Cancer Nanotechnology Symposium
Nanotechnology: Visualizing and Targeting Cancer

National Cancer Institute Symposium

Frederic de Hoffmann Auditorium
Salk Institute for Biological Studies
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Executive Summary

On March 3, 2004, the NCI held the second of what is expected to be a series of Nanotechnology Cancer Symposia whose primary purpose is to foster the interdisciplinary teamwork needed to leverage the promise of nanotechnology to detect, prevent and treat cancer. This symposium, *Nanotechnology: Visualizing and Targeting Cancer*, was held at the Salk Institute for Biological Sciences in La Jolla, CA, and was hosted by Dr. Geoffrey Wall, Professor of Biology at the Salk Institute and chaired by Dr. Mauro Ferrari, Special Expert on Nanotechnology for the NCI and Professor of Biomedical Engineering and Internal Medicine at Ohio State University. The day-long meeting, attended by over 100 cancer biologists, engineers, chemists and oncologists from NCI's cancer center in San Diego, CA, along with investigators from other leading research institutions in the San Diego area, included lectures on cancer biology and nanotechnology, and produced an active exchange of ideas with the goal of developing a common understanding of nanotechnology and its potential applications to cancer. Additionally, the NCI sought input from symposia participants as the Institute puts the final touches on its Cancer Nanotechnology Plan (CNP), a strategic initiative aimed at rapidly translating promising nanotechnologies into clinical and research advances.

Two keynote speakers gave broad overviews of cancer and nanotechnology, with one lecture on the growing role of systems biology approaches to studying cancer and the other outlining some of the different ways in which nanotechnology can impact cancer research and clinical oncology. The symposium featured several talks on nanoscale laboratory-on-a-chip type applications that would benefit both basic research and cancer diagnostics, as well as nanodevices designed for *in vivo* and *ex vivo* use in deciphering cancer genomics and proteomics for diagnosing and characterizing cancer. Symposium speakers also discussed several different types of nanoparticles that could prove useful in creating multifunctional imaging, diagnostic and therapeutic devices.

The ensuing roundtable discussions highlighted some of the important features needed from any nanoscale device developed for either research or clinical use. These included the ability to target multiple types of cancer with a high degree of specificity and sensitivity. In particular, researchers express a strong wish for nanoscale devices with the ability to make multiple simultaneous measurements of molecules or pathways involved

in cancer, since the difference between malignant and normal cells are likely to be reflected in relative changes among various pathways relative to one another.

The roundtable discussions also stressed the need for multidisciplinary partnerships and for new mechanisms to both support them and to facilitate collaborations among laboratories that may not be at the same institution or even in the same city. Finally, there was a recognition that the current funding situation presents both challenges and opportunities to develop new mechanisms that more effectively and efficiently encourage the translation of basic research into clinical advances.

Introduction

If the Nation is to meet the 2015 goal of eliminating death and suffering from cancer, clinicians will need new ways to detect, treat and prevent cancer and metastases. One expectation of the field is that this era of post-genomic science, with its emphasis on genomic and proteomic analysis and systems biology, will generate unprecedented advances in knowledge, fostering paradigm-changing diagnostics, therapeutics and preventatives. At the same time, nanotechnology is rapidly making a mark among a small but growing group of NCI-funded cancer researchers as a disruptive set of tools capable of leveraging scientific advances into a new generation of targeted clinical agents. Indeed, at least one nanotechnology based imaging product has demonstrated success in detecting micrometastases in humans and is awaiting FDA approval, and several other nanoparticulate imaging and therapeutic agents are showing remarkable promise in advanced animal models of cancer.

As a key component of its Cancer Nanotechnology Initiative, the NCI desires to boost the number of cancer researchers who are interested in applying nanotechnology to the problem of diagnosing, treating and preventing cancer. To help accomplish this, the NCI is holding a series of symposia aimed at introducing nanotechnology and its potential uses to as broad an audience as possible within the cancer and nanotechnology communities. An ancillary goal of these symposia is to solicit broad scientific input to provide direction to research and engineering applications and to identify barriers that are or may impede progress.

