**Meeting Report**

**Nano in Cancer: Linking Chemistry, Biology, and Clinical Applications In Vivo**

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**Abstract**

Development of nanoparticle agents for cancer therapeutics and diagnostics is steadily progressing and was the subject of the inaugural conference entitled, "Nano in Cancer," held during January 12–15, 2011, in Miami, FL. The meeting program was developed by co-chairs David Piwnica-Worms (Washington University in St. Louis), Jan Schnitzer (Proteogenomics Research Institute for Systems Medicine, San Diego), and Karen Wooley (Texas A&M University). Topics discussed for nanoparticle platforms under development included: nanotechnologies for cancer diagnostics and imaging, overcoming in vivo barriers, therapeutic nanoparticles and clinical prospects, and safety issues for nanotechnologies. Two important concepts emerged from this meeting. The first was the urgent need for uniform standards and protocols for nanoparticle characterization in vitro and in vivo, and the second was the continued need for discovery of definitive targets for tumor-directed nanoparticles across various cancers. Cancer Res; 71(17): 1–5. ©2011 AACR.

**Introduction**

Cancer is a highly personalized disease as evidenced by the patient-to-patient heterogeneity that exists for a given cancer type (1). Furthermore, nontargeted cytotoxic agents commonly used to treat cancers give rise to dose-limiting toxicities and side effects that are themselves a challenge to manage individually. Therefore, there is a consensus that cancer requires highly effective treatments that are tailored to specific needs of each patient. Progress toward personalization of cancer diagnosis and treatment is being made by the highly interdisciplinary group of scientists who study nanomedicine and was the subject of this AACR special conference on nanotechnology.

In general, the term nanoparticle refers to a synthetic molecular assembly that is 1 to 100 nm in at least one dimension (2). It is envisioned that nanoparticles would be of similar size as biological molecules and interactions between them would offer a means to directly impact physiology, or more often, nanoparticles could serve as carriers to deliver compounds to the tumor microenvironment, or to cross biological barriers, such as vascular endothelial cells or the tumor cell membrane. Nanoparticles can be used to deliver imaging agents or cytotoxic payloads, or both. Perhaps just as important as the scale is their synthetic nature, which provides the ability to manipulate their physical and chemical properties to achieve the necessary targeting to biological surfaces or molecules therein and facilitate highly specific biological interactions. Nanoparticles can comprise a variety of formulations for various applications, perhaps conferring some advantage over traditional small molecule agents in vivo. For instance, nanomaterials can be synthesized from various core materials (e.g., iron oxides, lipids, gold, silica, etc.) that provide tractable platforms for physical and chemical modification, in different shapes (e.g., nanospheres, nanorods, nanotubes, etc.) that can effect surface interactions, and various outer coatings [e.g., polyethylene glycol (PEG), peptides or proteins, nucleic acid polymers, etc.] that effect bioavailability, targeting, and in vivo stability.

The main goals of this inaugural conference were to provide an opportunity for researchers in the field to exchange information and ideas, and to assess where the field stands with respect to the intended goal of safely and effectively personalizing cancer medicine by using nanotechnology. The conference opened with a compelling presentation by Nobel laureate Roger Tsien (University of California, San Diego), who began by pointing out many of the issues one faces in developing an effective and clinically useful diagnostic agent. As the keynote speaker, Dr. Tsien spoke about his current research involving the creation of activatable multimodal nanoprobes for use as NMR diagnostic tools and in fluorescence-guided resection of tumors and preservation of peripheral nerves. He aptly ended his talk by encouraging and reminding the audience that the ultimate goal of translational research is to work in vivo and shepherd that work to the clinic. This was followed with sessions discussing diagnostic platforms, overcoming in vivo barriers, therapeutic and clinical prospects, and toxicity issues. The following is a summary of the main topics discussed and examples from the work...
presented at this meeting. The intent of this summary is to not overwhelm the reader with details of various nanoparticle formulations and assemblies being explored, but rather to focus on overarching themes under discussion within the field.

