and liver cancers and leukemia.

Targeting SKY Kinase in B-Lineage ALL with CD-19 Specific C-61 Nanoparticles, Children's Hospital Los Angeles (Fatih Uckun, M.D., Ph.D.)

Goal: To develop effective and paradigm-shifting treatment strategy for B-lineage Acute Lymphoblastic Leukemia, the most common form of childhood cancer.

Theranostic Nanoparticles for Targeted Treatment of Pancreatic Cancer, Emory University (Lily Yang, M.D., Ph.D., and Hui Mao, Ph.D.)

Goal: To develop a novel theranostic magnetic iron oxide nanoparticle (IONP) platform that enables both tumor-targeted imaging and drug delivery for effective treatment of pancreatic cancer.

Toxicity and Efficacy of Gold Nanoparticle Photothermal Therapy in Cancer, Emory University, (Dong Shin, M.D., and Mostafa El-Sayed, Ph.D.)

Goal: To enhance the limited knowledge about a new generation of optimized gold nanorod-assisted photothermal therapy for the evaluation of toxicity using animal models and xenografted tumor ablation with low doses of near-infrared light.

Tumor Targeted Nanobins for the Treatment of Metastatic Breast and Ovarian Cancer, Northwestern University, (Thomas O'Halloran, Ph.D., and Vincent Cryns, M.D.)

Goal: To develop a translational pipeline of nanoparticle-based anticancer drugs for the treatment of rare and difficult to treat cancers such as ovarian and metastatic breast cancers.

Cancer Nanotechnology Training Centers

Boston University Cross-Disciplinary Training in Nanotechnology for Cancer (Bennett B. Goldberg, Ph.D., and Douglas Faller, M.D., Ph.D.)

This Training Center applies nanotechnology in the training of pre- and post-doctoral fellows for early cancer detection/cancer prevention through identification of rare circulating tumor cells; use of proteomics to detect nuclear matrix proteins and new biomarkers for screening of early stage tumors; and uses nanowires and nanocantilever arrays for the early detection of precancerous and malignant lesions from biological fluids.

Integrative Cancer Nanoscience and Microsystems Training Center at the University of New Mexico (Janet M. Oliver, Ph.D., and Abhaya Datye, Ph.D.)

This Training Center accelerates the recruitment of interdisciplinary graduate students and postdoctoral fellows and the development of interdisciplinary teams to perform research that combines novel nanoprobes with *in vitro* fluorescence and electron microscopy to address altered membrane

organization and vesicular trafficking in cancer cells; develops and applies nano- and microdevices for DNA sequencing and analyzes chromatin remodeling in cancer; generates novel probes and instruments for *in vivo* cancer detection; and focuses on cancer drug discovery and the synthesis of multifunctional nanoprobes for targeted drug delivery.

Midwest Cancer Nanotechnology Training Center (M-CNTC) at the University of Illinois Urbana-Champaign (*Rashid Bashir, Ph.D., and Ann Nardulli, Ph.D.)*

This Training Center creates a highly interdisciplinary environment for students, post-doctoral engineers, physical scientists and biologists working in the areas of *ex vivo* diagnostic nanotechnology, *in vivo* imaging nanotechnology, therapeutic nanotechnology, and mechanobiology.

The Johns Hopkins Cancer Nanotechnology Training Center (Denis Wirtz, Ph.D.)

This Training Center develops programs to train pre-doctoral fellows at the interface between nanotechnology and cancer medicine to develop novel nanoscale therapeutic and diagnostic tools for the detection, treatment, and cure of human cancer.

The University of Kentucky Cancer Nanotechnology Training Center (Bradley D. Anderson, Ph.D., and B. Mark Evers, M.D.)

This Training Center develops cancer nanotechnology projects for multidisciplinary, focus-area teams with the goal of training future researchers in the areas of early detection and diagnosis in lung, colon and ovarian cancer; treatment of gastrointestinal tumors and metastases; lung cancer treatment; and glioma therapy.

UCSD Cancer Nanotechnology Training Center at the University of California San Diego (Robert F. Mattrey, M.D., and Andrew Kummel, Ph.D.)

This Training Center provides training in cancer nanotechnology to pre-doctoral students, post-doctoral researchers and physicians with tailored tracks for physical scientists/engineers and biological/life scientists, marked by a well-developed plan for minority recruitment and retention.

K99/R00 Pathway to Independence Awards in Cancer Nanotechnology

Enzyme-Responsive Nanoemulsions as Tumor-Specific Ultrasound Contrast Agents, University of Colorado, Boulder (Andrew P. Goodwin, Ph.D.)

Inhibition of Metastasis-Initiating Cells by Chimeric Polypeptide Nanoparticles,_University of Utah (Mingnan Chen, Ph.D.)

Nanoplatform Based, Combinational Therapy against Breast Cancer Stem Cells, University of Georgia (Jin Xie, Ph.D.)

Nanotechnology for Minimally Invasive Cancer Detection and Resection, Wake Forest University Health Sciences (Aaron M. Mohs, Ph.D.)

Next-Generation Quantum Dots for Molecular and Cellular Imaging of Cancer, University of Illinois at Urbana-Champaign (Andrew M. Smith, Ph.D.)

Theranostic Nanomedicine for Breast Cancer Prevention and Image-Guided Therapy, University of Massachusetts, Lowell (Prakash R. Rai, Ph.D.)

Tumor Targeting and Diagnostic Applications of Glycosylated Nanotubes, Wake Forest University School of Medicine (Ravi N. Singh, Ph.D.)

Appendix B

Alliance Progress Towards caNanoPlan Milestones

Milestone met

Milestone not met

In progress

No relevant Alliance research

Challenges to Developing New Nanomaterials	3 Year Milestones	References
	Adopt standardized techniques for the characterization of nanoparticles both *in vitro* and *in vivo*	http://ncl.cancer.gov/working_assay-cascade.asp (Smith et al., 2012, Chu et al., 2013, Adriani et al., 2012, Frieboes et al., 2013)
	Design nanoparticle compositions with reproducible activated, release properties in vivo	(Ding et al., 2013, Parrott et al., 2012, Shen et al., 2013)
	Conduct clinical trials of a variety of nanoparticles	(Hrkach et al., 2012, Weiss et al., 2013, Tabernero et al., 2013)
	5 Year Milestones	**References**
	Determine the effects of surface regiochemistry on nanoparticle internalization and biodistribution	(Perry et al., 2012)
	Expect the first polymer-based, nanoparticle therapeutic to be approved by the FDA	BIND-014 IND (NCT01300533)
Nanotherapeutic Delivery Systems	**3 Year Milestones**	**References**
	Synthesize 20-30 tumor-targeted nanotherapeutic delivery systems with high quality and yield	(Huang et al., 2012b, Parrott et al., 2012, Ashley et al., 2011b, Lee et al., 2013, Uckun et al., 2013, Yang et al., 2011b, Han et al., 2012, Parodi et al., 2013, Shen et al., 2013, Ashley et al., 2011a, Dam et al., 2012, Kim et al., 2012, Mann et al., 2011, Liu et al., 2012, Shu et al., 2013, Lee et al., 2012, Zheng et al., 2012)
	Demonstrate successful delivery of highly potent, toxic therapeutics using nanoparticle platforms.	(Karve et al., 2012, Cely et al., 2012, Uckun et al., 2013, Han and Davis, 2013)
	5 Year Milestones	**References**
	Perform PK/PD studies of the best nanotherapeutic systems	(Hrkach et al., 2012, Anders et al., 2013, Chu et al., 2013, Milane et al., 2011c)

