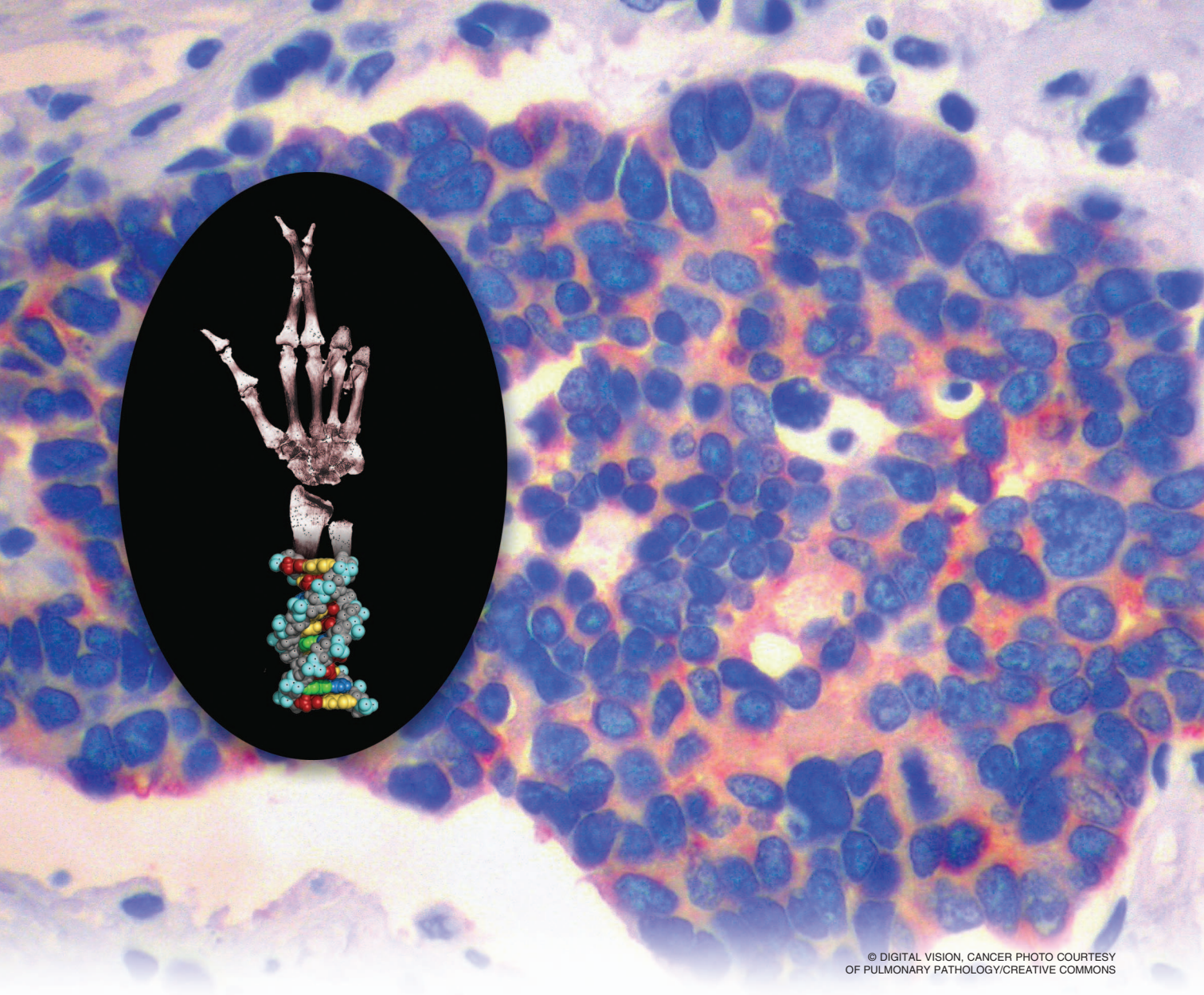

Cancer Therapy Through Nanomedicine

The National Cancer Institute's plan to defeat cancer through engineered design.

