Chapter 8
Nanooncology

Introduction

Application of nanotechnology in cancer can be termed nanooncology and includes both diagnostics and therapeutics (Jain 2008). Various applications in diagnosis and drug delivery for cancer are discussed in this chapter. Two nanotechnology-based products are already approved for the treatment of cancer – Doxil (a liposome preparation of doxorubicin) and Abraxane (paclitaxel in nanoparticle formulation). Approximately 150 drugs in development for cancer are based on nanotechnology. Some of the nanotechnologies and their applications in developing cancer therapies are described in this section. The most important factor in the fight against cancer, besides prevention, is early detection.

Nanobiotechnology for Detection of Cancer

Nanobiotechnology offers a novel set of tools for detection of cancer. It will contribute to early detection of cancer as follows:

- It can complement existing technologies and make significant contributions to cancer detection, prevention, diagnosis, and treatment.
- It would be extremely useful in the area of biomarker research and provide additional sensitivity in assays with relatively small sample volumes.
- Examples of applications of nanobiotechnology in cancer diagnostics include quantum dots and use of nanoparticles for tumor imaging.

Dendrimers for Sensing Cancer Cell Apoptosis

Poly(amidoamine) (PAMAM) dendrimers have been used as a platform for the targeted delivery of chemotherapeutic drugs in cancer. A PAMAM nanodevice can be used to monitor the rate and extent of cell-killing or apoptosis caused by the

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delivered chemotherapeutic drug, which is important for predicting clinical efficacy (Myc et al. 2007). Whereas other approaches to detect apoptosis rely on the human protein annexin V, which binds to a hidden cell membrane component revealed in the initial stages of apoptosis, this method detects caspase-3, an enzyme activated early in the apoptosis process. This enzyme cleaves the bond between two specific amino acids, and this specificity has been exploited to design fluorescence resonance energy transfer (FRET)-based assays for caspase-3. The fluorescence appears only when caspase-3 breaks a valine–aspartic acid bond in a specially designed substrate for this enzyme. To create a tumor-specific apoptosis detector, folic acid and the caspase-3 substrate were attached to a PAMAM dendrimer. Folic acid acts as a tumor-targeting agent, binding to folic acid that many types of tumor cells produce in abundance. Apoptotic tumor cells bearing this folic acid receptor take up the dendrimer and fluoresce brightly. In contrast, apoptotic cells lacking the folic acid receptor do not fluoresce. An optical fiber device, capable of detecting FRET emissions in tumors, has been used to quantify apoptosis in live mice with tumors bearing the folic acid receptor.

Detection of Circulating Cancer Cells

A method has been described for magnetically capturing circulating tumor cells (CTCs) in the bloodstream of mice followed by rapid photoacoustic detection (Galanzha et al. 2009). Magnetic nanoparticles, which were functionalized to target a receptor commonly found in breast cancer cells, bound and captured circulating tumor cells under a magnet. To improve detection sensitivity and specificity, gold-plated carbon nanotubes conjugated with folic acid were used as a second contrast agent for photoacoustic imaging. By integrating in vivo multiplex targeting, magnetic enrichment, signal amplification, and multicolor recognition, this approach enables circulating tumor cells to be concentrated from a large volume of blood in the vessels of tumor-bearing mice and has potential applications for the early diagnosis of cancer and the prevention of metastasis in humans.

A nano-Velcro technology, engineered into a 2.5×5-cm microfluidic chip is a second-generation CTC-capture technology, which is capable of highly efficient enrichment of rare CTCs captured in blood samples collected from prostate cancer patients (Wang et al. 2011). It is based on the research team's earlier development of "flypaper" technology that involves a nanopillar-covered silicon chip whose stickiness resulted from the interaction between the nanopillars and nanostructures on CTCs known as microvilli, creating an effect much like the top and bottom of Velcro. The new device adds an overlaid microfluidic channel to create a fluid flow path that increases mixing. In addition to the Velcro-like effect from the nanopillars, the mixing produced by the microfluidic channel's architecture causes the CTCs to have greater contact with the nanopillar-covered floor, further enhancing the device's efficiency. The device features high flow of the blood samples, which travel at increased speed, bouncing up and down inside the channel; get slammed against the surface; and get caught.

Differentiation Between Normal and Cancer Cells by Nanosensors

Rapid and effective differentiation between normal and cancer cells is an important challenge for the diagnosis and treatment of tumors. A nanoparticle array-based system has been described for identification of normal and cancer cells based on a "chemical nose/tongue" approach that exploits subtle changes in the physicochemical nature of different cell surfaces (Bajaj et al. 2009). Differential interactions with functionalized nanoparticles are transduced through displacement of a multivalent polymer fluorophore that is quenched when bound to the particle and fluorescent after release. This sensing method can rapidly (minutes/seconds) and effectively distinguish (1) different cell types; (2) normal, cancerous, and metastatic human breast cells; and (3) isogenic normal, cancerous, and metastatic murine epithelial cell lines.

Gold Nanoparticles for Cancer Diagnosis

Gold nanoparticles conjugated to anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies (MAbs) specifically and homogeneously bind to the surface of the cancer cells with 600% greater affinity than to the noncancerous cells. This specific and homogeneous binding is found to give a relatively sharper SPR absorption band with a red-shifted maximum compared to that observed when added to the noncancerous cells (El-Sayed et al. 2005). The particles that worked the best were 35 nm in size. These results suggest that SPR scattering imaging or SPR absorption spectroscopy generated from antibody-conjugated gold nanoparticles can be useful in molecular biosensor techniques for the diagnosis and investigation of living oral epithelial cancer cells in vivo and in vitro. Advantages of this technique are:

- It is not toxic to human cells. A similar technique with QDs uses semiconductor crystals to mark cancer cells, but the semiconductor material is potentially toxic to the cells and humans.
- It does not require expensive high-powered microscopes or lasers to view the results. All it takes is a simple, inexpensive microscope and white light.
- The results are instantaneous. If a cancerous tissue is sprayed with gold nanoparticles containing the antibody, the results can be seen immediately. The scattering is so strong that a single particle can be detected.

An animal study has successfully demonstrated the safety of diagnostic use of Raman-silica-gold-nanoparticles (R-Si-Au-NPs), which overcome the inherently weak nature of Raman effect by producing larger Raman signals through surface-enhanced Raman scattering (Thakor et al. 2011). R-Si-Au-NPs were bound to PEG molecules to improve biological tolerance. Molecules that home in on cancer cells can be attached to PEG-R-Si-Au-NPs, and the overall diameter is 100 nm. Photoimaging with these nanoparticles holds the promise of very early disease detection in colorectal cancer (CRC), even before any gross anatomical changes show up, without physically removing any tissue from the patient. Both rectal and

intravenous administration of the particles did not show any systemic toxicity in experimental animals. Furthermore, the nanoparticles were quickly excreted. The intravenously administered nanoparticles were rapidly sequestered by scavenger cells resident in organs such as the liver and spleen. This opens the door to human tests of intravenous injections of these nanoparticles to search for tumors throughout the body. Molecules targeting breast, lung, or prostate cancer can be attached to these nanoparticles. The investigators are now filing for FDA approval to proceed to clinical studies of the nanoparticles for the diagnosis of CRC.

Chemiluminescence resonance energy transfer (CRET) with gold nanoparticles (AuNPs) has been used as an efficient long-range energy acceptor in sandwich immunoassays. A CRET-based sandwich immunoassay has been developed for alpha-fetoprotein (AFP) cancer biomarker (Huang and Ren 2011). In immunoassay, two antibodies (anti-AFP-1 and anti-AFP-2) are conjugated to AuNPs and horseradish peroxidase, respectively. The sandwich-type immunoreactions between the AFP (antigen) and the two different antibodies bridge the donors (luminol) and acceptors (AuNPs), which leads to the occurrence of CRET from luminol to AuNPs upon chemiluminescent reaction. We observed that the quenching of chemiluminescence signal depended linearly on the AFP concentration within a range of concentration from 5 to 70 ng mL^{-1} and the detection limit of AFP was 2.5 ng mL^{-1}. This method was successfully applied for determination of AFP levels in sera from cancer patients, and the results were in good agreement with ELISA assays. This approach is expected to be extended to other assay designs, i.e., using other antibodies, analytes, and chemiluminescent substance.

Gold nanoparticles can be heated rapidly whenever exposed to infrared light of the right wavelength. Heating of gold nanoparticles results in variations in pressure surrounding them, which in turn is expressed in the generation of ultrasound – a phenomenon called plasmon resonance. The shape of the particles determines the wavelength at which this happens. In this way, light from a laser results in sound. By attaching MAbs to gold nanoparticles or nanorods, which can recognize a specific cancer cell, the heating phenomenon can be used in cancer detection. This acoustic signal gives valuable information about the presence of cancer cells. Scientists at the University of Twente (UT) in the Netherlands expect better results with this approach than is currently possible with imaging techniques. The temperature rise can be up to 100 °C. Photothermal therapy would use the heated gold to destroy the tumor. Another option would be to include gold particles in capsules filled with cancer medication: the capsule attaches to the cancer cell, is heated and the medicine is released locally. Both diagnostic and therapeutic applications will be investigated by the UT scientists together with colleagues from the Erasmus Medical Center in Rotterdam and two companies: Esoate Europe and Luminostix.

Gold Nanorods for Detection of Metastatic Tumor Cells

Scientists at Purdue University have developed a technique for producing biocompatible gold nanorods of various sizes to which antibodies can be attached.

Gold nanorods interact with light to produce plasmons, a wavelike motion of electrons on the surface of the nanorods. Depending on the ratio of a nanorod's length to its diameter, these plasmons trigger light emission at a specific frequency that is easily detected using SPR spectroscopy. An antibody that recognizes one specific cancer cell surface biomarker is attached to each nanorod of a given length and diameter. A gold nanorod-antibody construct that recognizes a biomarker found on all cell surfaces serves as an internal reference control that enables calculation of relative amounts of the various tumor biomarkers on a given cancer cell. Using a panel of three different antibody-labeled gold nanorods, the investigators were able to characterize breast tumors according to their cellular composition and correlate their findings to the metastatic potential of each given cell type. These results were validated using flow cytometry, the standard technique used to classify cells according to surface biomarkers. Gold nanorods enabled monitoring of as many as 15 different antibody-nanorod constructs simultaneously.

Implanted Biosensor for Cancer

An implant for biosensing of cancer, developed at the Massachusetts Institute of Technology (Cambridge, MA), contains nanoparticles that can be designed to test for different substances, including metabolites such as glucose and oxygen that are associated with tumor growth. It can also be used to test the effects of anticancer drugs in individual patients; the implant could reveal how much of a drug has reached the tumor. The nanoparticles are encased in a silicone delivery device, enabling their retention in patients' bodies for an extended period of time. The device can be implanted directly into a tumor, allowing a more direct look at what is happening in the tumor over a period of time. The technique makes use of detection nanoparticles composed of iron oxide and coated with dextran. Antibodies specific to the target molecules are attached to the surface of the nanoparticles. When the target molecules are present, they bind to the particles and cause them to clump together. That clumping can be detected by MRI. The nanoparticles are trapped inside the silicone device, which is sealed off by a porous membrane. The membrane allows molecules smaller than 30 nm to get in, but the detection particles are too large to get out. In addition to monitoring the presence of chemotherapy drugs, the device could also be used to check whether a tumor is growing or shrinking, or whether it has spread to other locations, by sensing the amount and location of tumor biomarkers. Preclinical testing is being done for this device for human chorionic gonadotropin that can be considered a biomarker for cancer because it is produced by tumors but not normally found in healthy individuals except pregnant women.

Nanotubes for Detection of Cancer Proteins

Single-wall carbon nanotubes (SWCNTs) are being developed for monitoring cancer-specific proteins. These are hundreds of times smaller than nanocantilevers,

highly sensitive to single-protein binding events, and can be massively multiplexed with millions of tubes per chip for proteomic profiling. The tubes have extraordinary strength, unique electronic properties, and the ability to tag cancer-specific proteins to their surface. These tubes can be fabricated by decomposition of carbon-based gas in a furnace, using iron nanoparticles as catalyst material. With diameter of 1 nm and length of 1 μm, these tubes are smaller than a single strand of DNA. In other words, such a tube is an atomic arrangement of one layer of carbon atoms, which are on the surface. Protein binding events occurring on the surface of these tubes produce a measurable change in the mechanical and electrical properties.

By coating the surfaces of SWCNTs MAbs, it is possible to detect circulating tumor cells (CTCs) in the blood. SWCNTs covered with MAbs, particularly those for insulin-like growth factor-1 receptor (IGF-1), which is commonly found at high levels on cancer cells, home in on target protein "antigens" on the surface of CTCs. This method can be used for detection of recurring CTCs or residual micrometastases from the originally treated tumor. The technique could be cost-effective and could diagnose whether cells are cancerous or not in seconds versus hours or days required for conventional histology examination. It will enable large-scale production methods to make thousands of biosensors and have microarrays of these to detect the fingerprints of specific kinds of CTCs. Eventually, it may be possible to design an assay that can detect CTCs on a handheld device no bigger than a cell phone. Limitation of the technique is that it may not detect more than one antigen at a time on a single cell.

Nanobiochip Sensor Technique for Analysis of Oral Cancer Biomarkers

A pilot study has described a nanobiochip sensor technique for analysis of oral cancer biomarkers in exfoliative cytology specimens, targeting both biochemical and morphologic changes associated with early oral tumorigenesis (Weigum et al. 2010). Oral lesions from dental patients, along with normal epithelium from healthy volunteers, were sampled using a noninvasive brush biopsy technique. Specimens were enriched, immunolabeled, and imaged in the nanobiochip sensor according to previously established assays for the epidermal growth factor receptor (EGFR) biomarker and cytomorphometry. Four key parameters were significantly elevated in both dysplastic and malignant lesions relative to healthy oral epithelium, including the nuclear area and diameter, the nuclear-to-cytoplasmic ratio, and EGFR biomarker expression. Further examination using logistic regression and receiver-operating characteristic curve analyses identified morphologic features as the best predictors of disease individually, whereas a combination of all features further enhanced discrimination of oral cancer and precancerous conditions with high sensitivity and specificity. Further clinical trials are necessary to validate the regression model and evaluate other potential biomarkers. Nanobiochip sensor technique is a promising tool for early detection of oral cancer, which could enhance patient survival.