On March 3, 2004, the NCI convened the second of these symposia[1] at the Salk Institute for Biological Studies in La Jolla, CA, titled *Nanotechnology: Visualizing and Targeting Cancer*. Over 100 investigators from academia, industry and government participated in this symposium. Dr. Geoffrey Wall of the Salk Institute, who hosted the meeting, gave a brief introduction, explaining that the purpose of the day's proceedings was to solve a key barrier in applying nanotechnology to cancer research: most cancer biologists and clinicians do not know much about nanotechnology.

To set the stage for remaining talks, Dr. Mauro Ferrari, who shares a joint appointment with the NCI and the Ohio State University, first laid out the objectives of the meeting:

[1] The first in the series, *Building the Interface of Nanotechnology and Cancer Imaging Research* was held January 28, 2004, in Bethesda, MD.

- Fostering exchange of information between cancer and nanotechnology researchers;
- Facilitating regional self-assembly of multidisciplinary teams; and
- Obtaining conceptual feedback on strategic directions for integration in Cancer Nanotechnology Plan.

He then presented the outlines of the Cancer Nanotechnology Plan, which as it currently stands is evolving along with three key components: extramural research, intramural research, and the soon to open National Standardization Laboratory (NSL). He also listed the seven challenge areas that the plan will address:

- Fundamental science, such as creating nanodevices that can pick up molecular signatures to provide the evolution over time of molecular signals and pathways;
- Prevention and control, such as bioengineered vaccines;
- Early detection, a key area, that will focus on topics such as enhancing mass spectroscopy-based proteomics and selectively harvesting molecules from blood and other tissues;
- Imaging, including developing injectable nanoparticles as targeted smart contrast agents that can at the simultaneously release therapeutics;
- Multifunctional devices, such as cellular factories and particles that can image, treat and report on the efficacy of treatment;
- Quality of life, which will examine using nanodevices to deliver medication to control pain, nausea and other side-effects, particularly in cancer patients whose cancers are beyond the help of therapy; and
- Cross-disciplinary training, which is central to meeting the other challenges

Dr. Ferrari then elaborated further on the function of the NSL, which will create the ideal environment for the confluence of nanotechnology and cancer research, as well as providing the field with a facility that will develop multi-station protocols for validation, comparison, objective evaluation of nanodevices designed for eventual clinical use. The objectives of the NSL are three-fold: to develop multi-station protocols for validation, comparison, and developing indications for a wide variety of nanoscale materials; collaborate with the FDA to develop a rigorous, accelerated pathway towards clinical translation; and to provide a place for the synergistic engagement of the private sector in developing cancer-directed nanotechnologies.

Cancer Biology and Nanotechnology Keynote Addresses

Dr. Leroy Hood of the Institute for Systems Biology gave the first of two keynote addresses, focusing of a systems biology approach to cancer, with its emphasis on multiparameter analysis, enables new strategies that will make it possible to meet the NCI's challenge goal of eliminating suffering and death from cancer by 2015. But to get the most out of systems biology, nanotechnology must yield advances in nanoscale laboratory analysis, or chip-based nanolabs, and molecular imaging. He also stressed the need for new organizational structures for enabling multidisciplinary research and for new computational tools capable of analyzing complex, multiparameter data.