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OVER THE COURSE OF THE LAST hundred years, great strides have been made through biomedical research to alleviate the disease burden of many of the scourges of humanity. At the turn of the 20th century, infectious diseases were largely responsible for limiting the life expectancies in the United States to fewer than 50 years. Since then, groundbreaking discoveries in vaccines and antibiotics have decreased the deaths due to infectious disease in the developed world by nearly 100-fold [1]. By effectively removing this mortality factor from the equation, people began to live much longer. As a result, chronic diseases associated with the cumulative effects of life became more and more prominent.

Most prevalent among these are cardiovascular disease and cancer, which respectively have been the top two killers in the United States since the heyday of infectious disease. However, after experiencing dramatic increases in incidence and mortality, cardiovascular disease-related deaths have decreased upward of 50% since 1950 [1]–[3]. These dramatic declines can be largely attributed to novel drug therapies, advanced surgical techniques and devices, and preventative efforts. Meanwhile, although great strides have been made in the understanding of the science of cancer, cancer mortality has surprisingly remained recalcitrant to the point that it is now the top killer of persons younger than 85 years [4].

Infectious and cardiovascular diseases are largely systemic in nature and foreign in composition. These characteristics make them easier to identify and characterize and their treatment specific to the cause. As a result, pharmaceutical treatments of these diseases are typically generally administered, and surgeries or devices address specific local problems with a high degree of resolution.

However, cancer has the unique challenges of being of the self, individually diverse, and heterogeneous. Its treatment has not changed much over the decades, where a regimen of surgery, radiation, and/or chemotherapy continue to be the therapeutic option, each with its own limitations. In surgical resection, it is often difficult to identify the boundaries of a tumor and/or the location of growing cancer before it spreads to other tissues. Chemotherapeutic treatments themselves are toxic to the body's cells, albeit more toxic to the growing tumor, but nonetheless poisonous to normal tissue, making systemic delivery wrought with secondary morbidities.

Furthermore, a treatment that works well on one patient may be ineffective on a patient with a histologically similar tumor because of differences on the molecular scale. The individual tumor may be composed of cells that are vulnerable to a drug, but others readily expel the drug before it can effect treatment. As a result, where an estimated 193.9 per 100,000 people died of cancer in 1950, 186.9 per 100,000 people are expected to die from cancer in 2010, a modest 3.6% decrease in 60 years [1], [4].

We at the National Cancer Institute's Office of Cancer Nanotechnology Research (NCI, OCNR) believe that nanotechnology will provide the platforms from which a revolution in cancer therapy is possible (Figure 1). In this review, we will highlight some of the promising work, particularly those involving engineered devices and particles, emerging from our Alliance for Nanotechnology in Cancer (Alliance) that address the shortcomings in today's cancer diagnosis and treatment regimen. The Alliance is composed of many of the nation's leaders in the field of nanomedical research and is a model of success at the National Institutes of Health (NIH), where it has had among the highest and most impactful scientific output per grant dollar spent since its inception [5].

At the core of the Alliance are nine large multidisciplinary Centers for Cancer Nanotechnology Excellences (CCNEs), where diverse teams focus on developing clinically relevant nanotechnology-based solutions to the challenges in cancer care. Complementing the CCNEs are 12 single-project Cancer Nanotechnology Platform Partnerships (CNPPs), where more basic research is developed with a preclinical focus and seven Pathway to Independence Awards in Cancer Nanotechnology