	Determine the lowest non-toxic dose using the best nanotherapeutic system in humans. Study nanoparticle biodistribution and toxicity to identify those that are most efficacious and least toxic	http://clinicaltrials.gov/show/NCT01300533, (Hrkach et al., 2012) http://clinicaltrials.gov/ct2/show/NCT00333502, (Weiss et al., 2013)
	Extend preclinical toxicology studies of the best nanotherapeutic systems from mice to rats and dogs. Conduct phase 0, I, and II clinical trials.	http://clinicaltrials.gov/ct2/show/NCT00333502, (Weiss et al., 2013) http://clinicaltrials.gov/ct2/show/NCT01380769
	Gain FDA approval of at least one nanoparticle-based targeted therapeutic	BIND-014 (NCT01300533), CRLX101 (NCT00333502, NCT01380769)
Targeted Drug Delivery	**3 Year Milestones**	**References**
	Develop new targeted therapeutic nanoparticles focusing on the tumor microenvironment as well as metastatic disease	(Mann et al., 2011, Lee et al., 2013, You et al., 2012, von Maltzahn et al., 2011, Huang et al., 2012b, Yokoi et al., 2013)
	Release and biodistribution animal studies for targeted nanoparticles to provide better insight into how targeted therapeutic nanoparticles work *in vivo*	(Lee et al., 2013, Milane et al., 2011c, Davis et al., 2013, Sexton et al., 2013)
	5 Year Milestones	
	Conduct phase 0/I/II clinical trials of new targeted nanoparticle therapies	http://clinicaltrials.gov/show/NCT01300533, (Hrkach et al., 2012)
siRNA Therapeutics	**3 Year Milestones**	**References**
	Expand the repertoire of chemical modifications to the siRNAs themselves as well conjugation to other carbohydrates, lipids, proteins, etc. to increase stability, bioavailability, and intracellular processing	(Shu et al., 2013, Lee et al., 2012, Vivas-Mejia et al., 2011, Dunn et al., 2012)
	Increase research on catalytic oligonucleotides capable of cleaving the target RNAs	
	5 Year Milestones	**References**
	Test new nanotechnology-based delivery vehicles for siRNA	(Shen et al., 2013, Zheng et al., 2012, Ren et al., 2012, Hasan et al., 2012, Cho et al., 2013)

	Develop formulations containing multiple siRNAs to target multiple signal transduction pathways	
	Conduct late stage clinical trials for siRNA delivery	
Nanotechnology To Overcome Tumor Drug Resistance	**3 Year Milestones**	**References**
	Develop animal models of refractory disease that recapitulate human disease in terms of location, genotypic and phenotypic heterogeneity, etc.	(Milane et al., 2011a)
	Characterize the effect of tumor microenvironmental factors on the development of clinically-relevant refractory disease.	Charo et al., 2013—Prostaglandin E2 regulates pancreatic stellate cell activity via the EP4 receptor, *Pancreas,*42(3): 467-74. PMID 23090667
	Identify and validate drug targets and strategies to overcome resistance through a multi-factorial approach that utilizes efficiency in drug delivery, residence, and intracellular penetration as well as approaches to overcome cellular resistance.	(Wang et al., 2011, Shen et al., 2013, Zhao et al., 2013, Milane et al., 2011b)
	5 Year Milestones	**References**
	Establish robust pre-clinical programs to develop and test multi-functional nanoparticulate drug delivery systems in appropriate models of refractory diseases.	
New Contrast Agents with Improved Spatial and Temporal Resolution	**3 Year Milestones**	**References**
	Elucidate issues governing efficacy, safety, and clinical use compatibility	(Thakor et al., 2011, Smith et al., 2012, Zavaleta et al., 2013, Keng et al., 2012); [^{18}F]FAC, http://sofiebio.com/fac
	5 Year Milestones	**References**
	Develop software to optimize image acquisition and presentation for clinical interpretation	(Zhao et al., 2011, Huang et al., 2012a, Mohs et al., 2010)
	Devise guidelines for utilizing imaging information to improve health care management	
	Acceptance of new molecular	

	imaging data	
Nanotechnology in Theranostics	**3 Year Milestones**	**References**
	Theranostic platforms with improved biocompatibility and performance	(Lee et al., 2013, Cho et al., 2013, Zavaleta et al., 2013)
	Preclinical development of platforms targeting >4 pathways simultaneously	(Cho et al., 2013)
	5 Year Milestones	**References**
	Facilitate clear regulatory framework for multifunctional nanomaterials	(Thakor et al., 2011, Kircher et al., 2012)
	3-5 IND submissions for multifunctional nanotheranostics	
Multi-modal Imaging	**3 Year Milestones**	**References**
	Develop multi-modal small animal imaging scanners	
	5 Year Milestones	**References**
	Translate these scanners into clinical application of multi-modal imaging	
	Complete large animal studies of at least five multi-modal agents	
Nanotechnology for Image-Guided Interventions	**3 Year Milestones**	**References**
	Targeted nanoparticle agents incorporating fluorescent dyes and targeting ligands	(Cho et al., 2013, Mohs et al., 2010)
	Develop non-photobleaching nanoparticles for tumor margin delineation	(Kircher et al., 2012, Zhao et al., 2011, Zhou et al., 2013)
	5 Year Milestones	**References**
	Study *in vivo* toxicity in model systems	(Thakor et al., 2011)
	Begin clinical trial evaluation of nanoparticles with non-invasive delivery	(Zavaleta et al., 2013)
Development of	**3 Year Milestones**	**References**