Nanodots for Tracking Apoptosis in Cancer

Apoptosis is a hallmark effect triggered by anticancer drugs. Researchers at Seoul National University (Korea) have developed a biocompatible, fluorescent nanoparticle that could provide an early sign that apoptosis is occurring as a result of anticancer therapy (Yu et al. 2007). The team created their fluorescent surface-enhanced Raman spectroscopic (F-SERS) nanodots to boost the optical signal generated by typical, biocompatible fluorescent dyes. The nanodots consist of silver nanoparticles embedded in a silica sphere. Attached to the silica core are fluorescent dye molecules and molecules known as Raman labels that enhance the electronic interactions between the silver nanoparticles and the dye molecules. The researchers also linked annexin-V, a molecule that binds specifically to a chemical that appears on cells undergoing apoptosis, to the silica–silver nanoparticle construct. Toxicity tests showed that the silica–silver nanodots were not toxic to various human cells growing in culture. The investigators then added the nanodots to cells triggered to undergo apoptosis and were able to image those cells as they went through programmed cell death. Based on these results, the researchers prepared other nanodots containing antibodies that bind to other molecules involved in apoptosis. They then added these antibody-linked nanodots and the annexin-V-linked nanodots to cultured human lung cancer cells. The investigators were able to track the appearance of all three molecules simultaneously, which has been difficult to do using conventional cell-staining techniques.

Nanolaser Spectroscopy for Detection of Cancer in Single Cells

Nanolaser scanning confocal spectroscopy can be used to identify a previously unknown property of certain cancer cells that distinguishes them with single-cell resolution from closely related normal cells (Gourlay et al. 2005). This property is the correlation of light scattering and spatial organization of mitochondria; normally, it is well scattered, but in cancer cells, the mitochondria are disorganized and scatter light poorly. These optical methods are promising powerful tools for detecting cancer at an early stage.

Nanoparticles Designed for Dual-Mode Imaging of Cancer

Scientists at Yonsei University, Korea, have combined the best characteristics of QDs and magnetic iron oxide nanoparticles to create a single nanoparticle probe that can yield clinically useful images of both tumors and the molecules involved in cancer (Choi et al. 2006). They start by synthesizing 30-nm-diameter silica nanoparticles impregnated with Rhodamine, a bright fluorescent dye, and 9-nm-diameter water-soluble iron oxide nanoparticles. They then mix these two nanoparticles with a chemical linker, yielding the dual-mode nanoparticle. On average, ten magnetic iron oxide particles link to a single-dye-containing silica nanoparticle, and the resulting construct is approximately 45 nm in diameter. The combination nanoparticle performed

better in both MRI and fluorescent-imaging tests than did the individual components. In MRI experiments, the combination nanoparticle generated an MRI signal that was over threefold more intense than did the same number of iron oxide nanoparticles. Similarly, the fluorescent signal from the dual-mode nanoparticle was almost twice as bright as that produced by dye molecules linked directly to iron oxide nanoparticles. Next, the researchers labeled the dual-mode nanoparticles with an antibody that binds to molecules known as polysialic acids, which are found on the surface of certain nerve cell and lung tumors. These targeted nanoparticles were quickly taken up by cultured tumor cells and were readily visible using fluorescence microscopy.

Nanotechnology-Based Single-Molecule Assays for Cancer

Information about the biological processes in living cells is required for the detection and diagnosis of cancer for the following reasons:

- To recognize the important changes, which occur when cells undergo malignant transformation.
- There are situations when primary cells from a surgical procedure cannot be propagated due to the type of cell or the low number of cells available.
- Detection of cancer at an early stage is a critical step for improving cancer treatment.

Early detection will require sensitive methods for isolating and interrogating individual cells with high spatial and temporal resolution without disrupting their cellular biochemistry. Probes designed to penetrate a cell and report on the conditions within that cell must be sufficiently small, exceedingly bright, and stable for a long time in the intracellular environment without disrupting the cell's normal biochemical functioning. A series of silver nanoparticles have been prepared that meet many of the criteria listed above. Although smaller than 100 nm in diameter, these particles are bright enough to be seen by eye using optical microscopy. Unlike fluorophores, fluorescent proteins, or quantum dots, silver nanoparticles do not photodecompose during extended illumination. Therefore, they can be used as a probe to continuously monitor dynamic events in living cells during studies that last for weeks or even months. Because the color of the scattered light from nanoparticles depends upon their size, they have been used to measure the change in single-membrane pores in real time using dark-field optical microscopy. Intracellular and extracellular nanoparticles can also be differentiated by the intensity of light scattering. Next challenge is to develop methods for modifying the surface of the nanoparticles to make them more biocompatible, so that biological processes can be observed without disturbing or destroying the cell's intrinsic biochemical machinery. Ultimately, these probes may be combined to produce highly sensitive assays with high spatial and temporal resolution. This advance will enable researchers to study the interactions of multiple genes in the same cell simultaneously by using different colored reporter molecules. In addition to transcription and translation, similar live-cell single-molecule assays will offer the prospect of studying more

complex cellular processes, such as cell signaling. Continuous advances and evolution along these research fronts is necessary to unravel biochemical processes in vivo and to develop tools that can be used to detect and diagnose cancer using only a single cell from the patient.

QDs for Detection of Tumors

QD bioconjugates that are highly luminescent and stable can be used for studying gene and enable visualization of cancer cells in living animals. QDs can be combined with fluorescence microscopy to follow cells at high resolution in living animals. These offer considerable advantages over organic fluorophores for this purpose. QDs and emission spectrum scanning multiphoton microscopy have been used to develop a means to study extravasation of tumor cells in vivo.

QD-Based Test for DNA Methylation

DNA methylation contributes to carcinogenesis by silencing key tumor suppressor genes. An ultrasensitive and reliable nanotechnology assay, MS-qFRET (fluorescence resonance energy transfer), can detect and quantify DNA methylation (Bailey et al. 2009). Bisulfite-modified DNA is subjected to PCR amplification with primers that would differentiate between methylated and unmethylated DNA. QDs are then used to capture PCR amplicons and determine the methylation status via FRET. The specific target of the test is DNA methylation which occurs when methyl attaches to cytosine, a DNA building block. When this happens at specific gene locations, it can stop the release of tumor-suppressing proteins; cancer cells then more easily form and multiply. The method involves singling out the DNA strands with methyl attachments through bisulfite conversion, whereby all nonmethyl segments are converted into another nucleotide. Copies of the remaining DNA strands are made, two molecules (a biotin protein and a fluorescent dye) are attached at either end, and the strands are mixed with QDs that are coated with a biotin-attractive chemical. Up to 60 DNA strands are attracted to a single QD. An UV light or blue laser activates the QDs, which pass the energy to the fluorescent molecules on the DNA strands which then light up and are identifiable via a spectrophotometer, which both identifies and can count the DNA methylation.

Key features of MS-qFRET include its low-intrinsic background noise, high resolution, and high sensitivity. This approach detects as little as 15 pg of methylated DNA in the presence of a 10,000-fold excess of unmethylated alleles, enables reduced use of PCR (as low as eight cycles), and allows for multiplexed analyses. The high sensitivity of MS-qFRET enables one-step detection of methylation at PYCARD, CDKN2B, and CDKN2A genes in patient sputum samples that contain low concentrations of methylated DNA, which normally would require a nested PCR approach.

The direct application of MS-qFRET on clinical samples offers great promise for its translational use in early cancer diagnosis and prognostic assessment of tumor behavior, as well as monitoring response to therapeutic agents. Gene DNA methylation indicates a higher risk of developing cancer and is also seen as a warning sign

of genetic mutations that lead to development of cancer. Moreover, since different cancer types possess different genetic markers, e.g., lung cancer biomarkers differ from leukemia, the test should identify which cancer a patient is at risk of developing. This test could be used for frequent screening for cancer and replacing traditionally invasive methods with a simple blood test. It could also help determine whether a cancer treatment is effective and thus enable personalized chemotherapy.

Nanobiotechnology for Early Detection of Cancer to Improve Treatment

Cancer cells themselves may be difficult to detect at an early stage, but they leave a fingerprint, i.e., a pattern of change in biomarker proteins that circulate in the blood. There may be 20–25 biomarkers, which may require as many as 500 measurements, all of which should be made from a drop of blood obtained by pinprick. Thus, nanoscale diagnostics will play an important role in this effort. Nanowire sensors are in development at California Institute of Technology (Pasadena, CA) for very early diagnosis of cancer, when there are just a few thousand cells. Nanowires can electronically detect a few protein molecules along with other biochemical markers that are early signs of cancer. Nanowires in a set are coated with different compounds, each of which binds to a particular biomarker and changes the conductivity of the nanowire that can be measured. Thousands of such nanowires are combined on a single chip that enables identification of the type of cancer. Currently, such a chip can detect between 20 and 30 biomarkers and is being used for the early diagnosis of brain cancer.

Cancer is easier to treat and less likely to develop drug resistance when treatment is started very early. Cancer cells in very early stages are less likely to have mutations that make them resistant to treatment.

An automated gold nanoparticle biobarcode assay probe has been described for the detection of prostate-specific antigen (PSA) at 330 fg/mL, along with the results of a clinical pilot study designed to assess the ability of the assay to detect PSA in the serum of 18 men who have undergone radical prostatectomy for prostate cancer. Available PSA immunoassays are often not capable of detecting PSA in the serum of men after radical prostatectomy. This new bio-barcode PSA assay is approximately 300 times more sensitive than commercial immunoassays, and all patients in this study had a measurable serum PSA level after radical prostatectomy. Because the patient outcome depends on the level of PSA, this ultrasensitive assay enables (1) informing patients, who have undetectable PSA levels with conventional assays, but detectable and nonrising levels with the barcode assay, that their cancer will not recur; (2) earlier detection of recurrence, earlier because of the ability to measure increasing levels of PSA before conventional tools can make such assignments; and (3) use of PSA levels, which would otherwise not be detectable with conventional assays, to follow the response of patients to treatment.

Nanobiotechnology-Based Drug Delivery in Cancer

Drug delivery in cancer is important for optimizing the effect of drugs and reducing toxic side effects. Several nanobiotechnologies, mostly based on nanoparticles, have been used to facilitate drug delivery in cancer. A classification of the nanotechnologies for drug delivery in cancer is shown in Table 8.1.

Approximately 150 drugs in development for cancer are based on nanotechnology. Those approved are listed in Table 8.2, and several more are in clinical trials.

Table 8.1 Classification of nanobiotechnology approaches to drug delivery in cancer

Nanoparticles
Nanoparticle formulations of anticancer drugs, e.g., paclitaxel
Exosomes for cancer drug delivery
Nanoencapsulation and enclosure of anticancer drugs
Enclosing drugs in lipid nanocapsules
Encapsulating drugs in hydrogel nanoparticles
Micelles for drug delivery in cancer
Targeted delivery of anticancer therapy
Targeted drug delivery with nanoparticles
PEGylated nanoliposomal formulation
Folate-linked nanoparticles
Carbon magnetic nanoparticles for targeted drug delivery in cancer
Targeted drug delivery with nanoparticle–aptamer bioconjugates
Nanodroplets for site-specific cancer treatment
Lipid-based nanocarriers
Targeted antiangiogenic therapy using nanoparticles
Nanoparticles for delivery of drugs to brain tumors
Combination of nanoparticles with radiotherapy
Combination with boron neutron capture therapy
Nanoengineered silicon for brachytherapy
Combination with physical modalities of cancer therapy
Combination with laser ablation of tumors
Combination with photodynamic therapy
Combination with thermal ablation
Combination with ultrasound
Nanoparticle-mediated gene therapy
p53 Gene therapy of cancer
Immunolipoplex for delivery of p53 gene
Intravenous delivery of FUS1 gene
Strategies combining diagnostics and therapeutics
Nanoshells as adjuncts to thermal tumor ablation
Perfluorocarbon nanoparticles
Nanocomposite devices

Table 8.2 Approved anticancer drugs using nanocarriers

Trade name/compound	Manufacturer	Nanocarrier
Abraxane/paclitaxel	Abraxis Biosciences	Albumin-bound paclitaxel
Bexxar/anti-CD20 conjugated to iodine-131	Corixa/GlaxoSmithKline	Radio-immunoconjugate
DaunoXome/daunorubicin	Diatos, available in France	Liposome
Doxil/Caelyx/doxorubicin	Ortho Biotech	Liposome
Myoset/doxorubicin	Cephalon, available in Europe	Non-PEGylated liposome
Oncaspar/PEG-L-asparaginase	Enzon	Polymer–protein conjugate
Ontak/IL2 fused to diphtheria toxin	Eisai Inc.	Immunotoxic fusion protein
SMANCS/zinostatin	Yamanouchi Pharma	Polymer–protein conjugate
Zevalin/anti-CD20 conjugated to yttrium-90	Cell Therapeutics Inc	Radio-immunoconjugate
Zoladex/goserelin acetate	AstraZeneca	Polymer rods

SMANCS styrene maleic anhydride neocarzinostatin

Nanoparticle Formulations for Drug Delivery in Cancer

Anticancer Drug Particles Incorporated in Liposomes

Several injectable and biodegradable systems have been synthesized based on incorporation of antiestrogens (AEs) in nanoparticles and liposomes. Both nanospheres and nanocapsules (polymers with an oily core in which AEs were solubilized) incorporated high amounts of 4-hydroxytamoxifen (4-HT) or RU 58668. Liposomes containing various ratios of lipids enhanced the apoptotic activity of RU 58668 in several multiple myeloma cell lines tested by flow cytometry. These cell lines expressed both estrogen receptor alpha and beta subtypes. RU-loaded liposomes, administered intravenously in an animal model, induce the arrest of tumor growth. Thus, the drug delivery of antiestrogens enhances their ability to arrest the growth of tumors which express estrogen receptors and are of particular interest for estrogen-dependent breast cancer treatment. In addition, it represents a new potent therapeutic approach for multiple myeloma.