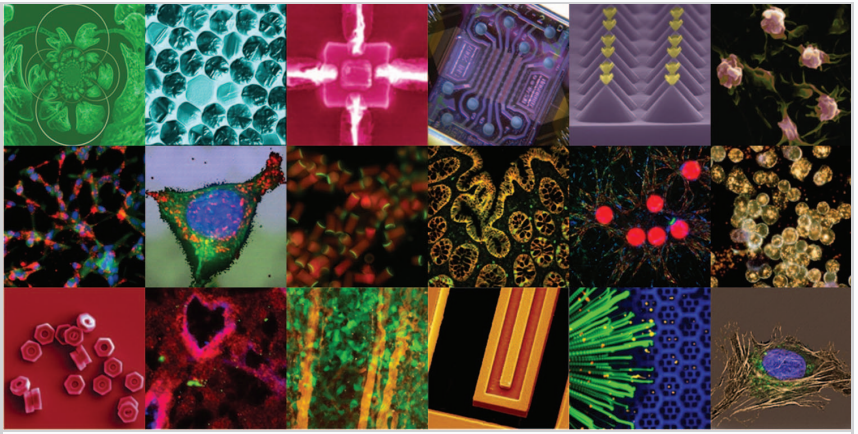

FIGURE 1 Collage of nanomedical particles and devices developed by Alliance members. Downloaded from http://www.nano.cancer.gov/learn/understanding/library.asp. (Photo courtesy of NCI Alliance for Nanotechnology in Cancer, Nanotechnology Image Library.)

for senior postdoctoral fellows transitioning into independent positions.

To facilitate this research, the NCI founded the Nanotechnology Characterization Laboratory (NCL) to perform expert standardized analysis and characterization of the nanoscale materials developed by Alliance researchers as well as the academic, government, and industrial researchers at large. The Alliance also supports six Cancer Nanotechnology Training Centers (CNTCs), where hopefully, the next leaders in nanobiomedical research will be forged.

Together, these groups comprise a nationwide network of research and training institutions that are driving this groundbreaking work (Figure 2). Major developments range between enhanced early diagnostics, novel therapeutic avenues, and improved monitoring and surveillance. By building novel devices on a nanoscale, researchers are taking advantage of recent cell and molecular biology discoveries and uniting them with cutting-edge materials science in a unifying, cross-disciplinary effort to transform cancer detection, diagnosis, treatment, and surveillance.

IMPROVING EARLY DETECTION AND SURVEILLANCE

As a tumor grows, it evolves. By catching a cancer early in its development, survival outcomes are dramatically improved. Currently, only a handful of cancers have screening protocols targeting higher risk populations to detect early-stage tumorigenesis [e.g., routine colonoscopy and/or mammography for patients more than 40 years and spiral computer tomography (CT) scans for older, long-term heavy smokers], and the net efficacy of these methods have been brought into question, typically because of limited scope and/or high false-positive diagnoses leading to unnecessary duress [6]–[8].

A parallel concept is posttreatment cancer surveillance, where a clinician acquires cellular information during and after a patient's treatment to determine the effectiveness of the given therapy, and again the patient would be screened for rare markers of disease. For both surveillance and screening, to detect a rare cancer cell that may initiate a tumor and/or circulating biomarkers, technologies need to be developed that reach several orders of magnitude of sensitivity greater than current practices. To this end, Alliance researchers have been developing devices and particles to detect cancer cells and biomarkers at unprecedentedly dilute levels.