Imaging Hardware Based on Nanotechnology	Development and clinical testing of stationary tomosynthesis scanners	Clinical trial NCT01773850
	Commercialize imaging systems for small animal models	http://sofiebio.com/genisys
	5 Year Milestones	**References**
	Study *in vivo* toxicity in model organisms	
	Conduct studies of therapeutic effect on small animal brain tumor models	
	Commercialize tomosynthesis imaging system	
Protein Based *In Vitro* Assays	**3 Year Milestones**	**References**
	Develop non-antibody-based protein biomarker detection	(Millward et al., 2011)
	Incorporate antibodies into microfluidic chips	(Ahmad et al., 2011, Chai et al., 2011)
	Integrate sample processing within assay platform	(Chung et al., 2012, Gaster et al., 2011, Ma et al., 2011)
	5 Year Milestones	**References**
	Develop multiplexed, integrated, miniaturized diagnostic assays	(Gaster et al., 2011, Haun et al., 2011, Ma et al., 2011)
	Conduct clinical trials on emerging diagnostic tests	(Ma et al., 2011), (NCT01752101, NCT01752114)
	Gain FDA approval of first cancer nanotechnology-based diagnostic test	
Tumor MicroRNA Profiling and Validation	**3 Year Milestones**	**References**
	Develop a multiplexed assay system to rapidly profile tumor miRNA	(Alhasan et al., 2012)
	5 Year Milestones	**References**
	Charaterize miRNA profiles of disease progression, aggressiveness, and refractivity	Sood's miRNA paper from Cancer Cell 2013
	Validate and correlate miRNA profiles with other genotypic and	(Alhasan et al., 2012)

	phenotypic profiling methods	
Nanotechnology and Cancer Prevention	**3 Year Milestones**	**References**
	Characterize natural products and their chemopreventive potential	
	Develop nanotechnology delivery systems for neutraceuticals and other chemopreventive agents	
	Carry out more prospective studies to identify genetic, behavioral, and environmental risks for various types of cancers.	
	5 Year Milestones	**References**
	Incorporate natural products with more standard therapeutic approaches in an increasing number of clinical trials	
	Conduct rational design experiments to improve on the potential therapeutic effects of existing neutraceuticals	
	Identify other potential targets for cancer vaccine development	

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Appendix C

Clinical Trials and IRB Approved Protocols

Alliance Therapeutics

Phase I (NCT # pending) **IND Approved (April 2013)**	**Title: An Open-Label, Phase I, Escalating Dose Study to Evaluate the Safety, Tolerability, and Pharmacodynamics of PDS0101 (ImmunoMAPK-RDOTAP/HPV-16 E6 & E7 peptides) in Subjects with Cervical Intraepithelial Neoplasia (CIN) and High-risk Human Papillomavirus (HPV) Infection**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
PDS Biotechnology Leaf Huang (UNC) NCL	Human papillomavirus (vaccine and treatment)	peptide antigen derived from E7 oncoprotein of human papillomavirus (HPV) type 16	PDS0101 (Versamune™; R-DOTAP liposome) (Chen et al., 2008)
Phase I (NCT00689065) Ongoing, not recruiting	**Title: Safety Study of CALAA-01 to Treat Solid Tumor Cancers**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Calando Pharmaceuticals (Acquired by Arrowhead Research Corporation) Mark Davis (Caltech)	Solid Tumors Refractory to Standard-of-Care Therapies	siRNA targeting the M2 subunit of ribonucleotide reductase (R2)	CALAA-01 (Cyclodextrin) (Davis et al., 2010)
Phase I/II (NCT00333502) Completed	**Title: Study of CRLX101 (Formerly Named IT-101) in the Treatment of Advanced Solid Tumors**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Cerulean Pharma Mark Davis (Caltech)	Advanced Solid Tumors	Camptothecin	CRLX101 (Cyclodextrin)(Davis, 2009)
Phase II (NCT01380769) Ongoing, not recruiting	**Title: A Phase 2 Study of CRLX101 in Patients With Advanced Non-Small Cell Lung Cancer**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Cerulean Pharma Mark Davis (Caltech)	Non-small Cell Lung Cancer	Camptothecin	CRLX101 (Cyclodextrin)(Davis, 2009)

Phase II (NCT01652079) Currently recruiting	Title: CRLX101 for Recurrent Ovarian/Tubal/Peritoneal Cancer		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Cerulean Pharma Mark Davis (Caltech)	Ovarian Cancer, Fallopian Tube Cancer, Primary Peritoneal Cancer	Camptothecin	CRLX101 (Cyclodextrin) (Davis, 2009)
Phase I (NCT01625936) Currently recruiting	**Title: CRLX101 Plus Bevacizumab in Advanced RCC**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Cerulean Pharma Mark Davis (Caltech)	Renal Cell Carcinoma	Camptothecin	CRLX101 (Cyclodextrin) (Davis, 2009)
Pilot Study (NCT01612546) Currently recruiting	**Title: Pilot Trial of CRLX101 in Treatment of Patients With Advanced or Metastatic Stomach, Gastroesophageal, or Esophageal Cancer That Cannot be Removed by Surgery**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Cerulean Pharma Mark Davis (Caltech)	Adenocarcinoma of the Esophagus, Adenocarcinoma of the Gastroesophageal Junction, Diffuse Adenocarcinoma of the Stomach, Intestinal Adenocarcinoma of the Stomach, Mixed Adenocarcinoma of the Stomach, Recurrent Esophageal Cancer, Recurrent Gastric Cancer, Squamous Cell Carcinoma of the Esophagus, Stage IIIB Esophageal Cancer, Stage IIIB Gastric Cancer, Stage IIIC Esophageal Cancer, Stage IIIC Gastric Cancer, Stage IV Esophageal Cancer, Stage IV Gastric Cancer	Camptothecin	CRLX101 (Cyclodextrin) (Davis, 2009)
Phase II (NCT01803269) Currently recruiting	**Title: Topotecan Hydrochloride or Cyclodextrin-Based Polymer-Camptothecin CRLX101 in Treating Patients With Recurrent Small Cell Lung Cancer**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Cerulean Pharma Mark Davis (Caltech)	Extensive Stage Small Cell Lung Cancer, Recurrent Small Cell Lung Cancer	Camptothecin	CRLX101 (Davis, 2009)