What is systems biology? Dr. Hood defined it as the identification of the elements in a system and the analysis of their interrelationships so as to explain the emergent properties of the system. In other words, taking a system, defining its elements and how they

interact with one another in the form of networks, and using the interactions of these networks to explain the biology that we actually see in a cell or organism. The two types of information that feed into this approach take the form of digital information encoded in the genome and environmental cues, both deterministic and stochastic, that impinge on the system. He then outlined the steps involved in taking a systems biology approach to cancer:

1. Define all elements – the discovery science phase.
2. Use all preexisting information to define a descriptive, graphical or mathematical model.
3. Perturb system to carryout functions and measure global relationships of elements one to another.
4. Integrate information; compare model and experimental observations; formulate hypotheses to explain disparities; perform simulations to check veracity of developing model.
5. Iterate steps 2-4, recasting the model each time until model and experimental observations are in accordance.
6. Formulate a mathematical model that will explain the origins of emergent or systems properties, predict systems behavior given any perturbation, and permit the system to be redesigned with new systems properties.

He then briefly showed two examples of how taking such an approach have lead to models of yeast physiology and sea urchin development.

Dr. Hood then began discussing how taking a systems biology approach can lead to advances in cancer diagnosis and treatment. Normal development and physiology, he said, are mediated by protein and gene regulatory networks, while cancer reflects abnormal regulation of networks that are perturbed by defective genes or pathologic environmental cues. Such disease-perturbed networks change dynamically during disease progression, and these changes should be detectable as unusual patterns of secreted proteins or protein fragments in blood or by altered patterns of gene expression in blood components. Multiparameter analysis is essential, however, to detecting these perturbations, and this involves analyzing thousands of mRNA or protein changes. The main challenges, then, are ones of sensitivity and signal-to-noise, given that 12 proteins constitute about 99 percent of the proteins in blood and changes in their levels are not likely to be that informative.

There is also the challenge of correlating specific parameter changes with specific diseases, but work at the Institute for Systems Biology is showing that such analyses are possible. Work in mouse models, for example, has shown that changes in the relative amounts of 100 different peptides, out of 3000 unique peptides identified in a single run using liquid chromatography-mass spectroscopy, can distinguish mice with cancer from those that are healthy. Studies using a second-generation gene fragment analytical system that is reportedly more sensitive than DNA microarray technology have identified differences between different human prostate cancer cell lines by analyzing two million gene signatures. Similarly, investigations using DNA microarrays and quantitative proteomics have identified over 100 potential gene and proteomic markers for human prostate and ovarian cancers.

Dr. Hood closed his talk by arguing that taking a systems biology approach to cancer will break down the artificial barriers that exist between diagnosis, treatment and prevention of cancer. Diagnosis, for example, will determine the molecular characterization of given tumor, which in turn will determine which therapy is appropriate. Diagnosis will delineate the state of tumor progression based on the networks that are perturbed, which in turn will identify the nodal points in those networks for therapeutic intervention. Diagnosis that becomes predictive then also leads to interventions that will stabilize networks before they lead to disease. He finished his talk by saying that none of this will happen, however, unless we develop a new research infrastructure that makes interdisciplinary collaboration the norm rather the exception.

Dr. James Baker of the Center for Biologic Nanotechnology at the University of Michigan School of Medicine presented the morning's second keynote address on nanotechnology applications for biology and medicine. What nanotechnology brings to cancer research is the ability to construct devices on the same operating scale as the central structures of life, ranging from DNA and proteins, to cellular components such as receptor complexes and mitochondria, and finally cells. And because of the benefits of working at this scale, nanotechnology, Dr. Baker predicted, will benefit cancer research in terms of facilitating real-time molecular analysis, creating *in vitro* and *in vivo* diagnostics that go beyond detection and actually monitor some of the systems inside cells that Dr. Hood discussed, and develop targeted, multifunctional therapeutics that will be able to enter cells and affect multiple pathways simultaneously. But these advances will only happen if nanotechnology applications provide unique capabilities, are biocompatible and non-toxic, and lead to cost savings through reduce utilization of the health care system and improved outcomes when treatment is needed.