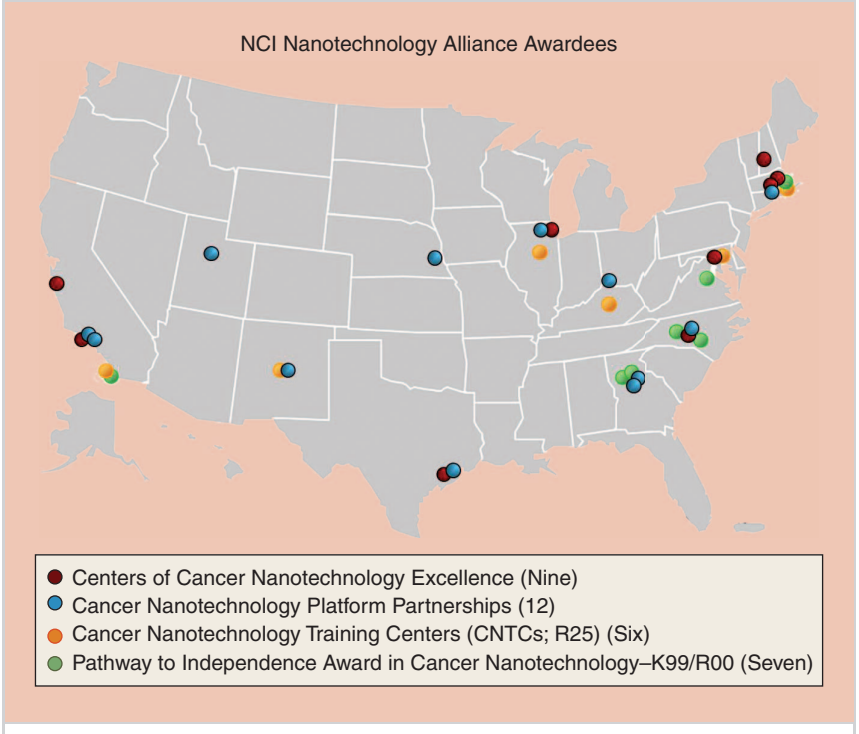

FIGURE 2 Map of the second phase of the Alliance for Nanotechnology in Cancer-funded centers and groups.

At present, the gold standard diagnostic technique for detecting low levels of proteins is the enzyme-linked immunosorbent assay (ELISA). This method involves an antibody fixed to a surface that captures a protein of interest. A second antibody, fused to a detection agent, is then used to bind to the interrogated protein and provide a route to quantify the target. This sandwich method necessitates restrictive buffering conditions, which often limit which proteins can be analyzed and, after a protocol of several hours, provides a lower detection limit of approximately 10 p*M* using colorimetric or fluorometric signals of a narrow linear range.

To circumvent this limitation, Dr. Shan Wang's group at the Stanford CCNE has developed a similar sandwiching protein quantitation method but one that is based on quantum principles of magnetism for detection [9]. Here, the capturing antibody is bound to a giant magnetoresistive (GMR) nanosensor, and the protein of interest gets sandwiched between the capture antibody and an antibody that binds to a magnetic nanotag (mNT). The external magnetic field of the specifically and proximally bound mNT induces a spin-dependent change in the electrical resistance at the GMR, which is detected by a readout device. The GMR nanosensor is an ideal platform for this approach owing to the linearity of its response, low noise, and general insensitivity to media enabling highly quantitative, rapid measurements directly of biological fluids.

The Wang group expanded the utility of this novel approach by integrating multiple GMR nanosensors bound to antibodies to different cancer biomarkers into an 8 $\times$ 8 array. Sequential addition of different secondary antibodies enabled multiplex interrogation of a heterogeneous sample and achieved resolution down to 50 a*M* that is linear over at least six logs of concentration. Remarkably, this procedure can be completed in just 15 min. These results were comparable across a

range of pH, salt balance, or serum in the testing media exacting promise toward future applications involving biological fluids.

Additionally, the sequential addition of interrogating antibodies into the test solution facilitates high-throughput screening for aberrant antibody–antigen interactions enabling better assay optimization [10]. The end result is a platform for protein detection that surpasses the accepted standard protocols by orders of magnitude of sensitivity using a protocol that takes a fraction of the time. Furthermore, the matrix insensitivity of this platform to various media demonstrated that the magnetic nanosensor technology can be directly applied to a variety of settings such as molecular biology or clinical diagnostics toward both early detection and post-treatment surveillance.

Another nanotechnology-based platform for monitoring cancer progression has come from work using gold nanoparticle (AuNP) probes for prostate-specific antigen (PSA) levels from the Northwestern University CCNE led by the research group of Dr. Chad Mirkin. Although the efficacy of PSA for cancer screening and initial diagnoses has come into question, its utility as an unambiguous metric of therapeutic response and postsurgical recurrence is established [8], [11]. Using current screening techniques, the limit of PSA detection is approximately 0.1 ng/mL; serum levels of patients who receive radical prostatectomy typically fall below this threshold, including both patients who remain disease free and those who relapse with deadly consequences.