SuperFluids™ technology (Aphios Corporation) involves biodegradable polymer nanospheres utilizing supercritical, critical, or near-critical fluids with or without polar cosolvents. These nanospheres are utilized to encapsulate proteins with controlled-release characteristics without usage of toxic organic solvent. The patented technology can be utilized to form stable biocompatible aqueous formulations of poorly soluble anticancer drugs such as paclitaxel and camptothecin. An improved process utilizing SuperFluids™ results in the formation of small, uniform liposomes (nanosomes) to improve the delivery and therapeutic efficacy of poorly water-soluble drugs while reducing their toxicities. The process has been

used for the nanoencapsulation of paclitaxel in a formulation called Taxosomes™, which has been tested in nude mice with breast cancer xenografts. Taxosomes™ will lead to (1) enhanced therapeutic efficacy, (2) elimination of premedication to counteract castor oil, (3) reduction of drug toxicity side effects, (4) prolonged circulation time and therapeutic effect, and (5) improved quality of life.

The process has also been used for the nanoencapsulation of camptothecin, a potent and exciting anticancer agent, in a stable aqueous liposomal formulation called Camposomes™. Water-soluble derivatives of camptothecin, a unique topoisomerase 1 inhibitor, have recently been approved by the FDA for use in colorectal cancer. Camposomes™ have been shown to be very effective against lymphomas in nude mice.

Nanocapsules, which are small aggregates of cisplatin covered by a single-lipid bilayer, have an unprecedented drug-to-lipid ratio and an in vitro cytotoxicity up to 1,000-fold higher than the free drug. Analysis of the mechanism of nanocapsule formation suggests that the method may be generalized to other drugs showing low water solubility and lipophilicity.

In Protein Stabilized Liposome (PSL™) nanotechnology of Azaya Therapeutics, the liposome product is prepared in a single step that encapsulates the active drug docetaxel (ATI-1123) in the lipid layer of the liposome while forming active nanoparticles in situ (100–130 nm). This process is geared toward the formulation of hydrophobic molecules that would otherwise have limited success as developmental drugs using traditional formulation methodologies. Azaya intends to use its PSL nanotechnology to improve the performance and reduce the nonspecific cytotoxicity of leading marketed chemotherapeutics such as Taxotere (docetaxel) and Camptosar®, as well as several experimental drugs that have been withdrawn from development due to their nonspecific cytotoxicity and formulation difficulties.

Cerasomes

Ceramide is a lipid molecule in plasma membrane of the cell and controls cell functions such as cell aging. Ceramide selectively kills cancer cells but is not toxic to normal cells. However, as a lipid, ceramide cannot be delivered effectively as a drug. To solve this limitation, cerasome is created to turn the insoluble lipid into a soluble form. Cerasomes are molecular-sized bubbles (size range from 60 to 200 nm) filled with C6-ceramide for use as anticancer agents. Paclitaxel-loaded cerasomes exhibit sophisticated controlled-release behavior for drug delivery in cancer (Cao et al. 2010). Cerasomes have already been shown to effectively treat cellular and animal models of breast cancer and melanoma. Systemic administration of nanoliposomal C6-ceramide to mice engrafted with SK-HEP-1 tumors reduced tumor vascularization and proliferation, induced tumor cell apoptosis, decreased phosphorylation of AKT, and ultimately blocked tumor growth (Tagaram et al. 2011). These studies show that nanoliposomal ceramide is an efficacious antineoplastic agent for the treatment of in vitro and in vivo models of human hepatocellular carcinoma.

Encapsulating Drugs in Polymeric Nanoparticles

Curcumin, an element found in the cooking spice turmeric, has long been known to have potent anticancer properties as demonstrated in several human cancer cell line and animal carcinogenesis models. Nevertheless, widespread clinical application of this relatively efficacious agent in cancer and other diseases has been limited due to poor aqueous solubility and, consequently, minimal systemic bioavailability. This problem has been overcome by encapsulating free curcumin with a polymeric nanoparticle, creating nanocurcumin (Bisht et al. 2007). Further, nanocurcumin's mechanisms of action on pancreatic cancer cells mirror that of free curcumin, including induction of cellular apoptosis, blockade of nuclear factor kappa-B (NF-kappaB) activation, and downregulation of steady-state levels of multiple pro-inflammatory cytokines (IL-6, IL-8, and TNF-α). No evidence of toxicity was found in tests with empty versions of the polymeric nanoparticle. Their findings show no evidence of weight loss, organ changes, or behavioral changes in live mice after administering a relatively large dosage of the empty nanoparticles. Nanocurcumin provides an opportunity to expand the clinical repertoire of this efficacious agent by enabling ready aqueous dispersion. Future studies utilizing nanocurcumin are warranted in preclinical in vivo models of cancer and other diseases that might benefit from the effects of curcumin.

Encapsulating Drugs in Hydrogel Nanoparticles

A versatile chemical technique has been developed for creating ultrafine nanosized hydrogels, essentially a network of polymer chains that absorb as much as 99% of their weight in water (Gao et al. 2007). Polyacrylamide was used to create nanoparticles of 2 nm diameter that have no charge on their surfaces. This lack of charge prevents blood proteins from sticking to the surface of the nanoparticles. Combined with the fact that these nanoparticles are too small to be recognized by the immune system, the result is a nanoscale drug-delivery vehicle with the ability to remain in circulation long enough to reach and permeate tumors before being excreted through the kidneys. These nanoscale hydrogels were first tested as a drug-delivery vehicle for a water-insoluble photosensitizer called meta-tetra(hydroxyphenyl) chlorin (mTHPC), which is approved in the European Union for use in treating head and neck cancer. mTHPC produces cell-killing reactive oxygen when irradiated with red light, but not without serious side effects resulting from the method now used to deliver this drug to tumors. When added to the chemical mixture used to create the nanoparticles, mTHPC becomes trapped within the polymer framework. Characterization experiments showed that this photosensitizer does not escape from the nanoparticles, but is still capable of producing the same amount of reactive oxygen as if it were free in solution. When added to human brain cancer cells growing in culture and irradiated with red light, this formulation kills the cells rapidly. Empty nanoparticles had no effect on the cells. Neither did drug-loaded nanoparticles added to the cells that were kept in the dark.

Exosomes

Exosomes are small (50–100 nm), spherical vesicles produced and released by most cells to facilitate intercellular communication. These vesicles are of endosomal origin and are secreted in the extracellular milieu following fusion of late endosomal multivesicular bodies with the plasma membrane. Exosomes have a defined protein composition, which confers specific biological activities contingent on the nature of the producing cell. Although exosomes express tumor antigens, leading to their proposed utility as tumor vaccines, they also can suppress and induce T-cell signaling molecules.

Exosomes produced by dendritic cells are called dexosomes and contain essential components to activate both adaptive and innate immune responses. Anosys is developing dexosome vaccines that use patient-specific dexosomes loaded with tumor antigen-derived peptides to treat cancer. Exosome research continues to reveal unique properties which broaden their fields of application. Anosys' Exosome Display Technology provides the ability to manipulate exosome composition and tailor exosomes with new desirable properties opening up opportunities in the field of recombinant vaccine and MAb preparation. This is achieved by generating genes coding for chimeric proteins linking an exosome addressing sequence to antigens or biologically active proteins. The resulting proteins are targeted to exosomal compartment and released in the extracellular milieu bound to exosomes.

Exosomes are emerging as novel approaches for cancer vaccine development. Safety of exosomes has been established in clinical trials that can be administered, but their potency for eliciting appropriate immune responses to kill cancer cells leaves much to be desired (Tan et al. 2010). Most of the investigational evidence is about solid tumors, and it has not been demonstrated that nonsolid tumors (e.g., hematological malignancies) can be treated using exosome technology. Moreover, exosomal immunotherapy relies on the immune system, and cancer patients, who are immunocompromised and/or immunosuppressed due to chemotherapy and radiotherapy, might not be able to overcome cancer with their immune system alone.

Folate-Linked Nanoparticles

PEG-coated biodegradable nanoparticles can be coupled to folic acid to target the folate-binding protein; this molecule is the soluble form of the folate receptor that is overexpressed on the surface of many tumor cells. The specific interaction between the conjugate folate nanoparticles and the folate-binding protein has been evaluated by surface plasmon resonance and confirmed a specific binding of the folate nanoparticles to the folate-binding protein. Thus, folate-linked nanoparticles represent a potential new drug carrier for tumor cell-selective targeting.

Iron Oxide Nanoparticles

A novel water-dispersible oleic acid (OA)–Pluronic-coated iron oxide magnetic nanoparticle formulation can be loaded easily with high doses of water-insoluble

anticancer agents (Jain et al. 2005). Drug partitions into the OA shell surrounding iron oxide nanoparticles and the Pluronic that anchors at the OA–water interface confers aqueous dispersity to the formulation. Neither the formulation components nor the drug loading affects the magnetic properties of the core iron oxide nanoparticles. Sustained release of the incorporated drug is observed over 2 weeks under in vitro conditions. The nanoparticles have further demonstrated sustained intracellular drug retention relative to drug in solution and a dose-dependent antiproliferative effect in breast and prostate cancer cell lines. This nanoparticle formulation can be used as a universal drug carrier system for systemic administration of water-insoluble drugs while simultaneously enabling magnetic targeting and/or imaging.

Lipid-Based Nanocarriers

LiPlasome Pharma's proprietary prodrug and drug-delivery technology is based on smart lipid-based nanocarriers (LiPlasomes) that can be applied for targeted transport of anticancer drugs (Andresen et al. 2005). The targeted drug-delivery principle consists of long-circulating nanoparticles such as liposomes or micelles that accumulate in porous cancer tissue with a high PLA2 activity. The carrier nanoparticles are composed of special prodrug lipids whose degradation products, after exposure to PLA2, are converted to active drugs such as anticancer lysolipids and/or fatty acid drug derivatives. The PLA2 hydrolysis products will furthermore act as locally generated permeability enhancers that promote the absorption of the released drugs across the cancer cell membranes into putative intracellular target sites. This innovative prodrug and drug-delivery concept allows for intravenous transport of high concentrations of anticancer drugs directly to the tumor target. It enables, without any prior knowledge of the position and size of the tumor, to release the anticancer drugs specifically at the tumor target site. The delivery system is formulated with PEG to prolong the serum half-life of the drugs and prodrugs and avoid the nanocarriers being removed by the reticuloendothelial system.

Micelles for Drug Delivery in Cancer

Block copolymer micelles are spherical supramolecular assemblies of amphiphilic copolymers that have a core-shell-type architecture. The core is a loading space that can accommodate hydrophobic drugs, and the shell is a hydrophilic brushlike corona that makes the micelle water soluble, thereby allowing delivery of the poorly soluble contents (Fig. 8.1).

However, a key issue with the contained cytotoxic drugs is an understanding of how the micelle and the micelle-incorporated agent are distributed. By using fluorescently labeled polymer and organelle-specific dyes in combination with confocal microscopy, it has been shown that the micelles localize in several cytoplasmic organelles, including the mitochondria, but not the nucleus. Furthermore, the micelles increase the amount of a drug delivered to the cells and have the potential to deliver drugs to particular subcellular targets. Antibodies can be attached to

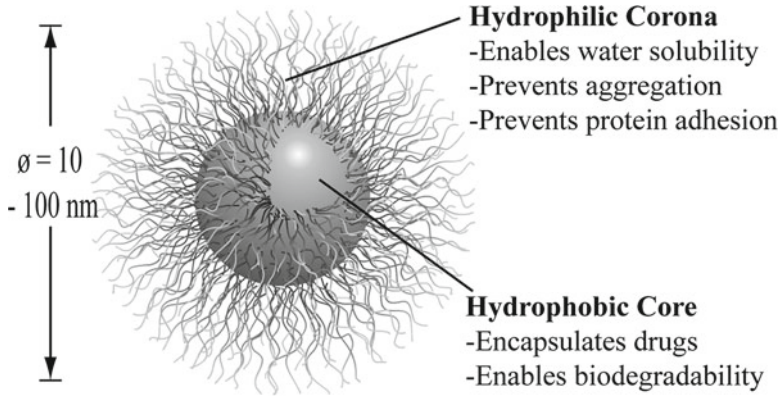

Fig. 8.1 Use of micelles for drug delivery (Source: Sutton et al. 2007)

the polymers that make up the micelles. Administering immunomicelles loaded with the sparingly soluble anticancer drugs. Paclitaxel micelle (NanoCarrier Ltd), a tumor-targeted drug-delivery system, has been investigated in clinical trials by Nippon Kayaku Co in Japan and is entering phase III in 2012. These micelles have a size of ~28 nm and exhibit a sustained drug release accompanied with the decay of the carrier itself in physiological saline. They show remarkably prolonged blood circulation and effectively accumulate in solid tumors indicating a potential for the targeted therapy of solid tumors.