Phase I (NCT01300533) Currently recruiting	Title: A Study of BIND-014 Given to Patients With Advanced or Metastatic Cancer		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
BIND Therapeutics Robert Langer & Omid Farokhzad (MIT/Harvard) NCL NCI SBIR Office	Advanced or Metastatic Cancer, Solid Tumor	docetaxel	BIND-014 (PLGA-PEG) (Hrkach et al., 2012)
Phase II (NCT01812746) Not yet open	**Title: A Phase 2 Study to Determine the Safety and Efficacy of BIND-014 (Docetaxel Nanoparticles for Injectable Suspension), Administered to Patients With Metastatic Castration-Resistant Prostate Cancer**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
BIND Therapeutics Robert Langer & Omid Farokhzad (MIT/Harvard) NCL NCI SBIR Office	Castration resistant prostate cancer (CRPC), Prostate Cancer	docetaxel	BIND-014 (PLGA-PEG) (Hrkach et al., 2012)
Phase II (NCT01792479) Currently recruiting	**Title: A Phase 2 Study to Determine the Safety and Efficacy of BIND-014 (Docetaxel Nanoparticles for Injectable Suspension) as Second-line Therapy to Patients With Non-Small Cell Lung Cancer**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
BIND Therapeutics Robert Langer & Omid Farokhzad (MIT/Harvard) NCL NCI SBIR Office	Non-small Cell Lung Cancer	docetaxel	BIND-014 (PLGA-PEG) (Hrkach et al., 2012)
Phase I (NCT01158079) Completed	**Title: Multi-center, Open Label, Extension Study of ALN-VSP02 in Cancer Patients Who Have Responded to ALN-VSP02 Treatment**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	Solid Tumors	siRNA targeting vascular endothelial growth factor (VEGF)-A and kinesin spindle protein (KSP)	ALN-VSP02 (lipid nanoparticle) (Maier et al., 2013)
Phase I (NCT00882180) Completed	**Title: Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Intravenous ALN-VSP02 In Patients With Advanced Solid Tumors With Liver Involvement**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	Solid Tumors	siRNA targeting vascular endothelial growth factor (VEGF)-A and kinesin spindle protein (KSP)	ALN-VSP02 (lipid nanoparticle) (Sahay et al., 2013)

Pilot Study (NCT01679470) Currently recruiting	Title: Efficacy Study of AuroLase Therapy in Subjects With Primary and/or Metastatic Lung Tumors		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Nanospectra Biosciences Naomi Halas & Jennifer West (Rice University) NCL NCI SBIR Office	Primary or Metastatic Lung Tumors	n/a (ablation)	Gold nanoshells (Bardhan et al., 2011)
Pilot Study (NCT00848042) Currently recruiting	**Title: Pilot Study of AuroLase Therapy in Refractory and/or Recurrent Tumors of the Head and Neck**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Nanospectra Biosciences Naomi Halas & Jennifer West (Rice University) NCL NCI SBIR Office	Head & Neck	n/a (ablation)	Gold nanoshells (Bardhan et al., 2011)
Phase I (NCT01159028) Not recently verified	**Title: A Phase I Clinical Trial to Study the Safety, Pharmacokinetics, and Efficacy of BP-100.1.01 (L-Grb-2 Antisense Oligonucleotide) in Patients With Refractory or Relapsed Acute Myeloid Leukemia, Philadelphia Chromosome Positive Chronic Myelogenous Leukemia, or Acute Lymphoblastic Leukemia, and Myelodysplastic Syndrome**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
M.D. Anderson Cancer Center Bio-Path Holdings, Inc. Gabriel Lopez-Berestein	Philadelphia Chromosome positive CML, AML, CLL and MDS	Antisense oliognucleotide against Growth Factor Receptor Bound Protein-2 (Grb-2)	BP-100-1.01 (Liposomal Grb-2 antisense oligonucleotide) (Tari et al., 2007)
Phase 1 (NCT01591356) Not yet open for recruitment	**Title: EphA2 Gene Targeting Using Neutral Liposomal Small Interfering RNA Delivery**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
M.D. Anderson Cancer Center Anil Sood (Univ. Texas)	Advanced Cancers	siRNA targeting EphA2, tyrosine kinase receptor in the ephrin family (siRNA-EphA2)	DOPC nanoliposomes (Nishimura et al., 2013)

Alliance Imaging and Diagnostics

Phase IV (NCT00920023) Ongoing, not recruiting	**Title: Pre-Operative Staging of Pancreatic Cancer Using Superparamagnetic Iron Oxide Magnetic Resonance Imaging (SPIO MRI)**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Ralph Weissleder (MIT/Harvard)	Pancreatic cancer	n/a (imaging)	Superparamagnetic Iron Oxide (Guimaraes et al., 2008)
Observational (NCT01773850) Currently recruiting	**Title: Comparison of Stationary Breast Tomosynthesis and 2-D Digital Mammography in Patients With Known Breast Lesions**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Xintek Inc. Otto Zhou (UNC)	Breast neoplasms	n/a (imaging)	multibeam field emission x-ray (MBFEX) technology using the carbon nanotube field emitters (Qian et al., 2009)
Phase I (NCT01626066) Enrolling by invitation only	**Title: Cathepsin Activatable Fluorescent Probe (LUM015)**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Moungi Bawendi (MIT/Harvard)	Sarcoma, Soft Tissue Sarcoma, Breast Cancer	n/a (imaging)	n/a (cathepsin-activated fluorescent probe) (Cuneo et al., 2013)
Stanford IRB-15766	**Title: Advanced Gastrointestinal Endoscopic Imaging**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Sanjiv Sam Gambhir (Stanford University)	Colon cancer	n/a (imaging)	fiber optic-based Raman spectroscopy device for detection of functionalized surface-enhanced Raman scattering (SERS) nanoparticles as molecular imaging contrast agents (Zavaleta et al., 2013)
Stanford IRB-19736	**Title: Detection of Serum Biomarkers for Patients with a Lung Nodule Undergoing FDG-PET imaging**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Shan Wang (Stanford University)	lung nodules	n/a (imaging)	giant magneto-resistive based magnetic nanoparticle protein sensor

IRB Protocol	Collection Of Peripheral Blood From Healthy Human Volunteers For The Optimization Of Magnetic Nanosensor-Based Analyses Of Circulation Microvesicles Collection Of Peripheral Blood From Healthy Human Volunteers For The Optimization Of Magnetic Nanosensor-Based Analyses Of Circulation Microvesicles		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Hakho Lee		n/a diagnostic	diagnostic magnetic resonance imaging (Shao et al., 2012)
IRB Protocol	**Title: Collection of specimens and peripheral blood from people with a suspicious lesion and suspected or known malignancy**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Ralph Weissleder (MIT/Harvard)		n/a (diagnostic)	diagnostic magnetic resonance device (Haun et al., 2011, Ghazani et al., 2012)
IRB Protocol	**Title: Collection of Peripheral Blood and Excess Tissue from Women with Ovarian Cancer for Tumor Cell Detection and Analyses**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Ralph Weissleder (MIT/Harvard)	Ovarian cancer	n/a (diagnostic)	diagnostic magnetic resonance device (Ghazani et al., 2012, Haun et al., 2011)
IRB Protocol	**Title: Nanotechnology-based crculating tumor cell detection and real time profiling of pathway inhibition in patients with metastatic gynecologic cancers**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Cesar Castro (MIT/Harvard)	gynecologic cancers	n/a (diagnostic)	diagnostic magnetic resonance device (Ghazani et al., 2012, Haun et al., 2011)
IRB Protocol	**Title: Collection of excess peripheral blood and lymphatic tissue from patients with melanoma for DMR analysis of tumor biomarkers**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Michael Gee (Massachusetts General Hospital)	melanoma	n/a (diagnostic)	diagnostic magnetic resonance device (Ghazani et al., 2012, Haun et al., 2011)
Observational (NCT01752101) Currently recruiting	**Title: Identification of a Plasma Proteomic Signature for Lung Cancer**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
James Heath and Leroy Hood (NSBCC) Integrated Diagnostics	lung cancer	n/a (diagnostic)	multiple reaction monitoring mass spectroscopy