Findings Presented

Nanotechnology for cancer diagnostics and imaging

One primary means for diagnosing cancers of tissues and organs not easily biopsied is to use molecular imaging techniques such as positron emission tomography (PET), SPECT, optical, or MRI (3). Because of the limits of resolution for these techniques, the smallest tumors identified are several cubic millimeters in size and thus, are composed of millions of cancer cells that likely utilize an established blood supply. Even at this small size, the tumor has nearly everything needed for dissemination and metastasis formation. It is for these reasons and others (e.g., high cost) that there is great need for the development of improved means for the early detection of cancers, a niche that may be fulfilled by using nanotechnology.

Besides the various core compositions and coatings required to increase circulation time and to enhance targeting, nanoparticle diagnostic probes require a signal-generating moiety such as a magnetic probe, radioisotope, or fluorophore. For example, MRI contrast agents hold significant promise as imaging agents because they are not hindered by tissue depth as with optical imaging or limitations associated with radiation from radioisotopes. However, conventional MR relaxivity agents are often limited by the mass required to measurably impact water relaxation times within a target tissue. To address these sensitivity challenges, Thomas Meade (Northwestern University) reported the creation of gadolinium(III)-chelated nanodiamonds that possess T1-relaxivities that are among the highest reported on a per gadolinium basis (4). The creation of these gadolinium-conjugated nanodiamonds has added MRI capabilities to nanoparticles that had previously only been studied by using optical techniques. Importantly, early in the conference, Dr. Meade also raised the issue of standards for characterization of nanoparticles, a theme which remained throughout the conference. He mentioned that his lab had directly tested 30 different literature methods for creating nanoparticles and found that after characterizing the size and composition of each, they could only reproduce the assembly and characterization of 3 formulations. He provided a take-home message encouraging scientists to validate their systems and highlighted the need when reporting nanoparticles for standards that are similar to those for small molecules.

The facile methods for conjugating a variety of molecules to nanoparticles enable pathways to the creation of multifunctional probes. Karen Wooley reported the use of polymer chemistry strategies to develop multifunctional nanoparticles that enable controlled release of either chemotherapeutic drugs or fluorescent molecules. For instance, acrylic acid or styrene cores, which have well defined and low-temperature glass and molten transitions, allowed for tuned release of diagnostic or therapeutic agents simply by changing temperature. David Piwnica-Worms reported a novel imaging modality for improving the optical imaging of Cerenkov radiation generated by radiopharmaceutical tracers which may promote the creation of multimodal probes for both PET and optical imaging (5). Cerenkov radiation occurs upon β or α decay, wherein the initial velocity of sufficiently high-energy particles can momentarily exceed the speed of light in tissue. Optical imaging of Cerenkov radiation in vivo is challenging because the ultraviolet/blue spectral output is highly absorbed in living organisms. Thus, to improve optical imaging, Cerenkov radiation is spectrally coupled by energy transfer to high Stokes-shift semiconductor nanoparticles (quantum dots) to produce highly red-shifted photonic emissions. In this context, quantum dot nanoparticles act as sensors of high-energy radiation and thus may expedite studies of PET radiopharmaceuticals and radiobiology in biomaterials, tissues, and live animals.

The ideal diagnostic technique would allow a physician to reliably detect, for primary and metastatic tumors, low-abundance targets that exist on just a few cells after noninvasively collecting a small sample from an ambulatory patient. Work presented by Ralph Weissleder (Massachusetts General Hospital) on clinical use of a point-of-care hand-held NMR device for the detection of cancer may provide an advance for diagnostics. The diagnostic magnetic resonance device, termed µNMR, uses cross-linked iron oxide nanoparticles conjugated to targeting antibodies to detect in less than 1 hour the presence of protein markers associated with scarce cancer cells in fine-needle aspirates. The µNMR device consists of a chip-sized sensor coupled to fluidic channels handling microliter volumes, a portable 0.5 T permanent magnet, and custom NMR hardware. Dr. Weissleder reported the ability to accurately and quantitatively profile markers of cancer; however, he also reported high patient-to-patient and within-tumor variability for a group of 10 markers when assessing fine-needle tumor aspirates (6). Thus, the need to identify definitive markers for the various cancers is ever pressing and was discussed heavily throughout the conference.