To distinguish these groups, the Mirkin group designed a detection platform that again uses the antibody sandwich technique but incorporates AuNPs and nucleic acids in the constructs to facilitate detection and reading of analytes [11]. Here, 30-nm AuNPs are coated in functionalized barcode DNA, a fraction of which is conjugated to PSA-specific antibodies. Magnetic microparticles (MMPs) of 1 μm are also coated in PSA antibodies such that, in the presence of PSA, a sandwich of AuNP–barcode-antibody–PSA-antibody–MMP is created that can be magnetically sorted from a solution. The barcodes are then released from the AuNPs, and half of each barcode sequence binds to complementary oligonucleotides immobilized on a glass slide. As there are many barcodes bound to each AuNP, this release serves as an important amplification step.

> Infectious and cardiovascular diseases are largely systemic in nature and foreign in composition.

The other half of the now immobilized barcode DNA is recognized by a universal scanometric DNA probe linked to a detection-AuNP. PSA levels can be quantitatively extrapolated from the intensity of light scattered by the barcode-mediated probe-bound AuNPs using a proprietary Verigene ID system developed and marketed by a company established by the investigator. Using this biobarcode assay, the Mirkin group could reproducibly detect PSA levels almost three orders of magnitude more sensitive than the current methods. This technology enables the delineation of cured patients from those with gradually increasing PSA levels, indicative of relapse, nearly one year sooner than current ELISA assays permit.

Cancer diagnosis is typically performed on tissue samples that have been surgically removed, but such biopsies only provide a snapshot of a tumor's physiology. Often, by the time test results are produced, they may no longer be relevant. Hence, there is a goal to create a diagnostic platform that could continually monitor patients longitudinally to track how they are responding to treatment. Toward this goal, Dr. Michael Cima and his colleagues from MIT Harvard CCNE have developed an implantable device with the potential to measure biomarker concentrations as indicators of the local tumor environment [12].

The device, which uses nanoparticle magnetic relaxation switches (MRSws) as reporters, is a cylindrical, 5×1.5 mm implant made of high-density polyethylene encased in a polycarbonate membrane with 10-nm pores. The semipermeable membrane that covers the MRSw reservoir allows circulating cancer biomarkers or chemotherapeutic agents to diffuse into the device and interacts with the MRSw while preventing diffusion of the MRSw into the tissue environment.

The switches themselves are magnetic 4-nm nanoparticles with a superparamagnetic iron oxide core in a dextran shell bearing functional groups that can react with biological targets, such as nucleic acids, receptor ligands, proteins, small molecules, and antibodies. The MRSw aggregate reacts with an analyte, causing a decrease in the transverse relaxation time ($T2$) that is detectable by magnetic resonance imaging (MRI).

Dr. Cima's group has recently extended their in vitro work and demonstrated detection of a model cancer biomarker, the beta subunit of human chorionic gonadotrophin (hCG-β, a soluble biomarker that is elevated in testicular and ovarian cancer patients), in proof-of-principle in vivo sensing experiments using mice [13]. The devices were implanted at the tumor site and successfully monitored hCG-β levels over a period of several days and the data verified using ELISA analysis of plasma. However, the detection limit of the device was shown to be 0.5 μg/mL, which is significantly higher than hCG-β levels found in many ovarian and testicular cancer patients. Therefore, this platform will require further refinement before clinical implementation. Regardless, this is a promising start in the development of an implantable, continuous monitor for cancer biomarkers.

Cancer has the unique challenges of being of the self, individually diverse, and heterogeneous.

TREATING THE DISEASE: NOVEL CANCER IMAGING AND THERAPIES

In many solid tumor cases, the most effective treatment remains surgical resection. However, today's surgeons must rely on visual and tactile recognition of tumor locations and boundaries to determine what tissue to remove. Therefore, in many cases, too little or too much tissue is cut out leading to tumor regrowth or unnecessary secondary morbidities. Alliance researchers are developing novel nanoparticle-based contrast agents to improve the existing imaging modalities such as MRI, and one group has developed a novel twist on a current imaging platform and is making headway toward its commercialization.