Observational (NCT01752114) Currently recruiting	Title: Early Diagnosis of Pulmonary Nodules Using A Plasma Proteomic Classifier, Protocol Number 1001-12		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
James Heath and Leroy Hood (NSBCC) Integrated Diagnostics	Precancerous Conditions, Carcinoma	n/a (diagnostic)	multiple reaction monitoring mass spectroscopy
Phase II (NCT00910650) Currently recruiting	**Title: Study of Gene Modified Immune Cells in Patients With Advanced Melanoma (F5)**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
James Heath and Antoni Ribas (NSBCC)	melanoma	n/a (diagnostic)	single cell barcode chip (Ma et al., 2011)
Caltech IRB JH-228	**Title: Measurement of Blood Serum Proteins Using Miniaturized Highly-Multiplexed Platform**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
James Heath (NSBCC)	melanoma and glioblastoma multiforme	n/a (diagnostic)	integrated blood biobarcode chip based on DEAL arrays (Fan et al., 2008)
UCLA IRB approval #10-000655	**Translating SCBC tissue assays into the clinic for identifying targeted therapy combinations for GBM**		
James Heath and Timothy Cloughesy (NSBCC)	glioblastoma multiforme	n/a (diagnostic)	single cell barcode chip (Ma et al., 2011, Shi et al., 2012)

Alliance Non-Cancer Trials

Phase II (NCT01617967) Currently recruiting	**Title: Trial to Evaluate Safety and Tolerability of ALN-TTR02 in Transthyretin (TTR) Amyloidosis**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	TTR-mediated Amyloidosis	RNAi-mediated TTR knockdown	ALN-TTR02 (Nakayama et al., 2012)
Phase II (NCT00496821) Completed	**Title: Intranasal ALN-RSV01 Administered to Adult Volunteers Experimentally Inoculated With Respiratory Syncytial Virus**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	Respiratory Syncytial Virus Infections	RNAi targeting nucleocapsid "N" gene of the RSV genome	ALN-RSV01 (Maier et al., 2013)
Phase I (NCT01814839) Currently recruiting	**Title: A Phase 1, Single- and Multi-Dose, Dose Escalation Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Subcutaneously Administered ALN-TTRSC in Healthy Volunteers**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	TTR-mediated Amyloidosis	RNAi-mediated TTR knockdown	ALN-TTRSC (Foster et al., 2012)
Phase I (NCT01437059) Completed	**Title: Trial to Evaluate Safety and Tolerability of ALN-PCS02 in Subjects With Elevated LDL-Cholesterol (LDL-C)**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	Elevated LDL-Cholesterol (LDL-C)	Stable nucleic acid lipid particles (SNALP)-formulated RNAi Therapeutic targeting PCSK9	ALN-PCS02 (Maier et al., 2013)

Phase I (NCT01559077) Completed	Title: Trial to Evaluate Safety, Tolerability, and Pharmacokinetics of ALN-TTR02 in Healthy Volunteer Subjects		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	TTR-mediated Amyloidosis	RNAi-mediated TTR (targeting transthyretin) knockdown	ALN-TTR02 (Maier et al., 2013)
Phase I (NCT01148953) Completed	**Title: Trial to Evaluate Safety and Tolerability of ALN-TTR01 in Transthyretin (TTR) Amyloidosis**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	Transthyretin Mediated Amyloidosis (ATTR)	RNAi-mediated TTR (targeting transthyretin) knockdown	ALN-TTR01 (Maier et al., 2013)
Phase II (NCT01065935) Completed	**Title: Phase 2b Study of ALN-RSV01 in Lung Transplant Patients Infected With Respiratory Syncytial Virus (RSV)**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	Respiratory Syncytial Virus Infections	RNAi targeting the nucleocapsid "N" gene of the RSV genome	ALN-RSV01 (Maier et al., 2013)
Phase II (NCT00658086) Completed	**Title: Phase 2 Study of ALN-RSV01 in Lung Transplant Patients Infected With Respiratory Syncytial Virus (RSV)**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Alnylam Pharmaceuticals Philip Sharp (MIT/Harvard)	Respiratory Syncytial Virus Infections	RNAi targeting the nucleocapsid "N" gene of the RSV genome	ALN-RSV01 (Maier et al., 2013)
Phase I/II (NCT01224262) Completed	**Title: A Study Evaluating the Safety and Tolerability of a Seasonal Influenza Vaccine Containing LIQ001 (LIfT)**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Joseph DeSimone (UNC) Liquidia Technologies	Influenza	?Influenza hemagglutinin?	Cylindrical PLGA-based PRINT nanoparticles (Galloway et al., 2013)

IRB Protocol	Title: Bacterial Phenotyping of Discarded Specimens Using Nanotechnology		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Hakho Lee (MIT/Harvard)	infectious disease	n/a (diagnostic)	diagnostic magnetic resonance (Chung et al., 2013, Liong et al., 2013)
IRB Protocol	**Title: Exosome analysis of Tb antigens in human serum**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Technology**
Ralph Weissleder (MIT/Harvard)	tuberculosis	n/a (diagnostic)	diagnostic magnetic resonance

Trials on materials characterized by NCL

Phase I (NCT01041235) **Completed**	**Title: Safety Study of a Liposomal Docetaxel Formulation in Patients With Solid Tumors Who Have Failed Previous Therapies**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Azaya Therapeutics NCL	Breast, Ovarian, Pancreatic Cancers, Solid Tumor, Non-Small Cell Lung	docetaxel	ATI-1123 (liposomal formulation of docetaxel)
Phase I (NCT01715168) **Currently recruiting**	**Title: A Crossover Bioequivalence Study of Intravenously Administered ATI0918 and DOXIL/CAELYX in Patients With Ovarian Cancer**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
Azaya Therapeutics NCL	Ovarian Cancer, Malignant Female Reproductive System Neoplasm, Ovarian Epithelial Cancer Recurrent	doxorubicin	ATI-0918 (liposomal doxorubicin)
Phase 0 (NCT00436410) **Completed**	**Title: Tumor Necrosis Factor in Patients Undergoing Surgery for Primary Cancer or Metastatic Cancer**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
CytImmune Sciences, Inc. Larry Tamarkin NCL	Adrenocortical Carcinoma, Melanoma, Sarcoma; Breast, Colorectal, Gastrointestinal, Kidney, Liver Cancer, Ovarian or Pancreatic Cancer	TNF	TNF-Bound Colloidal Gold (CYT-6091) (Libutti et al., 2010)
Phase I (NCT00356980) **Completed**	**Title: TNF-Bound Colloidal Gold in Treating Patients With Advanced Solid Tumors**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
CytImmune Sciences, Inc. Larry Tamarkin NCL	Unspecified Adult Solid Tumor, Protocol Specific	TNF	TNF-Bound Colloidal Gold (CYT-6091) (Libutti et al., 2010)