DACH-platin-PEG-polyglutamic acid (DACH Platin Medicelle™) from NanoCarrier, based on Medicelle™ technology, has demonstrated enhanced permeability and retention of the compound in the tumor, leading to improved efficacy and toxicity profiles in animal experiments. The mechanism of action of Medicelle™ delivery system is based on the formation of micelles, including hydrophilic–hydrophobic block copolymers, with a hydrophobic inner core and hydrophilic outer shell. This allows the chemical entrapment of various drugs into the micelles. The drugs are then released slowly into the organism. This product is being developed for clinical application.

Camptothecin (CPT) is a topoisomerase I inhibitor that is effective against cancer, but clinical application of CPT is limited by insolubility, instability, and toxicity problems. Biocompatible, targeted sterically stabilized micelles (SSM) have been used as nanocarriers for CPT (CPT-SSM). CPT solubilization in SSM is reproducible and is attributed to avoidance of drug aggregate formation. Furthermore, SSM composed of polyethylene glycol (PEGylated) phospholipids are attractive nanocarriers for CPT delivery because they are sufficiently small (~14 nm) to extravasate through the leaky microvasculature of tumor and inflamed tissues for passive targeting of solid cancers in vivo, resulting in high drug concentration in tumors and reduced drug toxicity to the normal tissues (Koo et al. 2006).

Stealth micelle formulations have stabilizing PEG coronas to minimize opsonization of the micelles and maximize blood circulation times. Clinical data have been

reported on three stealth micelle systems: SP1049C, NK911, and Genexol-PM (Sutton et al. 2007). SP1049C is formulated as doxorubicin (DOX)-encapsulated Pluronic micelles, NK911 is DOX-encapsulated micelles from a copolymer of PEG and DOX-conjugated poly(aspartic acid), and Genexol-PM is a paclitaxel-encapsulated PEG–PLA micelle formulation. Polymer micelles are becoming a powerful nanotherapeutics platform that affords several advantages for targeted drug delivery in cancer, including increased drug solubility, prolonged circulation half-life, selective accumulation at tumor sites, and a decrease in toxicity.

Minicells for Targeted Delivery of Nanoscale Anticancer Therapeutics

Indiscriminate drug distribution and severe toxicity of systemic administration of chemotherapeutic agents can be overcome through encapsulation and cancer cell-specific targeting of chemotherapeutics in 400-nm minicells (EnGeneIC Delivery Vehicle). Targeted minicells enter the cancer cells via receptor-mediated endocytosis, while the bacteria carrying nanoparticles enter the mammalian cells in a nonspecific manner, i.e., via phagocytosis. Scientists at EnGeneIC discovered that minicells can be packaged with therapeutically significant concentrations of chemotherapeutics of differing charge, hydrophobicity, and solubility (MacDiarmid et al. 2007). Targeting of minicells via bispecific antibodies to receptors on cancer cell membranes results in endocytosis, intracellular degradation, and drug release. Doses of drugs delivered via minicells are ~1,000 times less than the dose of the free drug required for equivalent or better tumor shrinkage. It produces significant tumor growth inhibition and regression in mouse xenografts and lymphoma in dogs despite administration of minute amounts of drug and antibody, a factor critical for limiting systemic toxicity that should allow the use of complex regimens of combination chemotherapy. Phase I clinical trials are in progress in Australia.

In a further study, minicells were shown to specifically and sequentially deliver to tumor xenografts siRNA- or shRNA-encoding plasmids to counteract drug resistance by knocking down a multidrug resistance protein (MacDiarmid et al. 2009). Subsequent administration of targeted minicells containing cytotoxic drugs eliminates formerly drug-resistant tumors. The dual sequential treatment, involving minicells loaded with both types of payload, enables complete survival without toxicity in mice with tumor xenografts, while involving several-thousand-fold less drug, siRNA, and antibody than needed for conventional systemic administration of cancer therapies.

Nanocarriers Enhance Doxorubicin Uptake in Drug-Resistant Cancer

Resistance to anthracyclines and other chemotherapeutics due to P-glycoprotein (pgp)-mediated export is a frequent problem in cancer treatment. Iron oxide–titanium dioxide (TiO_2) core-shell nanocomposites can serve as efficient carriers for doxorubicin to overcome this common mechanism of drug resistance in cancer cells (Arora et al. 2012). Doxorubicin nanocarriers (DNC) increased effective drug

uptake in drug-resistant ovarian cells. Doxorubicin binds to the TiO_2 surface by a labile bond that is severed upon acidification within cell endosomes. Upon its release, doxorubicin traverses the intracellular milieu and enters the cell nucleus by a route that evades pgp-mediated drug export. Confocal and X-ray fluorescence microscopy and flow cytometry have been used to show the ability of DNCs to modulate transferrin uptake and distribution in cells. Increased transferrin uptake occurs through clathrin-mediated endocytosis, indicating that nanocomposites and DNCs may both interfere with removal of transferrin from cells. Together, these findings show that DNCs not only provide an alternative route of delivery of doxorubicin to pgp-overexpressing cancer cells but also may boost the uptake of transferrin-tagged therapeutic agents.

Nanoconjugates for Subcutaneous Delivery of Anticancer Drugs

Most of the anticancer drugs are administered intravenously. Nanoformulation of anticancer drugs is being developed for subcutaneous delivery of existing and new chemotherapeutics. Subcutaneous nanocarrier delivery of hyaluronan-conjugated doxorubicin or cisplatin has demonstrated significantly improved efficacy with decreased toxicity compared with standard agent combination therapy at all doses tested, achieving complete pathologic tumor response in mice implanted with human tumors (Cohen et al. 2011). Advantages of subcutaneous anticancer drug delivery are:

- Avoids complicated and expensive intravenous infusions
- Improves safety and efficacy for existing chemotherapy drugs
- Highly localized drug delivery to primary tumor sites to prevent recurrence
- Enables development of multidrug combinations to overcome drug resistance
- Incorporation of imaging agents to monitor penetration of the drug into tumor

Nanomaterials for Delivery of Poorly Soluble Anticancer Drugs

Nanomaterials have been successfully manipulated to create a new drug-delivery system that can solve the problem of poor water solubility of most promising currently available anticancer drugs and thereby increase their effectiveness. The poorly soluble anticancer drugs require the addition of solvents in order for them to be easily absorbed into cancer cells. Unfortunately, these solvents not only dilute the potency of the drugs but create toxicity as well. A novel approach has been devised using silica-based nanoparticles to deliver the anticancer drug camptothecin and other water-insoluble drugs into human cancer cells (Lu et al. 2007). The method incorporates a hydrophobic anticancer drug camptothecin into the pores of fluorescent mesoporous silica nanoparticles and delivers the particles into a variety of human cancer cells to induce cell death. The results suggest that the mesoporous silica nanoparticles might be used as a vehicle to overcome the insolubility problem of many anticancer drugs.

Nanoparticle Formulation for Enhancing Anticancer Efficacy of Cisplatin

Cisplatin is first-line chemotherapy for most types of cancer. However, its use is dose-limited due to severe nephrotoxicity. Rational engineering of a novel nanoplatinate has been reported, which self-assembles into a nanoparticle at unique platinum-to-polymer ratio and releases cisplatin in a pH-dependent manner (Paraskar et al. 2010). The nanoparticles are rapidly internalized into the endolysosomal compartment of cancer cells and exhibit an IC50 comparable to that of free cisplatin and superior to carboplatin. The nanoparticles showed significantly improved anticancer efficacy in terms of tumor growth delay in breast and lung cancers. Furthermore, the nanoparticle treatment resulted in reduced systemic and nephrotoxicity, validated by decreased biodistribution of platinum to the kidney. Given the need for a better platinate, this coupling of nanotechnology and structure-activity relationship to rationally reengineer cisplatin is anticipated to have a major impact on the treatment of cancer.

Nanoparticle Formulations of Paclitaxel

Paclitaxel is active and widely used to treat multiple types of solid tumors. The commercially available paclitaxel formulation uses Cremophor/ethanol (C/E) as the solubilizers. Other formulations including nanoparticles have been introduced. A study evaluated the effects of nanoparticle formulation of paclitaxel on its tissue distribution in experimental animals (Yeh et al. 2005). The nanoparticle and C/E formulations showed significant differences in paclitaxel disposition; the nanoparticles yielded 40% smaller area under the blood concentration-time curve and faster blood clearance of total paclitaxel concentrations (sum of free, protein-bound, and nanoparticle-entrapped drug). Tissue specificity of the two formulations was different. The nanoparticles showed longer retention and higher accumulation in organs and tissues, especially in the liver, small intestine, and kidney. The most striking difference was an eightfold greater drug accumulation and sustained retention in the kidney. These data indicate that nanoparticulate formulation of paclitaxel affects its clearance as well as distribution in tissues with preferential accumulation in the liver, spleen, small intestine, and kidney. Solid tumors have unique features, such as leaky tumor blood vessels and defective lymphatic drainage, that promote the delivery and retention of macromolecules or particles, a phenomenon recognized as the enhanced permeability and retention effect. Tissue specificity of the gelatin nanoparticles warrants further investigations before using nanoparticle formulations of anticancer drugs for tumors in various organs.

AI-850 (Acusphere Inc.) is a rapidly dissolving porous particle formulation of paclitaxel, created by using the company's Hydrophobic Drug Delivery Systems. The patented spray-drying technology embeds small drug particles inside hydrophobic water-soluble matrices so that the whole composition is a mixture of microparticles and nanoparticles. AI-850 was compared to Taxol following intravenous

administration in a rat pharmacokinetic study, a rat tissue distribution study, and a human xenograft mammary tumor model in nude mice (Straub et al. 2005). The volume of distribution and clearance for paclitaxel following intravenous bolus administration of AI-850 were sevenfold and fourfold greater, respectively, than following intravenous bolus administration of Taxol. There were no significant differences between AI-850 and Taxol in tissue concentrations and area under the curve for the tissues examined. Nude mice implanted with mammary tumors showed improved tolerance of AI-850, enabling higher administrable dose of paclitaxel, which resulted in improved efficacy as compared to Taxol administered at its maximum-tolerated dose.

Gold nanoparticles (2 nm) have been covalently functionalized with paclitaxel (Gibson et al. 2007). The synthetic strategy involves the attachment of a flexible hexaethylene glycol linker at the C-7 position of paclitaxel followed by coupling of the resulting linear analogue to phenol-terminated gold nanocrystals. The reaction yields the product with a high molecular weight, while exhibiting an extremely low polydispersity index. The organic shell of hybrid nanoparticles contains 67% by weight of paclitaxel, which corresponds to ~70 molecules of the drug per one nanoparticle. High-resolution TEM was employed for direct visualization of the inorganic core of hybrid nanoparticles, which were found to retain their average size, shape, and high crystallinity after multiple synthetic steps and purifications. The interparticle distance substantially increases after the attachment of paclitaxel as revealed by low-magnification TEM, suggesting the presence of a larger organic shell. Thus organic molecules with exceedingly complex structures can be covalently attached to gold nanocrystals in a controlled manner and fully characterized by traditional analytical techniques. In addition, this approach gives a rare opportunity to prepare hybrid particles with a well-defined amount of drug and offers a new alternative for the design of nanosized drug-delivery systems. Follow-up studies will determine the potency of the paclitaxel-loaded nanoparticles. Since each ball is loaded with a uniform number of drug molecules, it will be relatively easy to compare the effectiveness of the nanoparticles with the effectiveness of generally administered paclitaxel. This technique could help to deliver more of the drug directly to the cancer cells and reduce the side effects of chemotherapy. The aim is to improve the effectiveness of the drug by increasing its ability to stay bound to microtubules within the cell.

Albumin nanoparticle technology enables the transportation of hydrophobic drugs such as paclitaxel without the need of potentially toxic solvents. Nab-paclitaxel can be administered without premedication, in a shorter infusion time and without the need for a special infusion set. Moreover, this technology allows the selective delivery of larger amounts of anticancer drug to tumors, by exploiting endogenous albumin pathways. Nab-paclitaxel is approved for the treatment of metastatic breast cancer, after the failure of first-line standard therapy, when anthracyclines are not indicated. Efficacy and safety data, along with a more convenient administration, confirm the potential for nab-paclitaxel to become a reference taxane in breast cancer treatment (Guarneri et al. 2012).

Nanoparticles Containing Albumin and Antisense Oligonucleotides

Nanoparticles consisting of human serum albumin (HSA) and containing different antisense oligonucleotides (ASO) have been used for drug delivery to tumors. The preparation process has been optimized regarding the amount of solving agent, stabilization conditions, as well as nanoparticle purification. The glutaraldehyde cross-linking procedure of the particle matrix is a crucial parameter for biodegradability and drug release of the nanoparticles. The drug-loading efficiency increases with longer chain length and employment of a phosphorothioate backbone. The resulting nanoparticles can be tested in cell cultures for cytotoxicity and cellular uptake. All cell lines show a significant cellular uptake of HSA nanoparticles. The entrapment of a fluorescent-labeled oligonucleotide within the particle matrix can be used for the detection of the intracellular drug release of the carrier systems. Confocal laser scanning microscopy reveals that nanoparticles cross-linked with low amounts of glutaraldehyde rapidly degrade intracellularly, leading to a significant accumulation of the ASO in cytosolic compartments of the tumor cells.

PEGylated Nanoliposomal Formulation

PEG-coated nanoparticles remain in the tumors and bloodstream longer compared to gelatin nanoparticles. The coating prevents the nanoparticles from removal by the reticuloendothelial system. This property has led to more effective nanoparticles with tumor-targeting properties.