Overcoming in vivo barriers

Diagnostic and therapeutic agents both require similar properties for passing safely through the body to arrive at a tumor. Most nanoparticles, by virtue of their size, primarily make use of the enhanced permeability and retention (EPR) effect to gain access to the interstitial space of tumors. The EPR effect reflects a tendency for any large molecular weight particle to accumulate in tumors because of the impact of leaky tumor-associated blood vessels without corresponding lymphatic vessels for clearance (7). It has been estimated that accumulation of nontargeted nanoparticles in tumors is greater than 10-fold in small molecule drugs as a result of the EPR effect per se (7, 8). However, such a strategy is not enough to increase the resolution or specificity of diagnostic and therapeutic agents to the levels that are needed. Intracellular-targeted nanoparticles must cross at least 2 barriers once injected: extravasating between endothelial cells lining blood vessels and then traversing the plasma membrane of tumor cells. However, nanoparticles must do so without
triggering an immune response that would prematurely clear them from the blood. This is a challenging situation because one promising strategy for gaining active targeting to tumor cells is conjugation to an antibody, but this can also trigger an immune response. For this reason, small peptides perhaps offer a promising solution to the problem because of the ability to synthesize them as d-isomers that are resistant to proteolytic cleavage and processing, and thus, often unrecognized by the immune system (9). Toward this end, several researchers, including the groups of Roger Tsien and Ranata Pasqualini (MD Anderson Cancer Center), have turned to using in vivo phage-display libraries to discover useful targeting peptides. Other researchers like Sangeeta Bhatia (Massachusetts Institute of Technology) are solving the delivery problem by developing novel strategies to mask targeting agents until needed and activate cell killing when appropriate. For instance, Dr. Bhatia reported the development of a protease-triggered unveiling of a targeting peptide that was found by using phage display. Breakthroughs in the Joseph DeSimone laboratory (University of North Carolina) by using specifically-designed materials for imprint lithography have enabled a versatile and high-throughput method for the direct fabrication of monodisperse nanoparticles. Cancer cell targeting can be accomplished by attaching cell-specific ligands to the surface of the particles in shape and size-specific manners.

It has become standard in the field to mask the surface of nanoparticles with PEG to prevent opsinization by the immune system and reduce liver clearance, thereby increasing circulation time and providing particles a chance to passively accumulate in tumors. However, work presented by the Ishida Tatsuhiro group (University of Tokushima) found that PEGylated liposomes, when administered repeatedly at specific intervals, trigger a T cell-independent immune response. A first dose of PEGylated liposomes triggers secretion of an anti-PEG IgM antibody from the marginal zone B cells of the spleen in a response that is similar to the response mounted against TNF-Ficoll and bacterial polysaccharides. This serves to increase liver clearance of the particles by activation of the complement system when a second dose is administered within certain intervals. Therefore, it might be of interest for those using nontargeted nanoparticles to explore other coatings as a means of increasing circulation time and passive tumor uptake.

The group of Jan Schnitzer is using novel isolation techniques coupled with mass spectrometry to identify cancer-induced protein expression changes on the endothelial surfaces of lung and solid tumors in which caveolae are present. Caveolae are markers of clathrin-independent endocytosis from the luminal side of the vasculature and can be exploited as a means of transport of exogenous materials from the blood stream to the interstitial spaces of the lung. To identify caveolae-associated proteins, the vascular surface is coated with silicon nanoparticles and then cross-linked by using polyacrylate. The cells are removed and subjected to gradient centrifugation to isolate the caveolae–nanoparticle containing fraction. After analysis of known markers of caveolae to confirm the isolation of caveolar vesicles, mass spectrometry is used to identify proteins that are needed in caveolae-dependent transcytosis across the cell. This group identified the protein aminopeptidase P that is present on the endothelial surface and regulates caveolae-mediated transport. An antibody to the protein was generated and tested successfully in lung tissue to move across the endothelium.