Phase I (NCT01191775) Completed	Title: A Study of PNT2258 in Patients With Advanced Solid Tumors		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
ProNAi Therapeutics NCL	Cancer Lymphoma, Prostate Cancer, Melanoma	PNT100, a 24-mer bcl-2 targeted oligonucleotide	PNT2258 (liposome encapsulated PNT100)
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
ProNAi Therapeutics NCL	Lymphoma, Non-Hodgkin's	PNT100, a 24-mer bcl-2 targeted oligonucleotide	PNT2258 (liposome encapsulated PNT100)
Phase I (NCT00470613) **Currently recruiting**	**Title: Safety Study of Infusion of SGT-53 to Treat Solid Tumors**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
SynerGene Therapeutics, Inc. Esther Chang (Georgetown) NCL	Neoplasm	wild type p53 gene (plasmid DNA)	SGT-53 (wild type p53 gene encapsulated in a liposome targeted to tumor cells via an anti-transferrin receptor single-chain antibody fragment, TfRscFv) (Senzer et al., 2013)
Phase I (NCT01517464) **Currently recruiting**	**Title: A Phase I Study of Systemic Gene Therapy With SGT-94 in Patients With Solid Tumors (SGT94-01)**		
Key Investigator(s)	**Indication**	**Therapeutic Agent**	**Particle**
SynerGene Therapeutics, Inc. Esther Chang (Georgetown)	Neoplasm	RB94 gene (plasmid DNA)	SGT-94 (RB94 gene encapsulated in a liposome targeted to tumor cells via an anti-transferrin receptor single-chain antibody fragment, TfRscFv) (Pirollo et al., 2008)

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Appendix D

Alliance affiliated INDs and IDEs

Typically, Investigational New Drug (IND) and Investigational Device Exemption (IDE) applications are filed with the FDA by partner companies, and these applications are not public records. However, the Alliance program office asks awardees to inform us of IND and IDE applications submitted for materials or devices developed or studied with Alliance support. The following tables list Alliance or NCL affiliated imaging agents, drugs and devices that have received IND/IDE approval and/or initiated clinical trials or Institutional Review Board (IRB) approved protocols.

More information about clinical testing within the Alliance is given in Chapter 4 of the Program Book, and a full list of Alliance affiliated and NCL supported clinical testing is given in Appendix C.

Imaging

Platform	Material/ Device	Sponsor	IND/IDE status Testing status
[18F]FHBG	PET imaging agent	CellSight Technologies (Stanford Center Partner)	CellSight personnel have been granted FDA "Investigational New Drug" (IND) to image PET reporter gene expression in patients using [18F]FHBG.
18F-L-FMAC	PET imaging agent	Sofie Biosciences	IND 110725 issued March 8, 2011. Clinical testing is being administered through the Department of Molecular and Medicinal Pharmacology (DMMP) at UCLA.
18F-L-FAC	PET imaging agent	Sofie Biosciences	IND 113879 issued November 9, 2011. Clinical testing is being administered by the UCLA DMMP.
18F-D-FAC	PET imaging agent	Sofie Biosciences	IND 112419 issued May 26, 2011. Clinical testing is being administered by the UCLA DMMP.
18F-FAC	PET imaging agent	Sofie Biosciences	Commercially available from Sofie.
LUMO15	Fluorescent imaging probe	David Kirsch (Duke) and ASCO	Phase 1 study to determine safe dose of cathepsin activated fluorescent probe LUMO15.
SPIO	MRI contrast agent	Massachusetts General Hospital	Interventional study, “Pre-Operative Staging of Pancreatic Cancer Using Superparamagnetic Iron Oxide Magnetic Resonance Imaging (SPIO MRI)”

Therapeutics

Platform	Material/ Device	Sponsor	IND/IDE status Testing status
BIND-014	Polymeric drug delivery vehicle	BIND Therapeutics	PSMA targeted docetaxel, currently in Phase 2 trials for castration resistant prostate cancer (NCT01812746) and non-small cell lung cancer (NCT01792479).
CALAA-01	Cyclodextrin nanoparticle drug delivery	Calando Pharmaceuticals	Polymer nanoparticle vehicle for siRNA delivery, currently in Phase 1b safety study in patients with solid tumors (NCT00689065).
CRLX101	Cycoldextrin vehicle for siRNA delivery	Cerulean Pharma	Polymeric formulation of camptothecin, currently in a number of Phase 1 and 2 studies for cancer indications.
ALN-VSP02	Lipid nanoparticle for siRNA delivery	Alnylam Pharmaceuticals	Completed Phase 1 trial of RNAi delivery in liver involved cancers (NCT00882180).
AuroLase	Gold nanoshells for thermal ablation	Nanospectra Biosciences	Pilot studies in patients with refractory head and neck tumors and with primary or metastatic lung tumors.
PDS0101	Versammune R-DOTAP liposome	PDS Biotechnology	Delivery of a peptide antigen derived from E7 oncoprotein of human papillomavirus (HPV) type 16 in a Phase 1 study (IND approved April 2013).
BP-100.1.01	Liposomal Grb-2 antisense oligonucleotide	BioPath Holdings, Inc.	Delivery of antisense oligonucleotide in Phase 1 testing in patients with Philadelphia Chromosome positive CML, AML, CLL and MDS. Trial not verified since 2010.
DOPC liposome	Delivery of anti-EphA2 siRNA	MD Anderson Cancer Center	Phase 1 clinical trial slated to start recruiting in late 2013.
PRINT nanoparticles	Vaccine containing LIQ001 (LIfT)	Liquidia Technologies	Safety study completed of seasonal influenza vaccine containing LIQ001 (LIfT)

Diagnostic Devices and Instruments

Platform	Material/ Device	Sponsor	IND/IDE status Testing status
IBBC	In vitro device for blood proteomics	Cal Tech	Being tested through IRB approved protocols.
SCBC	In vitro device for single cell proteomics	Cal Tech	Being tested through IRB approved protocols.
DMR – magnetic NP based device	Handheld diagnostic NMR device	MIT-Harvard Center/T2 Biosciences	Being tested through at least five IRB approved protocols.
GMR based protein sensor	Handheld diagnostic device	Stanford Center/ MagArray	Giant magneto-resistive based magnetic nanoparticle protein sensor being tested in a Stanford IRB approved study, "Detection of Serum Biomarkers for Patients with a Lung Nodule Undergoing FDG-PET imaging"
CNT source CT scanner	Stationary Digital Breast Tomography	UNC Center/ Xintek	Observational study comparing stationary breast tomography and 2D digital breast tomography in patients with known breast lesions (NCT01773850).
Raman endoscope	Diagnostic imaging	Stanford Center	Stanford IRB approved study "Advanced Gastrointestinal Endoscopic Imaging "

Additional NCL characterized nanomaterials that have received INDs and entered clinical trials.