Ceramide, an antimitogenic and proapoptotic sphingolipid, accumulates in cancer tissues and helps to kill cancer cells when patients undergo chemotherapy and radiation. Although the mechanism remains unknown, ceramide is inherently attracted to tumor cells. In vitro tumor cell culture models have shown the potential therapeutic utility of raising the intracellular concentration of ceramide. However, therapeutic use of systemically delivered ceramide is limited by its inherent insolubility in the blood as it is a lipid as well as its toxicity when injected directly into the bloodstream. Packaging ceramide in nanoliposome capsules allows them to travel through the bloodstream without causing toxicity and release the ceramide in the tumor. Systemic intravenous delivery of C6-ceramide (C6) in a PEGylated liposomal formulation significantly limited the growth of solid tumors in a syngeneic BALB/c mouse tumor model of breast adenocarcinoma (Stover et al. 2005). A pharmacokinetic analysis of systemic liposomal-C6 delivery showed that the PEGylated liposomal formulation follows first-order kinetics in the blood and achieves a steady-state concentration in tumor tissue. Intravenous liposomal-C6 administration was also shown to diminish solid tumor growth in a human xenograft model of breast cancer. In this study, in mice, the ceramide bundles targeted and destroyed only breast cancer cells, sparing the surrounding healthy tissue. Together, these results indicate that bioactive ceramide analogues can be incorporated into PEGylated liposomal vehicles for improved solubility, drug delivery, and antineoplastic efficacy. The next step is to explore how additional chemotherapeutic agents could be incorporated into the liposomes for a more lasting effect.

Peptide-Linked Nanoparticle Delivery

The coupling chemistry and surface charge effects of peptide labeling in nanoparticle drug-delivery strategies are more difficult to control than using folate. Chemical conjugation to peptides reduces colloidal stability, which is a limiting factor in the development of targeting nanoparticles. However, the successful peptide targeting of structural, hormonal cytokine and endocrine receptors in the delivery of therapeutic and diagnostic radionuclides provides justification for finding methods to synthesize peptide-targeted nanoparticles (Franzen 2011). Although most of the work so far has been done using gold nanoparticles, biological and polymer nanoparticles are more colloidally stable and present enormous opportunities for coupling to peptides. Further studies are needed to develop peptide targeting for nanoparticles to rival the selectivity that has been achieved with the small-molecule folate.

Poly-2-Hydroxyethyl Methacrylate Nanoparticles

Poly-2-hydroxyethyl methacrylate nanoparticles can potentially be used for the controlled release of the anticancer drug doxorubicin and reduction of its toxicity (Chouhan and Bajpai 2009). Suspension polymerization of 2-hydroxyethyl methacrylate (HEMA) results in the formation of swellable nanoparticles of defined composition. Release profiles of doxorubicin can be greatly modified by varying the experimental parameters such as percent loading of doxorubicin and concentrations of HEMA, cross-linker, and initiator. Swelling of nanoparticles and the release of doxorubicin increases with the increase in percentage loading of drug. Absorption spectra of doxorubicin do not change following its capture and release from the nanoparticles, indicating that chemical structure of the drug is likely to be unaffected by the procedure.

Polypeptide–Doxorubicin-Conjugated Nanoparticles

Artificial recombinant chimeric polypeptides (CPs), produced from genetically altered *Escherichia coli*, have been shown to spontaneously self-assemble into 50-nm nanoparticles on conjugation with various chemotherapeutics regardless of their water solubility (MacKay et al. 2009). CPs contain a biodegradable polypeptide that is attached to a short Cys-rich segment. Covalent modification of the Cys residues with a structurally diverse set of chemotherapeutics leads to spontaneous formation of nanoparticles over a range of CP compositions and molecular weights. Attachment to one of CPs induces characteristics that the drug alone does not possess. Most chemotherapeutics do not dissolve in water, which limits their ability to be taken in by cells, but attachment to a nanoparticle makes the drug soluble. When used to deliver chemotherapeutics to a murine cancer model, CP nanoparticles have a fourfold higher maximum-tolerated dose than free drug and induce nearly complete tumor regression after a single dose. After delivering the drug to the tumor, the delivery vehicle breaks down into harmless by-products, markedly decreasing

the toxicity for the recipient. This simple as well as inexpensive strategy can promote co-assembly of drugs, imaging agents, and targeting moieties into multifunctional nanomedicines. Since blood vessels supplying tumors are more porous, or leaky, than normal vessels, the nanoformulation can more easily enter and accumulate within tumor cells. This means that higher doses of the drug can be delivered, increasing its anticancer effects while decreasing the side effects associated with systematic chemotherapy.

Protosphere Nanoparticle Technology

Protosphere™ nanoparticle technology (Abraxis Bioscience Inc), also referred to as nanoparticle albumin-bound or nab™ technology, was used to integrate biocompatible proteins with drugs to create the nanoparticle form of the drug having a size of about 100–200 nm. SPARC (secreted protein acidic and rich in cysteine), a protein overexpressed and secreted by cancer cells, binds albumin to concentrate albumin-bound cytotoxic drugs at the tumor.

The product Abraxane (ABI-007) is a patented albumin-stabilized nanoparticle formulation of paclitaxel (nab-paclitaxel) designed to overcome insolubility problems encountered with paclitaxel. The solvent Cremophor EL, used previously in formulations of paclitaxel, causes severe hypersensitivity reactions. To reduce the risk of allergic reactions when receiving Taxol, patients must undergo premedication using steroids and antihistamines and be given the drug using slow infusions. The active component (paclitaxel) can be delivered into the body at a 50% higher dose over 30 min. This contrasts with Taxol infusions, which can take up to 3 h. Because Abraxane is solvent-free, solvent-related toxicities are eliminated, premedication is not required, and administration can occur more rapidly. Abraxane also has a different toxicity profile than solvent-based paclitaxel, including a lower rate of severe neutropenia. In a randomized phase III trial, the response rate of Abraxane was almost twice that of the solvent-containing drug Taxol. Because Abraxane does not contain solvents, higher doses of paclitaxel could be given which may account in part for its increased anticancer activity. In addition, albumin is a protein that normally transports nutrients to cells and has been shown to accumulate in rapidly growing tumors. Therefore, Abraxane's increased effectiveness may also be due to preferential delivery of albumin-bound paclitaxel to cancer cells. In addition to the standard infusion formulation of Abraxane, oral and pulmonary delivery formulations are also being investigated.

A randomized controlled phase III clinical trial compared the safety and efficacy of 260 mg/m^2 of Abraxane to 175 mg/m^2 of Taxol administered every 3 weeks in patients with metastatic breast cancer (Gradishar et al. 2005). Abraxane was infused over 30 min without steroid pretreatment and at a higher dose than Taxol, which requires steroid therapy and infusion over 3 h. Abraxane was found to be superior to Taxol on lesion response rate as well as on tumor progression rate. In 2005, the FDA approved Abraxane for the treatment of metastatic breast cancer. Abraxane also is being evaluated in non-small-cell lung, ovarian, melanoma, and cervical cancers.

Zinc Oxide Nanoparticles for Drug Delivery in Cancer

Zinc oxide (ZnO) nanomaterials provide versatile platforms for biomedical applications and therapeutic intervention, and recent studies demonstrate that they hold considerable promise as anticancer agents. Several of these are under development at the experimental, preclinical, and clinical stages. Through a better understanding of the mechanisms of action and cellular consequences resulting from nanoparticle interactions with cells, the inherent toxicity and selectivity of ZnO nanoparticles against cancer may be improved further to make them attractive new anticancer agents (Rasmussen et al. 2010).

Nanoparticles for Targeted Delivery of Anticancer Therapeutics

Nanosystems are emerging that may be very useful for tumor-targeted drug delivery: novel nanoparticles are preprogrammed to alter their structure and properties during the drug-delivery process to make them most effective for the different extra- and intracellular delivery steps (Wagner 2007). This is achieved by the incorporation of molecular sensors that are able to respond to physical or biological stimuli, including changes in pH, redox potential, or enzymes. Tumor-targeting principles include systemic passive targeting and active receptor targeting. Physical forces (e.g., electric or magnetic fields, ultrasound, hyperthermia, or light) may contribute to focusing and triggered activation of nanosystems. Biological drugs delivered with programmed nanosystems also include plasmid DNA, siRNA, and other therapeutic nucleic acids.

Drug-delivery systems are being developed that attempt to destroy tumors more effectively by using synthesized smart nanoparticles that target and kill cancer cells while sparing healthy cells. Such particles can be injected intravenously into the blood circulation. Each particle, chemically programmed to have an affinity for the cell wall of tumor, can recognize the cancer cell, anchor itself to it, and diffuse inside the cell. Once inside, the particle disintegrates, causing a nearly instantaneous release of the drug precisely where it is needed. To be effective, the nanoparticles must evade the body's immune system, penetrate into the cancer cells, and discharge the drugs before being recognized by the cancer cells. Advantages of such systems are:

- They can fool cancer cells, which are very good at detecting and rejecting drugs.
- Provide rapid drug delivery at sufficiently high concentration that can overwhelm the cancer cell's resistance mechanisms.
- Reduction of side effects because the cancer cells are targeted selectively, sparing the normal cells.

Another approach is to use a nanoparticle made of a hydrogen and carbon polymer with anticancer drug bound up in its fabric and attached to a substance that targets cancer cells. Following intravenous injection, the polymer would gradually

dissolve on reaching the target and gradually release the drug. One limitation of systemic introduction of such nanoparticles, unless properly targeted, is that they may end up in the liver and spleen. This is an unwanted side effect because once the nanoparticles dissolve in those organs, they release toxic levels of chemotherapy in healthy tissues.

Canine Parvovirus as a Nanocontainer for Targeted Drug Delivery

The canine parvovirus (CPV) utilizes transferrin receptors (TfRs) for binding and cell entry into canine as well as human cells. TfRs are overexpressed by a variety of tumor cells and are widely being investigated for tumor-targeted drug delivery. To explore the natural tropism of CPV to TfRs for targeting tumor cells, CPV viruslike particles (VLPs) produced by expression of the CPV-VP2 capsid protein in a baculovirus expression system were examined for attachment of small molecules and delivery to tumor cells (Singh et al. 2006a). Structural modeling suggested that six lysines per VP2 subunit are presumably addressable for bioconjugation on the CPV capsid exterior. Between 45 and 100 of the possible 360 lysines/particle could be routinely derivatized with dye molecules depending on the conjugation conditions. Dye conjugation also demonstrated that the CPV-VLPs could withstand conditions for chemical modification on lysines. Attachment of fluorescent dyes neither impaired binding to the TfRs nor affected internalization of the 26-nm-sized VLPs into several human tumor cell lines. CPV-VLPs therefore exhibit highly favorable characteristics for development as a novel nanomaterial for tumor targeting.

Carbon Magnetic Nanoparticles for Targeted Drug Delivery in Cancer

Using dense medium plasma technology, carbon magnetic nanoparticles (CMNP) have been synthesized at room temperature and atmospheric pressure. Results of X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, and scanning electron microscopy show that these nanoparticles are composed of spherical particles, 40–50 nm in diameter, with iron/iron oxide particles dispersed in a carbon-based host structure (Ma et al. 2004). Thermal gravimetry/differential thermal gravimetry analysis shows these nanoparticles are stable to temperatures as high as 600 °C. The synthesized CMNP were treated by argon plasma, aminated with ethylene diamine and subsequently activated by generating aldehyde groups on them. Free doxorubicin (DOX) molecules are then immobilized onto the surfaces of activated CMNP particles to form CMNP–DOX conjugates. The in vitro antiproliferative activity of immobilized doxorubicin in the conjugates has been demonstrated in tumor cell cytotoxicity assays. It is suggested that this CMNP–DOX system can be used for targeted drug-delivery systems in cancer.

Carbon Nanotubes for Targeted Drug Delivery to Cancer Cells

CNTs with DNA and RNA wrapped around them to make them biocompatible can be targeted toward cancer cells by attaching additional molecules. Such CNT composites,

in combination with laser treatment, may be used to destroy the cancer cells. An improved delivery scheme for intracellular tracking and anticancer therapy uses a novel double functionalization of a carbon nanotube delivery system containing antisense oligodeoxynucleotides as a therapeutic gene and CdTe QDs as fluorescent-labeling probes via electrostatically layer-by-layer assembly (Jia et al. 2007).

Chemically functionalized SWCNTs have shown promise in tumor-targeted accumulation in mice and exhibit biocompatibility, excretion, and little toxicity. The anticancer drug paclitaxel (PTX) has been conjugated to branched PEG chains on SWCNTs via a cleavable ester bond to obtain a water-soluble SWCNT–PTX conjugate (Liu et al. 2008a). SWCNT–PTX is more efficient in suppressing tumor growth than Taxol in a murine 4 T1 breast cancer model, owing to prolonged blood circulation and tenfold higher tumor PTX uptake by SWCNT delivery, likely through enhanced permeability and retention. Drug molecules carried into the reticuloendothelial system are released from SWCNTs and excreted via biliary pathway without toxic effects on normal organs. Thus, CNT drug delivery is promising for enhancing treatment efficacy and minimizing side effects of cancer therapy by use of low drug doses. Water-dispersed carbon nanohorns, prepared by adsorption of polyethylene glycol–doxorubicin conjugate (PEG–DXR) onto oxidized single-wall carbon nanohorns, have been shown to be effective anticancer drug-delivery carriers when administered intratumorally to human NSCLC-bearing mice (Murakami et al. 2008). There was significant retardation of tumor growth associated with prolonged DXR retention in the tumor.

Although considerable further work is required before any new drugs based on CNTs are developed, it is hoped that it will eventually lead to more effective treatments for cancer. However, it is too early to claim whether carbon-based nanomaterials will become clinically viable tools to combat cancer, although there is definitely room for them to complement existing technologies.