Clinical trials are expected to begin soon on a new type of CT scanner developed by Dr. Otto Zhou at the University of North Carolina (UNC) CCNE that uses carbon nanotubes (CNTs) as an X-ray source. This new scanner, jointly developed with Siemens and Xintek (a spin-off company founded by UNC CCNE members), contains 52 CNT X-ray sources and detectors arranged in a ring [14]. The basic design of the X-ray tube has not changed significantly since X-rays were first translated into an imaging tool: a thermionic cathode emits electrons that strike a metal target generating X-rays. This design has several intrinsic drawbacks that have limited the effectiveness and advancement of X-ray technologies for decades. These limitations include the high operating temperature of the cathode (approximately 1,000 °C), which prevents miniaturization and novel source configurations that could increase imaging speed and accuracy; difficulty in targeting high imaging doses resulting in offsite radiation damage to sensitive tissues; and low temporal and spatial resolution affecting the size and accuracy of the features that can be detected.

The CNT-based field emission X-ray source has the potential to not only overcome these limitations but also enable new imaging modalities. For example, traditional CT scanners use a single X-ray source that takes hundreds of images from multiple angles by rotating the X-ray source about the subject at high speed. This dynamic micro-CT scanner has multiple X-ray sources and requires no mechanical motion; instead, it accomplishes the 360° angle of interrogation by switching rapidly among its multiple X-ray sources, each taking an image of the object from a different angle. This multiple X-ray source innovation also enables multiplexed imaging in which all the X-ray sources are turned on simultaneously to capture images from multiple views at the same time. This should reduce the imaging time and radiation dose while improving image resolution by reducing motion-induced blur. It has been validated as a promising tumor imaging technology, using breast phantom models, able to detect dense masses of just a few millimeters [15].

As previously mentioned, while the spectrum of cancer treatments has grown over the last several generations, the efficacy of many of these treatments has been modest and/or limited because of the adverse side effects and drug resistance. Engineering novel treatment modalities is a centerpiece of many of the research groups within the Alliance. These involve light-induced thermal ablation using targeted AuNPs, lipid-based carriers to specifically deliver potent drugs directly to the tumor diminishing offsite toxic effects, novel platforms for crossing biological barriers, RNA-based structures for the delivery of drug and nucleic acid-based therapies, and others.

Among the more confounding problems facing treatment of some cancers are biological barriers that isolate many tissues from the circulation environment, blocking drug delivery. Prominent among these is the blood–brain barrier (BBB) that separates circulating blood from cerebral spinal fluid via tight junctions between cells of blood vessel walls in the central nervous system (CNS). Only small hydrophobic molecules and dissolved gases can readily diffuse across the BBB, whereas transport of important large or polar biomolecules is facilitated by specific receptors on the cell membrane that actively carry these substrates into the CNS environment.

Glioblastoma multiforme (GBM) is the most common brain lesion, which is characterized by its aggressive growth and >95% mortality by five years post-diagnosis. Currently, the best treatment involves a combination of surgery, radiotherapy, and chemotherapy. Collectively, this standard regimen grants a median survival of 14.6 months and carries a significant risk of permanent secondary injury and profound neurological impairment [16].

To address this disease, the group led by Dr. Julia Ljubimova at the Cedars-Sinai Medical Center CNPP has been engineering a complex nanoplatform to pass through the BBB, target GBM, and deliver therapeutic agents. The foundation of their particle is a polymer from the slime mold *Physarum polycephalum* that exhibits a variety of appealing characteristics: water solubility, low immunogenicity, low toxicity, and covalent conjugability. Thus, the Ljubimova group has loaded particles with functional groups to specifically address roadblocks associated with GBM treatment [17].