Platform	Material/ Device	Sponsor	IND/IDE status Testing status
ATI-1123	Liposomal formulation of docetaxel	Azaya Therapeutics	Phase 1 trial "Safety Study of a Liposomal Docetaxel Formulation in Patients With Solid Tumors Who Have Failed Previous Therapies" (NCT01041235) completed.
ATI-0918	Liposomal doxorubicin	Azaya Therapeutics	Phase 1 trial "A Crossover Bioequivalence Study of Intravenously Administered ATI0918 and DOXIL/CAELYX in Patients With Ovarian Cancer" (NCT01715168) currently recruiting.
CYT-6091	TNF-Bound Colloidal Gold	CytImmune Sciences, Inc.	Phase 0 trial "Tumor Necrosis Factor in Patients Undergoing Surgery for Primary Cancer or Metastatic Cancer" (NCT00436410) and Phase 1 trial "TNF-Bound Colloidal Gold in Treating Patients With Advanced Solid Tumors" (NCT00356980) completed.
PNT2258	Liposome encapsulated PNT100	ProNAi Therapeutics	Phase 1 trial "A Study of PNT2258 in Patients With Advanced Solid Tumors" (NCT01191775) completed.
SGT-53	Liposome delivery of wild type p53 gene, with targeting agent TfRscFv	SynerGene Therapeutics, Inc.	Currently recruiting for Phase 1 trial "Safety Study of Infusion of SGT-53 to Treat Solid Tumors" (NCT00470613).
SGT-94	Liposome delivery of RB94 gene with targeting agent TfRscFv	SynerGene Therapeutics, Inc.	Currently recruiting for Phase 1 trial "A Phase I Study of Systemic Gene Therapy With SGT-94 in Patients With Solid Tumors (SGT94-01)" (NCT01517464).

Appendix E

Federal Register Notice establishing TONIC partnership

[Federal Register Volume 76, Number 209 (Friday, October 28, 2011)]
[Notices]
[Pages 66932-66933]
From the Federal Register Online via the Government Printing Office [www.gpo.gov]
[FR Doc No: 2011-27939]

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

The National Cancer Institute (NCI) Announces the Initiation of a Public Private Industry Partnership on Translation of Nanotechnology in Cancer (TONIC) To Promote Translational Research and Development Opportunities of Nanotechnology-Based Cancer Solutions

AGENCY: National Cancer Institute (NCI), Office of Cancer Nanotechnology Research (OCNR), National Institutes of Health (NIH), Department of Health and Human Services (HHS).

ACTION: Notice.

SUMMARY: The Alliance for Nanotechnology in Cancer of the National Cancer Institute (NCI) is initiating a public private industry partnership called TONIC (Translation Of Nanotechnology In Cancer) to promote translational research and development opportunities of nanotechnology-based cancer solutions. An immediate consequence of this effort will be the formation of a consortium involving government and pharmaceutical, and biotechnology companies. This consortium will evaluate promising nanotechnology platforms and facilitate their successful translation from academic research to clinical environment, resulting in safe, timely, effective and novel diagnosis and treatment options for cancer patients.

The purpose of this notice is to inform the community about the Alliance for Nanotechnology in Cancer of NCI's intention to form the consortium and to invite eligible companies (as defined in last paragraph) to participate.

DATES: Interested parties should contact Ms. Sonia Calcagno (calcagnosl@mail.nih.gov) and inform her of their intention to participate. This notice will remain open to accept the inquiries and letters of intent.

FOR FURTHER INFORMATION CONTACT: Ms. Sonia Calcagno (calcagnosl@mail.nih.gov).

SUPPLEMENTARY INFORMATION:
Background: The National Cancer Institute established the Alliance for Nanotechnology in Cancer (ANC) program in September 2004 to facilitate the discovery and development of innovative nanotechnologies

for applications in cancer prevention, diagnosis, and treatment and to address different stages of the developmental pipeline ranging from discovery, applied research through translation. The program has been providing funding to academic groups to support large multi-disciplinary projects--Centers for Cancer Nanotechnology Excellence (CCNEs) along with smaller Cancer Nanotechnology Platform Partnerships (CNPPs) and training programs. NCI also formed an intramural laboratory, the Nanotechnology Characterization Laboratory (NCL), to serve as a centralized facility to characterize nanomaterials. A proposed TONIC consortium will operate in parallel with the
Alliance program and will bring together individuals from sufficiently capitalized pharmaceutical, biotechnology and other healthcare-related companies and start-ups, which either have ongoing internal efforts within their organization or have strategic interest in evaluating the nanotechnology platforms for oncology care solutions, through participating in a academic-private partnership aimed at promoting translational opportunities.

Consortium Goals: Specifically, the TONIC consortium will undertake the key tasks of:

1. Creating a Discussion Forum for opportunities in the nanotechnology platform drug delivery, monitoring and imaging specifically in cancer, but may extend it to other therapeutic indications if an opportunity arises;
2. Developing a Roadmap for the development of nanotechnology-based cancer products;
3. Developing a robust translational model to move promising opportunities based on nanotechnology from academic research to the clinical environment;
4. Evaluating the most promising technology candidates within existing R&D developments and generating Case Studies based on them;
5. Recognizing and promoting translational efforts at every stage of development through appropriate partnerships among industry, academia, government, and philanthropy.

Consortium Membership: Membership to the TONIC consortium will be limited to companies which (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 should have (1) A corporate structure with centralized operations and, (2) the capability and resources to move along the translational efforts effectively and to provide feedback to the academic researchers on industry technological needs. Consortia members will be expected to attend regular meetings and \participate in the project evaluation funded through TONIC consortium.

The following information must be provided by parties interested in participating in the consortium:

(1) The company profile;
(2) The name and specific function of the company representative for the TONIC consortium; and
(3) A brief rationale and/or statement of intent for participating in the consortium.