Cyclosert System for Targeted Delivery of Anticancer Therapeutics

Cyclosert™ (Calando/Insert Therapeutics) is the first nanoparticle drug transport platform to be designed de novo and synthesized specifically to overcome limitations in existing technologies used for the systemic transport of therapeutics to targeted sites within the body. Based on small cyclic repeating molecules of glucose called cyclodextrins, Cyclosert promotes the ability of cytotoxic drugs to inhibit the growth of human cancer cells while reducing toxicity and remaining nonimmunogenic at therapeutic doses. In particular, the system is designed to reduce the toxicity of the drugs until they actually reach the targeted tumor cells where the active drug is released in a controlled fashion. Animal studies have shown that the Cyclosert system can safely deliver tubulysin A, a potent, but highly toxic, antitumor agent. In vitro studies have shown the tubulysin–Cyclosert conjugate to be effective against multiple human cancer cell lines. The conjugate is stable and 100 times more water soluble than the free drug. Calando is developing CALAA01, a siRNA, for anticancer use using Cyclosert as a delivery system.

IT-101 (Calando) is a de novo-designed experimental therapeutic comprised of linear, cyclodextrin(CD)-containing polymer conjugates of camptothecin (CPT)

that assemble into 40-nm-diameter nanoparticles via polymer–polymer interactions that involve inclusion complex formation between the CPT and the CD. Particle size, near-neutral surface charge, and CPT release rate were specifically designed into IT-101. Cyclosert platform forms nanoscale constructs with hydrodynamic diameters between 30 and 60 nm. This makes Cyclosert-based drugs ideal for effective delivery to solid tumors. Preclinical animal studies show extended circulation times, tumor accumulation, slow release of the CPT, and anticancer efficacy that directly correlate to the properties of the nanoparticle. Release of CPT can disassemble the nanoparticle into individual polymer chains ~10 nm in size that are capable of renal clearance. IT-101 has been evaluated in patients with relapsed or refractory cancer following two cycles of therapy by intravenous infusion. Interim analysis shows that IT-101 is well tolerated and pancytopenia is the dose-limiting toxicity (Yen et al. 2007). Pharmacokinetics data were favorable and consistent with results from preclinical animal studies. In the patients studied, IT-101 showed longer half-life, lower clearance, and lower volume of distribution than seen in patients treated with other camptothecin-based drugs. It is in phase II clinical trials.

DNA Aptamer–Micelle for Targeted Drug Delivery in Cancer

Design of a self-assembled aptamer–micelle nanostructure has been reported that achieves selective and strong binding of otherwise low-affinity aptamers at physiological conditions (Wu et al. 2009). Specific recognition ability is directly built into the nanostructures. The attachment of a lipid tail onto the end of nucleic acid aptamers provides these unique nanostructures with an internalization pathway. Other merits include extremely low off rate once bound with target cells, rapid recognition ability with enhanced sensitivity, low critical micelle concentration values, and dual-drug-delivery pathways. To prove the potential detection/delivery application of this aptamer–micelle in biological living systems, the authors mimicked a tumor site in the blood stream by immobilizing tumor cells onto the surface of a flow channel device. Flushing the aptamer–micelles through the channel demonstrated their selective recognition ability under flow circulation in human whole-blood sample. The aptamer–micelles show great dynamic specificity in flow channel systems that mimic drug delivery in the blood system. Therefore, DNA aptamer–micelle assembly has shown high potential for cancer cell recognition and for targeted in vivo drug-delivery applications.

Fullerenes for Enhancing Tumor Targeting by Antibodies

Although it was previously possible to attach drug molecules directly to antibodies, scientists have not been able to attach more than a handful of drug molecules to an antibody without significantly changing its targeting ability. That happens, in large part, because the chemical bonds that are used to attach the drugs – strong, covalent bonds – tend to block the targeting centers on the antibody's surface. If an antibody is modified with too many covalent bonds, the chemical changes will destroy its ability to recognize the cancer it was intended to attack.

In order to overcome this limitation, a new class of anticancer compounds have been created that contain both tumor-targeting antibodies and nanoparticles called fullerenes (C60), which can be loaded with several molecules of anticancer drugs like Taxol® (Ashcroft et al. 2006). It is possible to load as many as 40 buckyballs into a single skin-cancer antibody called ZME-018, which can be used to deliver drugs directly into melanoma tumors. Certain binding sites on the antibody are hydrophobic (water repelling) and attract the hydrophobic fullerenes in large numbers, so multiple drugs can be loaded into a single antibody in a spontaneous manner. No covalent bonds are required, so the increased payload does not significantly change the targeting ability of the antibody. The real advantage of fullerene immunotherapy over other targeted therapeutic agents is likely to be the fullerene's potential to carry multiple drug payloads, such as Taxol plus other chemotherapeutic drugs. Cancer cells can become drug resistant, and one can cut down on the possibility of their escaping treatment by attacking them with more than one kind of drug at a time. The first fullerene immunoconjugates have been prepared and characterized as an initial step toward the development of fullerene immunotherapy.

Gold Nanoparticles for Targeted Drug Delivery in Cancer

Gold and silica composite nanoparticles have been investigated as nanobullets for cancer. Gold atoms bind to silicon atoms with dangling bonds and serve as seeds for the growth of Au islands. The large electron affinity of gold causes a significant change in the electronic structure of silica resulting in a substantial reduction in the highest occupied and the lowest unoccupied molecular orbital and the optical gap, thus allowing it to absorb near-infrared radiation. This suggests that a small cluster can have a similar effect in the treatment of cancer as the large-size nanoshell, but with a different mechanism.

The unique chemical properties of colloidal gold make it a promising targeted delivery approach for drugs or genes to specific cells. The physical chemical properties of colloidal gold permit more than one protein molecule to bind to a single particle of colloidal gold. CytImmune Sciences Inc has shown that tumor necrosis factor (TNF) can be bound to gold nanocrystals and delivered safely and effectively to tumor-burdened mice and dogs. CytImmune scientists have characterized and modified the colloidal gold (cAu) particles to optimize binding of TNF to the nanocrystals and also the targeting of the particles to the tumor. The therapeutic compounds that CytImmune is developing are new formulations of the TNF-α, which causes the death of tumors but is toxic to healthy organs. Coupling TNF-α to colloidal gold is expected to improve the safety and effectiveness of anticancer therapy. Specifically, two drugs are in development: Aurimune-T and AuriTax. Aurimune-T is manufactured by covalently linking molecules of TNF-α and thiol-derivatized polyethylene glycol (PEG-THIOL) onto the surface of 25-nm colloidal gold. Intravenously administered Aurimune-T rapidly accumulates in solid tumors implanted in mice and shows little to no accumulation in the reticuloendothelial system or in other healthy organs. Coincident with the sequestration of gold is a tenfold accumulation of TNF-α in the tumor when compared to animals treated

with native TNF-α. By getting more TNF-α to the tumor, Aurimune-T improves the safety and efficacy of TNF-α treatment since maximal tumor responses were achieved at lower doses of the drug. The second nanoparticle drug, AuriTax, consists of TNF-α, a chemotherapeutic (paclitaxel), and PEG-THIOL, which are bound to the same cAu nanoparticle. Like Aurimune-T, AuriTax delivers tenfold more TNF-α and paclitaxel to the solid tumor when compared to each drug alone. These results support the continued development of the colloidal gold platform for cancer therapy and TNF-α as a tumor-targeting ligand.

Biocompatible and nontoxic PEGylated gold nanoparticles with surface-enhanced Raman scattering have been used for in vivo tumor targeting and detection (Qian et al. 2008). Colloidal gold has been safely used to treat rheumatoid arthritis for 50 years and has recently been found to amplify the efficiency of Raman scattering by 14–15 orders of magnitude. It has been shown that large optical enhancements can be achieved under in vivo conditions for tumor detection in live animals. An important finding is that small-molecule Raman reporters such as organic dyes are not displaced but were stabilized by thiol-modified polyethylene glycols. These PEGylated SERS nanoparticles are considerably brighter than semiconductor QDs with light emission in the near-infrared window. When conjugated to tumor-targeting ligands such as single-chain variable fragment antibodies (ScFv), the conjugated nanoparticles are able to target tumor biomarkers such as EGFRs on human cancer cells and in xenograft tumor models. ScFv peptides bind cancer cells and the gold particles latch onto tumors after their injection into a mouse. When illuminated with a laser beam, the tumor-bound particles emit a signal that is specific to the dye. The signal from the dye tags is very bright and the distinct peaks in the dye signal mean several different probes could be used at the same time. The tags' rich spectroscopic signatures provide the capability of using several probes at once, but that will require more sophisticated computational tools. The authors are developing data processing tools and making them available to the NCI's caBIG (cancer biomedical informatics grid) so that the research community can use them. Compared with QDs, the gold particles are more than 200 times brighter on a particle-to-particle basis, although they are about 60 times larger by volume. Covered with a nontoxic polymer, the gold particles are about 60–80 nm in diameter, that is, 150 times smaller than a typical human cell and thousands of times smaller than a human hair. The researchers were able to detect human cancer cells injected into a mouse at a depth of 1–2 cm. That makes the gold particles especially appropriate tools for gathering information about head or neck tumors, which tend to be more accessible. The technology will need further adaptation for use with abdominal or lung cancers deep within the body.

PEGylated gold nanoparticles are decorated with various amounts of human transferrin (Tf) to give a series of Tf-targeted particles with near-constant size and electrokinetic potential. Studies in experimental animals with tumors show that quantitative biodistribution of the nanoparticles 24 h after intravenous injections results in their accumulations in the tumors and other organs independent of Tf (Choi et al. 2010). However, the nanoparticle localization within a particular organ

is influenced by the Tf content. In tumor tissue, the content of targeting ligands significantly influences the number of nanoparticles localized within the cancer cells. In liver tissue, high Tf content leads to small amounts of the nanoparticles residing in hepatocytes, whereas most nanoparticles remain in nonparenchymal cells. These results suggest that targeted nanoparticles can provide greater intracellular delivery of therapeutic agents to the cancer cells within solid tumors than their nontargeted analogues.

Selective transport of gold nanoparticles to the nuclei of cancer cells has been achieved by properly conjugating them with specific peptides (Kang et al. 2010). Localization of gold nanoparticles at the nucleus of a cancer cell damages the DNA resulting in double-strand breaks. Dark-field imaging of live cells in real time revealed that the nuclear targeting of gold nanoparticles specifically induces cytokinesis arrest in cancer cells leading apoptosis.

Magnetic Nanoparticles for Remote-Controlled Drug Delivery to Tumors

Remotely controlled nanoparticles, when pulsed with an electromagnetic field, can release anticancer drugs into tumors (Derfus et al. 2007). The innovation could lead to the improved diagnosis and targeted treatment of cancer. In an earlier work, injectable multifunctional nanoparticles were designed to flow through the bloodstream, home to tumors, and clump together. Clumped particles help visualization of tumors by MRI. The system that makes it possible consists of particles that are superparamagnetic, a property that causes them to give off heat when they are exposed to a magnetic field. Tethered to these particles are active molecules, such as anticancer drugs. Exposing the particles to a low-frequency electromagnetic field causes the particles to radiate heat, which melts the tethers and releases the drugs. The waves in this magnetic field have frequencies between 350 and 400 kHz – the same range as radio waves. These waves pass harmlessly through the body and heat only the nanoparticles. The tethers in the system consist of strands of DNA. Two strands of DNA link together through hydrogen bonds that break when heated. In the presence of the magnetic field, heat generated by the nanoparticles breaks these, leaving one strand attached to the particle and allowing the other to float away with its cargo. One advantage of a DNA tether is that its melting point is tunable. Longer strands and differently coded strands require different amounts of heat to break. This heat-sensitive tunability makes it possible for a single particle to simultaneously carry many different types of cargo, each of which can be released at different times or in various combinations by applying different frequencies or durations of electromagnetic pulses. To test the particles, the researchers implanted mice with a tumorlike gel saturated with nanoparticles. They placed the implanted mouse into the well of a cup-shaped electrical coil and activated the magnetic pulse. The results confirm that without the pulse, the tethers remain unbroken. With the pulse, the tethers break and release the drugs into the surrounding tissue. The experiment is a proof of principle demonstrating a safe and effective means of tunable remote activation. However, work remains to be done before such therapies become viable in the clinic.

Mesoporous Silica Nanoparticles

Mesoporous silica nanoparticles (MSNs) have a considerable potential for drug-delivery applications due to their flexibility and high drug load potential. MSNs are biodegradable and mostly eliminated through renal clearance. Although numerous reports demonstrate sophisticated drug-delivery mechanisms in vitro, the therapeutic benefit of these systems for in vivo applications has been uncertain in the past. Recent preclinical data has demonstrated that MSNs are safe and a biocompatible technology platform for targeted drug delivery, passive as well as folate-directed, in cancer. MSN medical applicability in vivo has been demonstrated for both drug delivery and diagnostics. Therapeutic efficacy of MSNs has been shown in vivo following oral, local, subcutaneous, or intravenous administration, and MSNs have been approved for clinical trials. Incorporation of multiple therapeutic and diagnostic agents into MSNs is feasible, and this will enable diagnostic-guided therapy. There are still several issues to be considered before MSNs can be used in clinical practice (Rosenholm et al. 2012).

Nanobees for Targeted Delivery of Cytolytic Peptide Melittin

The in vivo application of cytolytic peptides for cancer therapeutics is hampered by toxicity, nonspecificity, and degradation. A specific strategy was developed to synthesize a nanoscale delivery vehicle for cytolytic peptides by incorporating the synthetic version of a toxin called melittin that is found in bees into the outer lipid monolayer of a perfluorocarbon (PFC) nanoparticle. The composite structures, called nanobees, are engineered to travel directly to tumor cells without harming any others. They spare the healthy cells but attach to tumor blood vessels, which express a particular protein to which a substance on the nanobees has a chemical affinity. Melittin, which would destroy red blood cells and other normal tissues if it is delivered intravenously, is completely safe when carried on a nanoparticle. Favorable pharmacokinetics of this nanocarrier have been demonstrated, which allow accumulation of melittin in murine tumors in vivo and a dramatic reduction in tumor growth without any apparent signs of toxicity (Soman et al. 2009). Furthermore, direct assays demonstrated that molecularly targeted nanocarriers selectively delivered melittin to multiple tumor targets, including endothelial and cancer cells, through a hemifusion mechanism. In cells, this hemifusion and transfer process did not disrupt the surface membrane but did trigger apoptosis and in animals caused regression of precancerous dysplastic lesions. Collectively, these data suggest that the ability to restrain the wide-spectrum lytic potential of a potent cytolytic peptide in a nanovehicle, combined with the flexibility of passive or active molecular targeting, represents an innovative molecular design for chemotherapy with broad-spectrum cytolytic peptides for the treatment of cancer at multiple stages. So far, nanobees have been tested only on mice, with promising results, but what works in mice does not always work in humans. If proven to be effective in humans, this therapy could become widely available in about 5–10 years.