Dr. Baker then turned his discussion to smart nanoscale devices. These, he said, are characterized by an ability to target to a particular site on or within a cell. They have an imaging function that documents the presence of cancer and can sense for pathophysiologic changes that then triggers release of one of several on-board therapeutic agents appropriate to treat a given change. Such devices may also release their therapeutic payload in response to an externally applied stimulus, such as a magnetic field. Finally, such devices will document the response to therapy and have the means to send that information to an external monitoring source.

As an example of what engineered nanoscale devices can do, Dr. Baker discussed some of his group's work with synthetic dendrimers, non-immunogenic, spherical polymers with defined, modifiable structures. Dendrimers can be synthesized in a variety of uniform sizes, allowing for the selection of particles that will clear through the kidney if they are not taken up by cells. Through a series of computational modeling studies, Dr. Baker's group was able to create a dendrimer that would carry multiple functionalities on its surface, and decorate it with methotrexate (the therapeutic agent), folate (a targeting agent), and fluorescein (an imaging/detection agent). Animal studies have shown that this agent specifically targets folate-bearing tumor cells, delivering methotrexate with a therapeutic index between 20- and 100-fold higher than with free drug. More importantly,

animals receiving the nanodevice survived their cancer, with tumors undergoing necrosis. Fluorescence imaging, using a fiber optic probe, was able to detect the cells being treated.

Dr. Baker then reviewed a number of potential uses for nanotechnology in the cancer arena. He showed, for example, how fluorescent imaging could be used to visualize dye-conjugated dendrimers in live animals, and how nano-textured gold surfaces could be prepared that selectively adhered to tumor cells. But each of these indications faces a number of scientific and social barriers. On the science side, he said that nanotechnologists have yet to show that nanoscale devices are non-toxic, and that they can work reproducibly in a complex biological system. Regulatory approval of nanoscale devices is not a given, nor are the economic advantages of nanotechnology apparent yet. If society is to accept nanoscale device, it is critical not to hype this technology, yet to be open about potential problems and limitations. There is also the possibility that not all nanoscale devices will be devoid of untoward uses – for example, would a device capable of monitoring brain function be a good thing?

Session 1: Advanced Technology to Reveal the Molecular Complexity of Cancer
This session began with a talk by Dr. Joe Gray of the Lawrence Berkeley National Laboratory, who highlighted efforts to use nanodevices in a variety of cancer-related applications. One such device, for example, would act as a molecular scavenger that would capture thousands of substances in blood for subsequent use in detecting cancer in its earliest stages, as well as characterize the effect of therapy. Another nanotechnology under development would analyze genome complexity, which varies dramatically across cancer genotypes. Early work on one such system, he said, has shown that early stage tumors expressing similar phenotypes can be distinguished on the basis of how each tumor selects a slightly different approach to derange its genome, something that can be detected. He also told the audience about an automated nanoanalytical system under development that would mimic the extracellular environment, which is critical to the behavior of malignant cells, while allowing researchers to gain detailed molecular profiles of multiple cancer cells simultaneously. One caveat to *in vivo* work, he said, was the remaining uncertainty about the toxicity of different types of nanoscale constructs. He then reiterated early comments that nanotechnology should prove useful if researchers can develop selective targeting approaches and if they can construct nanoscale therapeutics that can report back on the success of their deliveries.

Dr. James Heath of the California Institute of Technology, noted that diagnostics and therapeutics are becoming intimately coupled in this era of genomics and proteomics, and that in the this new world of stratifying cancer according to its molecular signature it is hard to imagine it would be possible to develop a therapeutic without a diagnostic to go along with it. Toward that end, he discussed various approaches to using nanotechnology to acquire multiparameter molecular and genomic data and then using software to integrate this data into a systems diagram that would inform both diagnosis and treatment of cancer. He then briefly talked about nanoscale laboratories, or nanolabs, that he and his colleagues are developing at Caltech. Currently, such nanolabs can analyze 16 inputs simultaneously, providing measurements on 1000 chemical environments at the level of the individual cell. Such devices, he said, can also be constructed to make

electrophysiological measurements, which when coupled with microfluidic technology can afford the possibility of sorting cells and then measuring their electrical behavior. Early experiments with such a system have been able to detect differences among individual leukemia cells, for example.