Dated: October 21, 2011.
Piotr Grodzinski,
Director, Office of Cancer Nanotechnology Research, Center for
Strategic and Scientific Initiatives, National Cancer Institute.
[FR Doc. 2011-27939 Filed 10-27-11; 8:45 am]
BILLING CODE 4140-01-P

Appendix F

Alliance Program Staff

Dr. Piotr Grodzinski is a Director of Nanotechnology for Cancer programs at NCI. He coordinates program and research activities of the Alliance for Nanotechnology in Cancer which has dedicated. The Alliance supports the formation of interdisciplinary centers as well as individual research and training programs targeting nanotechnology solutions for improved prevention, detection, and treatment of cancer.

Dr. Grodzinski is a materials scientist by training, but found bio- and nanotechnology fascinating. In the mid-nineties, he left the world of semiconductor research and built a large microfluidics program at Motorola Corporate Research & Development in Arizona. The group made important contributions to the development of integrated microfluidics for genetic sample preparation with its work being featured in *Chemical & Engineering News* and *Nature Reviews*. After his tenure at Motorola, Dr. Grodzinski joined the Bioscience Division of Los Alamos National Laboratory where he served as a Group Leader and an interim Chief Scientist for the Department of Energy Center for Integrated Nanotechnologies (CINT).

Dr. Grodzinski received his Ph.D. in Materials Science from the University of Southern California, Los Angeles in 1992. He is an inventor on 15 patents and has authored over 100 technical publications and conference presentations. He has been an invited speaker and has served on the committees of numerous bio- and nano-Micro-Electromechanical Systems conferences.

Dr. Dorothy Farrell is a member of program staff in the Office of Cancer Nanotechnology Research (OCNR) at NCI. In her role as a program manager for the NCI Alliance for Nanotechnology in Cancer, Dr. Farrell oversees and manages nanotechnology development projects, implements new nanotechnology development initiatives, and evaluates the effectiveness of Alliance programs. With a background in physical sciences and nanomaterials, she serves as a point of contact within the office for National Nanotechnology Initiative (NNI) programs and is heavily involved in the Nanotechnology for Sensors Signature Initiative within the NNI. She also works closely with the Alliance Nanoformulation and Nanosynthesis and Nanoparticle Biodistribution Working Groups.

Dr. Farrell received her doctorate in Physics from Carnegie Mellon University, where her thesis project focused on the synthesis and characterization of self-assembled arrays of magnetic nanoparticles. She then spent two years at University College London on a Royal Society USA Research Fellowship, where she worked on the preparation of nanoparticle-antibody conjugates for use in cancer therapy. She returned to the United States to work at the Naval Research Laboratory, as part of the National Research Council's Research Associate Program, before joining NCI. Dr. Farrell received her Bachelor of Science degree in Physics from Brooklyn College, City University of New York.

Dr. Lynn Hull is an American Association for the Advancement of Science (AAAS) Science and Technology Policy Fellow working as a projects manager for NCI's Office of Cancer Nanotechnology Research. Dr. Hull programmatically supports grantees in the Alliance. She has taken a lead role on the evaluation of Phase 2 of the Alliance including designing and analyzing a Request for Information to the cancer nanotechnology field, overseeing interviews of members of the Alliance as well as other leaders in the field, as well as bibliometric, portfolio and network analysis of the Alliance members and their research output. Dr. Hull also oversees the Alliance website and plays an important role in communication and integration efforts within the Alliance. She in particular works with the PIs of the Training Centers to organize information collection and sharing activities and best practices sharing.

Dr. Hull completed her graduate studies at Virginia Commonwealth University, earning her Ph.D. in Pharmacology and Toxicology, in August 2009. Her dissertation work examined two separate enzymatic mechanisms which play a role in opioid tolerance. Lynn then joined the Institute for Drug and Alcohol Studies at VCU for her Postdoctoral training where she researched clinical assessments and interventions for alcohol and substance abuse.

Dr. Stephanie A. Morris serves as a program manager for the National Cancer Institute's Alliance for Nanotechnology in Cancer program in the Office of Cancer Nanotechnology Research. She manages nanotechnology research projects overseen by the program and participates in the development of new research initiatives. Dr. Morris is also responsible for developing the nanoinformatics efforts supported by the office and does so by serving on interagency committees and NIH working groups such as NNI's Nanotechnology Knowledge Infrastructure team, and the NIH Leadership teams for the Nanomaterial Registry and the cancer Nanotechnology Laboratory data repositories. Dr. Morris has an interest in establishing opportunities for research collaborations between cancer genomics/proteomics and nanotechnology as well and is the project lead for the administrative supplements supporting Alliance/CTD^2 collaborations.

Prior to joining OCNR, Dr. Morris performed her postdoctoral work at the National Cancer Institute focusing on the genome-wide activity of chromatin remodeling enzymes involved in nuclear receptor function and oncogenesis, and was funded by a UNCF-Merck Postdoctoral Fellowship. She received her Ph.D. in Biochemistry and Biophysics from the University of North Carolina at Chapel Hill. Before pursuing her graduate studies, Dr. Morris worked at the Albert Einstein College of Medicine, where she ran an Analytical Ultracentrifugation Facility in the Laboratory of Macromolecular Analysis and Proteomics. She graduated from Wesleyan University in Middletown, Connecticut with a B.A. in Biology, and Neuroscience and Behavior.

Dr. Nicholas J. Panaro is a Senior Scientist at the Nanotechnology Characterization Laboratory (NCL) and holds a PhD in Chemical Engineering from the Rice University Biomedical Engineering Laboratory (now the Chemical and Biomolecular Engineering Department) and a BS in Chemical Engineering from Drexel University. Dr. Panaro leads the OCNR effort to reformulate failed chemotherapeutic agents including the development of Request for Proposals, overseeing the review of proposals and managing Leidos Biomedical Research subcontract proposals. He works closely with the NCI SBIR Development Center and has developed multiple contract topics for the center resulting in 37 contracts totaling more than $18 million being awarded to 32 cancer nanotechnology start-up companies. Dr. Panaro also serves as a business development manager for the NCL, identifying and recruiting small companies and academic groups with promising technologies to the Nanotechnology Characterization Laboratory for incorporation into their client portfolio. He also developed the scientific agenda for and identified prominent subject matter experts to participate in the inaugural Gordon Research Conference on Cancer Nanotechnology and the Best Practices in Cancer Nanotechnology workshop.

Prior to joining NCL, Dr. Panaro worked as a Patent Examiner at the United States Patent and Trademark Office specializing in the areas of biosensors, microarrays and nucleic acid technologies, as a Visiting Scholar at the University of Pennsylvania specializing in the design, fabrication and optimization of microfluidic devices for genetic analysis, and as a postdoctoral fellow at the National Cancer Institute studying the mechanism of action of Flavone Acetic Acid in endothelial cells, cancer cells and small animal models. He has also worked at E.I. du Pont deNemours as a process engineer and as a software engineer and consulted for start-up companies. Dr. Panaro enjoys learning and has completed training in bioinformatics, robotics and business development.