Nanovehicles for Targeted Delivery of Paclitaxel

Nanoparticles have been used to deliver paclitaxel, an antitumor drug, directly to tumors for targeted anticancer treatment. The nanoparticles were loaded with paclitaxel and then mixed with lipids to form nanoparticle-like clusters, which were then coated with a glycosaminoglycan (GAG). The nanovehicle was formed of clusters of loaded nanoparticles then coated with a GAG. When these clusters come into contact with cancerous cells, the paclitaxel is released from the individual nanoparticles directly into the cancerous cell. This targeted release allows the treatment to be focused to the cancerous cells, reducing the negative effects of the chemotherapy treatment.

The ability of the nanovehicles to specifically target the cancerous cells is due to the specific GAG used in the coating of the clusters. The researchers use hyaluronan, a sugar that is recognized by receptors on many different types of cancer cell. When the nanovehicle interacts with the cancer cell, the sugar is recognized by the receptors, triggering a structural change and the subsequent release of the paclitaxel directly into the cell. The release of the paclitaxel directly into the target cell also overcomes the issues associated with the solubility of the drug (Rivkin et al. 2010). The vehicle is similar to a cluster bomb; when the delivery vehicle, comprising of multiple nanoparticles, comes into contact with cancer cells, it releases the chemotherapeutic payload directly into the cell. Peer also mentions that the device can be used to treat a variety of cancers, such as blood, colon, breast, pancreatic, ovarian, and several types of brain cancer. The ability of the nanovehicle to be recognized by the cancer cells means that it increases the effectiveness of the treatment while healthy cells are unaffected by the treatment. Tests on tumor-bearing mice have demonstrated that the nanoclusters are more effective when compared with free paclitaxel and other albumin nanoparticles containing paclitaxel. The nanoclusters also demonstrated a high safety profile and a high level of accumulation within tumors.

The fabrication of the nanovehicles means that the treatment may potentially be safer than alternative current therapies. The nanocluster is formed of the naturally occurring lipid hyaluronan, a lipid that decomposes in the body once the nanoparticles have delivered the paclitaxel. The researchers hope that GAGs may provide vehicles for other chemotherapy drugs, such as taxanes, and may also be used as carriers for other therapeutic applications.

Nanocell for Targeted Drug Delivery to Tumor

Simultaneous delivery of chemotherapeutic and antiangiogenic drugs is clearly beneficial, but because chemotherapy is blood-borne, shutting down the tumor's blood supply with antiangiogenic drugs may decrease the delivery of drugs designed to kill the tumor cells. A more effective strategy would be to use a delivery vehicle that became concentrated in tumors before the vasculature shuts down and allows the staged release of the two drugs (Sengupta et al. 2005). The delivery of the antiangiogenic factor could lead to a collapse of the vascular network and imprison the

vehicle, which would still be carrying its second payload of chemotherapeutic drug, inside the tumor. The subsequent release of the latter drug within the tumor would kill the cancer cells. For example, a composite nanocell can be constructed with a solid biodegradable polymer core surrounded by a lipid membrane in which the outer membrane is loaded with the antiangiogenic drug combretastatin and the inner membrane with the chemotherapy drug doxorubicin. The nanocells are small enough to pass through tumor blood vessels, but they are too large to pass through the pores of normal vessels. Once inside the tumor, the nanocell's outer membrane can disintegrate, releasing the antiangiogenic drug and causing the collapse of the blood vessels feeding the tumor. The collapsed blood vessels trap the nanocell inside the tumor. The nanocell can then slowly release the chemotherapy drug. Although the effect of the sequential delivery of these two drugs on tumor growth is dramatic, these results cannot be quickly translated into therapy for humans. There is a concern that antiangiogenic drugs may promote the spread of tumors to other tissues. Also, in contrast to combretastatin, many antiangiogenic drugs require prolonged tissue exposure to shut down the vasculature and so may not be amenable to this particular approach with a short exposure time.

Nanodiamonds for Local Delivery of Chemotherapy at Site of Cancer

Nanodiamonds (NDs) with diameter of 2–8 nm, physically bound with doxorubicin, and sandwiched between a base and thin layer of polymer parylene enable extended targeted and controlled release at diseased areas in cancer, viral infection, and inflammation (Lam et al. 2008). A substantial amount of drug can be loaded onto clusters of nanodiamonds, which have a high surface area. Nanodiamonds have many other advantages for drug delivery. They can be functionalized with nearly any type of therapeutic. They can be suspended easily in water, which is important for biomedical applications. They are very scalable and can be produced in large quantities. In control experiments, where the drug was administered without the nanodiamonds, virtually the whole drug was released within 1 day. By adding the drug-laden nanodiamonds to the device, drug release was instantly lengthened to the month-long timescale. The FDA-approved polymer parylene displayed the stable and continuous slow release of drug for at least 1 month due to the powerful sequestration abilities of the DOX–ND complex. The device also avoids the massive initial release of the drug, which is a disadvantage of conventional therapy.

The nanodiamonds are quite economical and have already been mass-produced as lubrication components for automobiles and for use in electronics. Because the fabrication process is devoid of any destructive steps, the DOX–ND conjugates are unaffected and unaltered. The flexible microfilm device resembles a piece of plastic wrap and can be customized easily into different shapes. It can transform conventional treatment strategies and reduce patients' unnecessary exposure to toxic drugs. The biocompatible and minimally invasive device could be used to deliver chemotherapy drugs locally to sites where malignant tumors have been surgically removed.

Nanoimmunoliposome-Based System for Targeted Delivery of siRNA

Low transfection efficiency, poor tissue penetration, and nonspecific immune stimulation by in vivo administered siRNAs have delayed their therapeutic applications. Their potential as anticancer therapeutics hinges on the availability of a vehicle that can be systemically administered, safely and repeatedly, and will deliver the siRNA specifically and efficiently to both primary tumors and metastases. A nanosized immunoliposome-based delivery complex (scL) has been developed that will preferentially target and deliver molecules, including plasmid DNA and antisense oligonucleotides, to tumor cells following systemic administration (Pirollo et al. 2007). This tumor-targeting nanoparticle delivery vehicle can also deliver siRNA to both primary and metastatic disease. The efficiency of this complex has been enhanced by the inclusion of a pH-sensitive histidine–lysine peptide in the complex (scL–HoKC) and by delivery of a modified hybrid (DNA–RNA) anti-HER-2 siRNA molecule. Scanning probe microscopy confirms that this modified complex maintains its nanoscale size. More importantly, this nanoimmunoliposome anti-HER-2 siRNA complex can sensitize human tumor cells to chemotherapeutics, silence the target gene, affect its downstream pathway components in vivo, and significantly inhibit tumor growth in a pancreatic cancer model. This complex has the potential to help translate the potent effects of siRNA into a clinically viable anticancer therapeutic.

Nanoparticle-Mediated Targeting of MAPK Signaling Pathway

The MAPK signal transduction cascade is dysregulated in a majority of human tumors, and nanoparticle-mediated targeting of this pathway can optimize cancer chemotherapy. Nanoparticles engineered from a polymer that is chemically conjugated to a selective MAPK inhibitor, PD98059, are taken up by cancer cells through endocytosis and demonstrate sustained release of the active agent, resulting in the inhibition of phosphorylation of downstream extracellular signal-regulated kinase (Basu et al. 2009). Modification of the polymer, which is biocompatible as well as biodegradable and approved by the FDA, leads to a 20-fold increase in drug-loading capacity. Nanoparticle-mediated targeting of MAPK has been shown to inhibit the proliferation of melanoma and lung carcinoma cells and induce apoptosis in vitro. Administration of the PD98059-nanoparticles in melanoma-bearing mice inhibits tumor growth and enhances the antitumor efficacy of cisplatin chemotherapy. This study shows the nanoparticle-mediated delivery of signal transduction inhibitors is a potentially effective method of cancer chemotherapy

Nanoparticles for Targeted Antisense Therapy of Cancer

Antisense oligonucleotides (ASO) against specific molecular targets (e.g., Bcl-2 and Raf-1) are important reagents in cancer biology and therapy. Phosphorothioate modification of the ASO backbone has resulted in an increased stability of ASO in vivo without compromising, in general, their target selectivity. Although the

power of antisense technology remains unsurpassed, dose-limiting side effects of modified ASO and inadequate penetration into the tumor tissue have necessitated further improvements in ASO chemistry and delivery systems. Oligonucleotide delivery systems may increase stability of the unmodified or minimally modified ASO in plasma, enhance uptake of ASO by tumor tissue, and offer an improved therapy response. An overview of ASO design and in vivo delivery systems with focus on preclinical validation of a liposomal nanoparticle containing minimally modified raf antisense oligodeoxynucleotide (LErafAON) has been published (Zhang et al. 2007). Intact rafAON (15-mer) is present in plasma and in normal and tumor tissues of athymic mice systemically treated with LErafAON. Raf-1 expression is decreased in normal and tumor tissues of LErafAON-treated mice. Therapeutic benefit of a combination of LErafAON and radiation or an anticancer drug exceeds radiation or drug alone against human prostate, breast, and pancreatic tumors grown in athymic mice. Further improvements in ASO chemistry and nanoparticles are promising avenues in antisense therapy of cancer.

Nanoparticles for Delivery of Suicide DNA to Prostate Tumors

A prostate-specific, locally delivered gene therapy has been developed for the targeted killing of prostate cells using C32/DT-A, a degradable polymer a nanoparticulate system, to deliver a diphtheria toxin suicide gene (DT-A) driven by a prostate-specific promoter to cells (Peng et al. 2007b). These nanoparticles were directly injected to the normal prostate and to prostate tumors in mice. Nearly 50% of normal prostates showed a significant reduction in size, attributable to cellular apoptosis, whereas injection with naked DT-A-encoding DNA had little effect. A single injection of C32/DT-A nanoparticles triggered apoptosis in 80% of tumor cells present in the tissue. It is expected that multiple nanoparticle injections would trigger a greater percentage of prostate tumor cells to undergo apoptosis. These results suggest that local delivery of polymer/DT-A nanoparticles may have application in the treatment of benign prostatic hypertrophy and prostate cancer.

Nanoparticles for Targeted Delivery of Concurrent Chemoradiation

The development of chemoradiation – the concurrent administration of chemotherapy and radiotherapy – has led to significant improvements in local tumor control and survival. However, it is limited by its high toxicity. A novel NP therapeutic, ChemoRad NP, has been developed, which can deliver biologically targeted chemoradiation (Wang et al. 2010a). This is a biodegradable and biocompatible lipid–polymer hybrid NP that is capable of delivering both chemotherapy and radiotherapy. Using docetaxel, indium^{111}, and yttrium^{90} as model drugs, ChemoRad NP was shown to encapsulate chemotherapeutics (up to 9% of NP weight) and radiotherapeutics (100 mCi of radioisotope per gram of NP) efficiently and deliver both effectively. Targeted delivery of ChemoRad NPs and high therapeutic efficacy of ChemoRad NPs was demonstrated using prostate cancer as a disease model.

ChemoRad NP represents a new class of therapeutics that holds great potential to improve cancer treatment.

Nanoparticle-Based Therapy Targeted to Cancer Metastases

Early detection of metastases plays an important role in the management of metastatic cancer. In patients with prostate cancer who undergo surgical lymph node resection or biopsy, MRI with lymphotropic superparamagnetic nanoparticles can correctly identify all patients with nodal metastases. This diagnosis is not possible with conventional MRI alone and has implications for the management of men with metastatic prostate cancer, in whom adjuvant androgen-deprivation therapy with radiation is the mainstay of management.

Nanoparticle formulations of anticancer drugs may be more effective against cancer metastases. Nanoparticles have the ability to transport complex molecular cargoes to the major sites of metastasis, such as the lungs, liver, and lymph nodes, as well as targeting to specific cell populations within these organs (Schroeder et al. 2012). Oral administration of alpha-TEA formulated in liposome or biodegradable poly(D, L-lactide-co-glycolide) nanoparticle has been shown to significantly reduce tumor burden in a mammary cancer mouse model (Wang et al. 2007a). Both formulations reduced lymph node and lung micrometastatic tumor foci, but nanoparticle formulation was more effective in reducing metastases. Tumor targeting with nanoparticles facilitates systemic delivery of immunomodulatory cytokine genes to remote sites of cancer metastasis. Targeted delivery and localized expression of the intravenously administered nanoparticles bearing the gene encoding granulocyte/macrophage colony-stimulating factor was confirmed in a patient with metastatic cancer, as was the recruitment of significant tumor-infiltrating lymphocytes (Gordon et al. 2008).

Nanostructured Hyaluronic Acid for Targeted Drug Delivery in Cancer

Active targeting of bioactive molecules by nanoparticulate delivery systems that include hyaluronic acid (HA) in their structures is an attractive approach to drug delivery because HA is biocompatible, nontoxic, and noninflammatory. To make HA useful as an intravenous targeting carrier, strategies have to be devised to reduce its clearance from the blood, suppress its uptake by liver and spleen, and provide tumor-triggered mechanisms of release of an active drug from the HA carrier (Ossipov 2010).