Dr. Health then discussed the use of nanowires whose conductivity varies as substances bind to ligand on the wires' surface. The possibility exists, he said, to lay down a series of wires and coat each with a different receptor, ligand or antibody, allowing each to detect a single protein. Currently, however, there is no good manufacturing technology available to make such systems reproducibly in large numbers and to address each wire in such a device. As these obstacles are solved, such systems will become invaluable to systems biology research given the multiparameter nature of these devices and the data they will generate.

The final talk of the morning, by Dr. Richard Caprioli of Vanderbilt University School of Medicine, presented promising results using nanodevices to enable multiparameter MALDI-TOF mass spectroscopic analysis of tumor cell proteins. Such systems would perform molecular biopsies on tumor cells, which if correlated to clinical outcome would give physicians an important diagnostic tool that would inform their therapeutic approach for each patient. Preliminary comparisons of normal tissue versus cancerous lung tissue have shown it is possible to predict metastasis two years prior to its first appearance with 80 percent accuracy. Similar results with glioma and colon carcinoma samples suggests that with further refinement, mass spectroscopy could prove to be a paradigm-changing tool for cancer diagnosis and therapy.

A roundtable discussion followed the morning's presentations. Key points raised in this discussion were:

- Targeted delivery of nanoparticles is a reality, and utility of these nanoparticles will increase dramatically as proteomics, genetically defined mouse models and other research tools identify additional targeting moieties.
- Phenotypic characterization of tumors has to be specific, quantitative and based on multiple parameters recorded simultaneously in a manner that allows internal comparisons of relative pathway expression. Sensitivity of such measures is a critical issue, as is coupling molecular and pathway changes with phenotype.
- There is an urgent need for mechanisms to support multidisciplinary collaboration over the long term. The NSF technology hub initiative was given as an example of a successful mechanism that was unique because the funding lasted 11 years.
- It is necessary to leverage technology development through industrial collaboration, since NIH funding should only be for early stage research, with industry providing support for long-term development.
- NCI should use the current "difficult" funding environment as an opportunity to assess its current research portfolio with an eye toward creating new, more efficient mechanisms for funding technology development and applying that technology to the cancer field.
- The NSL should focus its initial efforts on developing standards for nanoscale particles and devices that will help further the field.

Session 2: Targeting Cancer Diagnostics and Therapeutics
After a lunchtime discussion of NCI technology funding opportunities, led by the NCI's Ed Monachino, Dan Gallahan, Paul Wagner, and Avi Rasooly, who also holds a joint appointment with the Food and Drug Administration (FDA), symposium attendees were treated to three talks that gave an exciting view of how nanotechnology will help create novel cancer diagnostics and therapeutics.

Dr. Abraham Lee of the University of California, Irvine, reviewed his group's efforts to create integrated microfluidics and nanoscale analytical tools capable of providing real-time molecular signatures of cancer cells. The goal of this work is to improve both diagnostics and therapeutics. Deciphering biological complexity, said Dr. Lee, will require the development and use of both physical and computational tools for biological problems. Thus, the field needs new devices capable of interrogating living cells and single molecules to help biologists characterize the physical properties of a cell's molecular components, determine their numbers and their location. There is also a need for new mathematical approaches for analyzing massive quantities of information and reducing the dimensionality of primary data. New computational tools are required to model and simulate the dynamic behavior of biological systems at appropriate levels of granularity while being faithful to the physical and chemical behavior of system components.