Debbie Liao (Scripps Research Institute) presented a strategy for gaining selective entry into tumor cells which involved appending an aza-peptide inhibitor of legumain to liposomal nanoparticles. Legumain is a protein found to be overexpressed in solid breast, colon, and prostate tumors and is absent from normal tissue. Overexpression of legumain was found to occur when cells undergo hypoxic stress and may provide a strategy for targeting nanoparticles to the cancer cell surface. Her nanoparticle formulation also included doxorubicin, which she found was efficacious at decreasing tumor burden and showed reduced nonspecific toxicity compared with doxorubicin alone in mice as evidenced by an increase in body weight posttreatment.

**Therapeutic nanoparticles and clinical prospects**

There exists great potential for using nanoparticles as therapeutics if investigators can couple the innate ability of nanoparticles to passively home to tumors with active targeting of tumor-specific markers and a molecule to facilitate cell death. In general, presenters at the conference discussed 1 of 3 types of molecules to facilitate a therapeutic effect for their nanoparticle formulations: cytotoxic small molecules, protein or peptide signaling ligands, and siRNAs. In the case of small molecule cytotoxic agents, many presenters at the conference chose to append DNA targeting or microtubule damaging agents to their nanoparticles because these agents are already approved for use. For example, nanoparticle-cytotoxic drug formulations that have been approved by the Food and Drug Administration (FDA) for the treatment of cancer include Doxil and Abraxane. Doxil is a liposomal formulation of doxorubicin and Abraxane is an albumin formulation of paclitaxel, both of which have greatly improved the pharmacokinetics of these conventional chemotherapeutics. The successes observed with these 2 nanoparticle formulations may lead to the revival of molecules that were previously set aside because of poor pharmacokinetic and pharmacodynamic properties. However, there are still improvements needed for Doxil and Abraxane because of side effects associated with poor selectivity.

The use of siRNAs would offer the greatest selectivity for treatment if one had sufficient genetic information about a given tumor, but use of siRNA is complicated given the low stability of the molecule and the changes that can occur when manipulating the expression of one gene that is part of an intricate network of cellular processes and pathways. Chad Mirkin (Northwestern University) reported the use of nanogold particles with oligonucleotides on the surface as opposed to the more traditional liposomal formulations. He reported that highly dense arrays of oligos bind complimentary DNA tightly and that increasing local salt concentration increases the stability of the particles. The nanoparticles were found to enter by endocytosis mediated by scavenger proteins and that 17% of endosomes are sufficiently leaky to...
facilitate endosomal escape and gene knockdown. A mouse flank breast tumor model was used in which the nanoparticles facilitated the knockdown of survivin, a caspase-3 inhibitor protein. Deep penetration of the particles into organs and throughout most cells was shown.

Rather than use of siRNA or cytotoxic drugs to kill tumors, Steven Curley (MD Anderson Cancer Center) makes use of the rapid heating that can be induced in quantum dots and gold, silver, and carbon nanoparticles upon induction with a non-ionizing radiofrequency generator. In one set of data, he presented selective treatment of tumors mediated by passive accumulation of highly polar carbon nanotubes and the ability to aim the RF generator at a specified location in the animal’s body. The inclusion of cetuximab conjugated to gold nanoparticles afforded active cell targeting. By using tumors placed in rabbit livers, he has been exploring what properties of various nanoparticles result in the best tumor ablation. He reported that nanoparticles with small size, increased charge, and increased surface area will heat faster. In addition, he mentioned that silver heats faster than gold. He pointed out that there is no need to achieve near boiling temperatures; heating to 60 °C is sufficient to treat tumor cells and tends to reduce apoptotic induction in normal cells. He also mentioned that multiple rounds of heating increases efficacy of treatment with continued low amounts of off-target cell death.

Lastly, Piotr Grodzinski of the Alliance for Nanotechnology in Cancer [National Cancer Institute (NCI)] highlighted what are the clinical prospects for nanoparticles in medicine. He outlined the Alliance’s view of the innovations that have occurred, key questions relating to cancer research that remain, NCI initiatives, and what phase 2 of the program will be. He mentioned that there have been more than a 1,000 papers published, 50 companies started, and 10 clinical trials initiated under the nanotechnology initiatives of NCI. He mentioned that with the obvious goal of moving nanoparticles to the clinic, there are several things to consider: let biology lead the discovery and do not overengineer, choose targets and disease applications wisely, and he encouraged incremental improvement as opposed to solving unsolved problems. He also pointed out the need to develop diagnostic agents for cancers other than the big 4 cancers (lung, colon, breast, and prostate). Lastly, he listed several goals to be achieved, including: develop early diagnostics, successfully deliver siRNA, and develop therapeutics with low toxicity and high efficacy.