Using a mouse model of GBM, this group injected their nanoconjugates into the vasculature, where they eventually reached the BBB. To traverse the BBB, an antibody was conjugated that recognizes endothelial transferrin receptor (TfR) such that particles bind the endothelium of the BBB and are actively transported through the capillary barrier

into the brain parenchyma. From here, tumor cells are targeted via another specific antibody tailored to activate the cancer cells' endosomal uptake pathway, where the external molecules are taken into the cell in an encapsulating vesicle.

Typically, such an endosome would gradually become an acidified digestive lysosome where uptaken molecules are destroyed. However, the Ljubimova group built in a novel endosomal escape route to their nanoconjugate: a virus-derived trileucine peptide sequence that lyses endosomes when their pH begins to drop. This releases the nanoconjugate into the cell's cytoplasm, where it releases its treatment payload. In this case, an RNA sequence leads to the specific degradation of gene transcripts coding for laminin-411, a protein that has been associated with GBM recurrence and poor prognosis. These experiments proved the first successful in vivo treatment of laminin-411.

Using these methods, tumors were efficiently and specifically targeted and their growth dramatically retarded. Excitingly, this platform has recently been translated into another disease model and has proven similarly effective in treating breast cancer [18].

The laboratory of Dr. Peixuan Guo at the University of Cincinnati CNPP is engineering an entirely new class of cancer therapy platforms based on the RNA skeleton of the bacteriophage phi29's DNA-packaging nanomotor. This motor is central to translocating the bacteriophage DNA genome and is built from stable dimers and trimers of packaging RNA known as pRNA [19]. Some of the advantages of using RNA as a foundation of nanoparticle engineering are their capacity for in vivo self-assembly, their defined structure and stoichiometry, multivalency, and nonimmunogenicity.

The Guo laboratory has spearheaded the research, showing how these pRNA moieties can serve as building blocks to create a menagerie of shapes and structures that are starting to find their purchase in biomedical research. Using different configurations of monomers, differently oriented dimers, and tetramers, this CNPP has constructed nanoparticles exhibiting enzymatic activity, specific aptameric binding, RNA silencing, and/or conjugation of receptor ligands and detection molecules.

FIGURE 3 Participants of the kick-off meeting of the Alliance for Nanotechnology in Cancer, Phase II, November 2010.

One of the successes in this field by the Guo laboratory has been to overcome the notorious sensitivity of RNA to RNase digestion by replacing the position 2-hydroxyl group with fluorine on the pyrimidines cytidine and uridine [20]. This substitution renders RNA oligomers that are virtually impervious to RNase degradation while maintaining the self-assembly and catalytic functions of the RNA even across a broad pH range. This chemical modification should increase the clinical utility of recent results, where pRNA-based constructs were used to deliver silencing RNA sequences to cancer cells resulting in cell death [21].

WHERE DO WE GO FROM HERE?

The first phase of the NCI's Alliance for Nanotechnology in Cancer, 2005–2010, heralded a new approach to biomedical research. The Alliance promoted a convergence of specialists in oncology, materials science, biomedical research, and engineering to work together toward developing transformative nanotechnology for cancer medicine. The program was a remarkable success, producing more than 1,300 publications (with an average impact factor of 7.4), more than 250 patent disclosures and applications, dozens of spin-off companies, and several clinical trials [5].

The Alliance, in its second phase (2010–2015), has no intent to rest on its laurels. Following another round of competitive, nonrenewal, grant reviews, the OCNR is funding another batch of enthusiastic investigators with many new faces (Figure 3). The foundations of this new phase are again grounded in research driven by the CCNEs and CNPPs with technical characterization support from the NCL while maintaining a goal of training the next-generation's leaders in nanomedical research at the CNTCs. Central themes of this second phase are increased interinstitutional collaboration and leveraging funding toward greater clinical development with a goal of each CCNE sending at least one product into clinical studies by the end of the phase.

Through this collective multiplatform expertise, collaboration, and ingenuity, we expect the contributions made by the Alliance toward fighting cancer to lead the way in breakthrough technology toward alleviating the burdens of cancer. We invite the reader to gain further insight and to follow the progress of the Alliance at our Web site at http://nano.cancer.gov/.

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