Today, said Dr. Lee, research still proceeds much as it has for centuries, with labor intensive, macro scale methods still the norm. He detailed two nanoscale platforms that his group is developing: a microfluidics system and a method to perform bioassays on droplets. He demonstrated a variety of nanoscale metering systems capable of creating droplets of various sizes with precise control. He also showed nanoscale systems for mixing such droplets, controlling their chemical composition, and sorting them, as well as for trapping isolated droplets for multiplexed analysis or for use as nanoscale drug delivery vesicles. Potential applications that his laboratory is pursuing include intelligent protein crystallization, multiplexed biosensors, droplet-based bioassays, and molecular motors for vesicle transport.

Dr. Marianne Manchester of The Scripps Research Institute, then presented promising data on the use of engineered viruses as nanoscale tumor imaging and drug delivery devices that would be targeted to specific tumors in the body. The goal of this project, which is aided by the interdisciplinary team that she works with at Scripps, is to develop virus-based nanoparticles that can specifically recognize tumor cells and function as image contrast agents and can deliver tumor-killing agents *in vivo*. The Scripps team has been working with two viruses, cowpea mosaic virus (CPMV) and flock house virus (FHV), both of which are easily and inexpensively produced in large quantities and whose coats can accommodate specific ligand attachment or insertion. Using her work with CPMV to illustrate the potential of these viruses, she showed the results of using genetically modified capsid proteins to create particles with 60 targeting peptides on their surfaces. Because of the large number of targeting molecules, binding to the chosen target increased 100-fold tanks to cooperative binding. Working with either CPMV or

FHV is easy because of the availability of well-established systems for modifying the viral genome.

Using genetic modification, Dr. Manchester and her colleagues were able to create virus particles with reactive groups on their surfaces, allowing for chemical attachment of a variety of ligands. As an example, she showed the results of efforts to add neuropeptide Y to the viral coat. This peptide binds to a receptor that is overexpressed in neuroblastoma tumor cells. She also showed that it was possible to conjugate herstatin, the naturally secreted protein that inhibits the HER2 oncogene, onto CPMV particles. Preliminary results showed that these particles are taken up orally, are not immunogenic, and that they bind to tumor cells expressing the respective ligands. Further studies are underway.

The day's last presentation, by Dr. Sangeeta Bhatia of the University of California, San Diego, discussed efforts to develop multifunctional nanoparticles. She began by discussing work with modified quantum dots to identify new targets for cancer diagnostic and therapeutics. She showed data demonstrating that these quantum dots could bind specifically to targeted tissues *in vitro* and *in vivo*. She also showed how coating these particles with polyethylene glycol kept the particles from being cleared by the reticuloendothelial system without affecting their ability to target tissue. It is even possible, she explained, to target these particles to specific subcellular compartments using canonical import peptides, which she illustrated with examples of quantum dots localized differentially to the nucleus and mitochondria using different trafficking molecules. One potential limitation to using quantum dots in humans is their potential toxicity – they are made of cadmium and selenium, both of which are highly toxic. Preliminary studies suggest that quantum dots can, however, be used *in vitro* without damaging cells.

She finished her talk by discussing work that other groups are doing with nanoscale particles. She singled out work with gold nanoshells, which are being developed for imaging and therapeutic applications, and nanoporous silicon, which can be machined into nanoparticles that appear to assemble into particles at the site of a tumor. Both of these technologies, she said, have the potential to create modular, multifunctional nanoparticles. One possibility for future work that she mentioned was to create nanoparticles that self-destruct over time, which might minimize toxicity.

The day closed with Dr. Anna Barker, NCI's Deputy Director for Advanced Technologies and Strategic Partnerships, leading a roundtable discussion of the challenges in advancing cancer biology and clinical oncology through the use of nanotechnology. A key point that she and others raised was the need to develop new mechanisms for encouraging researchers from disparate fields to work together at the intersections of nanotechnology and cancer research. One suggestion was to create a new type of grant that would enable NCI postdoctoral fellows to work in multiple laboratories on a central problem. Issue of manufacturing capabilities and toxicology studies were also raised as important to address today in order to pave the way for tomorrow's advances.