Safety and characterization of nanotechnologies

All of this work is helping to define what attributes and characteristics need to be included in nanoparticle formulations, especially if they are to be successfully translated into the clinic. However, this work will not be completed until we have a thorough understanding of the associated toxicities and biodistribution of these molecular assemblies. Accordingly, the organizers of this conference devoted a session to discussing these issues. Laura Braydich-Stole (Wright-Patterson Air Force Base) presented her work for the United States Air Force about toxicity of silver nanoparticle fuel additives that are coated either with a hydrocarbon chain or polysaccharide. She reported that silver nanoparticles are toxic in a size-dependent manner and that germline cells are more sensitive than somatic cell types. The silver nanoparticles she used were endocytosed and moved eventually into lysosomes. She suspended her nanoparticles in various artificial biological fluids and found that the coatings would degrade, expose the silver and thereby induce toxicity in cells. At the end of her talk, she summarized nanotoxicity considerations, including thorough physical and chemical characterization in vitro and in vivo, a definition of acceptable exposure levels, acceptable dosing, and biodistribution.

Scott McNeil (NCI-Frederick) spoke about many of the trends that he has observed as he leads the group that characterizes the various particles for researchers around the world. His talk was very useful for understanding toxicities and how the body will deal with certain types of particles. He mentioned several trends for any core having various surface chemistry: cationic particles have increased cytoxicity, large hydrophobic particles are recognized by the reticuloendothelial system, small anionic particles are retained in the liver and kidneys, and cationic dendrimers cause platelet aggregation. He reported that optimization of PEG length will reduce immune recognition, and over time, PEG will be released from the nanoparticle and increase the number of granulomas in vivo. Nearly all particles induce interleukin-8 secretion that is often greater than what is observed for endotoxin and will concomitantly recruit other immune cells.

As mentioned previously, a major topic of discussion concerned the characterization and reproducibility of nanoparticle formulations available from the literature. As such, the conference ended with a panel discussion about this topic. The panel consisted of Laura Braydich-Stole and Scott McNeil as well as the meeting co-chairs, who addressed questions from the audience and moderated the discussion. It was mentioned that physical and chemical characterization is the only thing that allows researchers to accurately compare biological outcome and that the chemical entity in vivo may not be the same as what one characterizes in vitro. Queries were made as to the status of future approval criteria for nanoparticle formulations at the FDA and Scott McNeil stated that, although he does not know exactly what will be decided, there must be boundaries set (e.g., plus or minus X number of ligands per particle, etc.), and he encouraged pre-IND talks with the FDA. The panel went so far as to make a list of parameters needed as a standard set for full characterization, including composition, size (by using multiple techniques, DLS, TEM, and AFM), flexibility (glass or melting temperature), serum stability with regard to aggregation, and in vivo bioavailability. Karen Wooley pointed out that changes to nanoparticles occur from in vitro to in vivo and thus, a different strategy may have to be sought for generating nanoparticles with known structure in vivo. For instance, a strategy might be to attach ligands of a given number on a scaffold and then attach that construct to a core. Lastly, it was suggested that a team be put together to synthesize formulation/toxicity data in the form of a book or review article to standardize what everyone should be doing to address these concerns.
Conclusion

There are 2 major themes that became apparent during the course of this conference: the need for standardized procedures for nanoparticle characterization in vitro and in vivo, and the need for identification of definitive biological markers for use in targeting nanoparticles to various cancers. The field has come a long way in a short while. Notable highlights are the discovery of appropriately useful sizes and shapes of particles. We know more about which compositions are stable and can handle specific accessory functions. The field has advanced with regard to specifically targeting various cancers. There is a promising future for using nanotechnologies for selective diagnosis and treatment of cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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