HA nanoparticles (HA-NPs), which are formed by the self-assembly of hydrophobically modified HA derivatives, have been tested for their physicochemical characteristics and fates in tumor-bearing mice after systemic administration (Choi et al. 2010a). Irrespective of the particle size, significant amounts of HA-NPs circulated for 2 days in the bloodstream and selectively accumulated into the tumor. The smaller HA-NPs were able to reach the tumor more effectively than larger HA-NPs. The concentration of HA-NPs in the tumor site was dramatically reduced when mice were pretreated with an excess of free HA. These results indicate that HA-NPs can accumulate into the tumor site by a combination of passive and active-targeting mechanisms.

Perfluorocarbon Emulsion for Targeted Chemotherapeutic Delivery

Kereos Inc's emulsion particles consist of a perfluorocarbon core surrounded by a lipid monolayer, which stabilizes the particle in addition to providing a virtually unlimited number of anchoring sites for targeting ligands and payload molecules. The result is an oil-in-water emulsion of particles with an average size of approximately 250 nm, referred to as "targeted nanoparticles." Delivered by injection, this approach offers the following advantages:

- High molecular specificity of MAbs, small-molecule ligands, and other targeting ligands for disease biomarkers translates directly into high specificity of the emulsion particles for disease sites.
- Although only 10–100 targeting ligand molecules are needed to direct and securely bind an individual emulsion particle to the disease site, each particle can carry 100,000 or more payload molecules. This "signal amplification" opens up opportunities not otherwise possible.
- Both in terms of size and composition, the emulsion particles are designed to be both safe and effective and to avoid potential problems with distribution, metabolism, or excretion.

Polymer Nanoparticles for Targeted Drug Delivery in Cancer

Cerulean Nanopharmaceuticals' CRLX101 (formerly IT-101), a cyclodextrin polymer-based nanoparticle containing camptothecin, is in phase IIa clinical development for the treatment of cancer. PET data from ^{64}Cu-labeled CRLX101 to quantify the in vivo biodistribution in mice-bearing tumors shows that ~8% of the injected dose is rapidly cleared as a low molecular weight fraction through the kidneys, and the remaining material circulates in plasma with a terminal half-life of 13.3 h (Schluep et al. 2009). A three-compartment model is used to determine vascular permeability and nanoparticle retention in tumors and is able to accurately represent the experimental data. The calculated tumor vascular permeability indicates that the majority of nanoparticles stay intact in circulation and do not disassemble into individual polymer strands. A key assumption to modeling the tumor dynamics is that there is a sink for the nanoparticles within the tumor. Histological measurements using confocal microscopy show that CRLX101 localizes within tumor cells and provides the sink in the tumor for the nanoparticles.

Several mechanisms have been proposed to explain nanoparticle retention in tumors:

1. Dextran-coated iron oxide nanoparticles accumulate in the interstitial fluid and are taken up by tumor vascular endothelial cells, which observed mostly in areas of neovascularization, whereas intracellular concentrations are highest in tumor cells.
2. Long-circulating liposomes accumulate predominantly in tumor stroma, either in the extracellular space or in tumor-associated macrophages in a breast cancer tumor model. A Her2-targeted version of the same liposomes achieves the same

over all tumor concentration, but more internalization by cancer cells through endocytosis is observed.
3. Cyclodextrin-based polymer (CDP) conjugates have been shown to be avidly taken up by cancer cells. This result may be a function of the unique surface characteristics of CDP nanoparticles, which contain hydrophobic pockets within the cyclodextrin molecules that have been shown to interact with lipid rafts of cell membranes.

Scientists at the MIT-Harvard Center for Cancer Nanotechnology Excellence (Cambridge, MA) have studied the effects of altering nanoparticle polymer composition, drug loading, and solvents on the ability of the resulting nanoparticles to target and deliver drugs to tumors. As a targeting agent for all the polymer nanoparticles studied, they used a molecule that recognizes the prostate-specific membrane antigen. The aim of this study was to develop formulation parameters that would control the size of the resulting polymer nanoparticles, which the investigators believe play a major role in optimizing tumor targeting. Nanoparticles were prepared from a biocompatible material. Experimenting with a variety of polymer concentrations and solvent mixtures, they found that they could systematically control the size of the resulting polymers. The results were so consistent that the investigators believe that they may have developed a broadly applicable approach to reproducibly tuning the size of polymer nanoparticles during their formulation. In a final experiment, the researchers added the targeting agent to their optimized nanoparticles. The targeted nanoparticles were able to significantly increase drug delivery to human prostate tumors growing in mice.

Polymersomes for Targeted Cancer Drug Delivery

Polymersomes, hollow shell nanoparticles, have unique properties of that allow them to deliver two distinct drugs, paclitaxel and doxorubicin, directly to tumors implanted in mice (Ahmed et al. 2006). Loading, delivery, and cytosolic uptake of drug mixtures from degradable polymersomes are shown to exploit both the thick membrane of these block copolymer vesicles and their aqueous lumen as well as pH-triggered release within endolysosomes. Drug-delivering polymersomes break down in the acidic environment of the cancer cells resulting in targeted release of these drugs within tumor cells. While cell membranes and liposomes (vesicles often used for drug delivery) are created from a double layer of fatty molecules called phospholipids, a polymersome is comprised of two layers of synthetic polymers. The individual polymers are degradable and considerably larger than individual phospholipids but have many of the same chemical features. The large polymers making up the shell allow paclitaxel, which is water insoluble, to embed within the shell. Doxorubicin, which is water soluble, stays within the interior of the polymersome until it degrades. The polymersome and drug combination is self-assembling and the structure spontaneously forms when all of the components are suitably mixed together. Recent studies have shown that cocktails of paclitaxel and

doxorubicin lead to better tumor regression than either drug alone, but previously, there was no carrier system that could carry both drugs as efficiently to a tumor. Polymersomes get around those limitations.

Another approach is by assembling diverse bioactive agents, such as DNA, proteins, and drug molecules into core-shell multifunctional polymeric nanoparticles (PNPs) that can be internalized in human breast cancer cells (Bertin et al. 2006). Using ring-opening metathesis polymerization, block copolymers containing small-molecule drug segments (>50% w/w) and tosylated hexaethylene glycol segments were prepared and assembled into PNPs that allowed for the surface conjugation of single-stranded DNA sequences and/or tumor-targeting antibodies. The resulting antibody-functionalized particles were readily uptaken by breast cancer cells that overexpressed the corresponding antigens.

Quantum Dots and Quantum Rods for Targeted Drug Delivery in Cancer

A single-particle QD conjugated with a tumor-targeting MAb (anti-HER2) has been tracked in tumors of live mice (Tada et al. 2007). The researchers used a dorsal skinfold chamber and a high-speed confocal microscope with a high-sensitivity camera to track the antibody-labeled QDs and made 30-frame-per-second movies of these nanoparticles (NPs) as they traveled through the bloodstream. The HER2 MAb binds to a protein found on the surface of certain breast and other tumors. This was injected, conjugated to the QDs, into mice with HER2-overexpressing breast cancer to analyze the molecular processes of its mechanistic delivery to the tumor. The investigators identified six distinct "stop-and-go" steps in the process involved in the antibody-labeled QDs traveling from the injection site to the cell where they bind HER2: within a blood vessel in the circulation, during extravasation, in the extracellular region, binding HER2 on the cell membrane, moving into the perinuclear region, and within the perinuclear region. The image analysis of the delivery processes of single particles in vivo thus provides valuable information on antibody-conjugated therapeutic nanoparticles, which will be useful in increasing therapeutic efficacy.

Water-soluble CdSe/CdS/ZnS quantum rods (QRs) have been developed as targeted probes for imaging cancer cell lines using two-photon fluorescence imaging. The researchers first developed a new method of creating QRs that would remain well dispersed in water and then refined the technique to allow the attachment of targeting molecules (in this case, transferrin, which binds to a receptor that is overexpressed in many types of cancer cells) to the QR surface. QRs, similar to the spherical QDs, fluoresce and can be made to fluoresce in a range of colors. However, since QRs have larger dimensions than QDs, they are easier to excite with incoming light than QDs. This research showed that the QRs were only taken up by targeted transferrin-positive cells and accumulated within these cells, being easily visible using low-intensity near-infrared light, which helps to protect cell integrity. If future research can further our understanding of QDs and QRs following these studies, it is hoped that we could then improve the ability of NPs to deliver drugs specifically to tumors, thus resulting in improved cancer diagnostics and therapeutics.

Remote-Controlled Drug Delivery from Magnetic Nanocrystals

Combination of magnetic nanocrystals with ability to exhibit hyperthermic effects when placed in an oscillating magnetic field and mesoporous silica nanoparticles that can contain and release drug cargos could provide a unique drug-delivery system for cancer, which is a nanosystem that incorporates zinc-doped iron oxide nanocrystals within a mesoporous silica framework that has been surface-modified with pseudorotaxanes (Thomas et al. 2010). Upon application of an AC magnetic field, the nanocrystals generate local internal heating, causing the molecular machines to disassemble and allowing the drug cargos to be released. Breast cancer cell (MDA-MB-231) death was achieved in vitro when doxorubicin-loaded particles were exposed to an AC field. This material has potential as a noninvasive, externally controlled drug-delivery system with cancer-killing properties.

Targeted Delivery of Nanoparticulate Drugs into Lymphatic System

The lymphatic system plays a major role in the defense cancer and is one of the main pathways for the metastasis of tumors. The regional lymph nodes, when invaded by cancer cells, act as reservoirs from where these cells spread to other parts of the body. The lymphatic system is not easily accessible by conventional intravenous infusion of chemotherapeutics, thus limiting the amount of drug that reaches lymphatic tissues including lymph node metastases. The lymphatics, however, can be exploited as a route for drug delivery as these channels can transport certain lipophilic compounds and chemotherapeutics.

Nanoparticles can be effectively taken up into lymphatics as well as retained in lymph nodes for several days, and without using any specific targeting ligand, they are internalized exclusively by nodal resident dendritic cells (DCs) and other antigen-presenting cells. Animal studies have demonstrated that nanoparticles made of natural or synthetic polymers and liposomal carriers have higher accumulation in the lymph nodes and surrounding lymphatics compared to conventional intravenous therapies (Xie et al. 2009). In vivo studies have shown that up to 40–50% of resident lymph node DCs internalize nanoparticles, further supporting the feasibility of this delivery strategy. Bioavailability and biodistribution can be controlled easily by varying the size of nanoparticles. Biodegradable nanoparticles of 20–45 nm have shown the potential for immunotherapeutic applications that specifically target DCs in lymph nodes, e.g., targeted delivery of immunomodulating formulations and vaccines (Reddy et al. 2006a). This can diminish toxicity of highly toxic active drugs.

Targeted Drug Delivery with Nanoparticle–Aptamer Bioconjugates

Nucleic acid ligands (aptamers) are potentially well suited for the therapeutic targeting of drug encapsulated controlled-release polymer particles in a cell- or tissue-specific manner. Scientists at the Massachusetts Institute of Technology (Cambridge, MA) have synthesized poly(lactic acid)-block-polyethylene glycol (PLA-PEG) copolymer with a terminal carboxylic acid functional group (PLA-PEG-COOH) and

encapsulated Rhodamine-labeled dextran (as a model drug) within PLA-PEG-COOH nanoparticles (Farokhzad et al. 2004). These nanoparticles have the following desirable characteristics:

- Negative surface charge, which may minimize nonspecific interaction with the negatively charged nucleic acid aptamers
- Carboxylic acid groups on the particle surface for potential modification and covalent conjugation to amine-modified aptamers
- Presence of PEG on particle surface, which enhances circulating half-life while contributing to decreased uptake in nontargeted cells

Nanoparticle–aptamer bioconjugates were generated with RNA aptamers that bind to the prostate-specific membrane antigen (PSMA), a well-known prostate cancer tumor marker that is overexpressed on prostate acinar epithelial cells. These bioconjugates could efficiently target and get taken up by the prostate epithelial cells, which express the PSMA protein. The uptake of these particles was not enhanced in cells that do not express the prostate-specific membrane antigen protein. This represents the first report of targeted drug delivery with nanoparticle–aptamer bioconjugates.

Numerous investigators have used aptamers as replacements for antibodies in various therapeutic and diagnostic applications, and now, a team at McMaster University has found a third use for these versatile molecules – as the heart of a DNA-protein nanoengine that can be programmed to release therapeutically useful molecules in response to a programmed molecular signal (Nutiu and Li 2005). To construct their nanoengine, the researchers first create an aptamer that binds to a molecule that would signal "release cargo here." The researchers call this signaling molecule the "input." The researchers then prepare a complementary piece of DNA that binds to the aptamer according to the Watson–Crick rules. A drug molecule, or even a therapeutic gene, can be linked to this piece of DNA, and the combination is called the "output." When the output piece of DNA is then mixed with the aptamer, the two bind to one another until the aptamer comes in contact with the input signal. The aptamer folds around the input signal, causing it to release its cargo, the output DNA-drug molecule combination. As an example, the researchers used this construct to carry and release an enzyme.

Use of T Cells for Delivery of Gold Nanoparticles to Tumors

Gold nanoparticles (AuNPs) are injected intravenously and are allowed to accumulate within the tumor via the enhanced permeability and retention (EPR) effect. Although reliance on the EPR effect for tumor targeting has proven adequate for vascularized tumors in small animal models, the efficiency and specificity of tumor delivery in vivo, particularly in tumors with poor blood supply, may not be adequate. Human T cells, loaded with 45-nm gold colloid nanoparticles, can be used as cellular delivery vehicles for AuNP transport into tumors, without affecting

viability or function (e.g., migration and cytokine production). Using a human tumor xenograft mouse model, it was demonstrated that AuNP-loaded T cells retain their capacity to migrate to tumor sites in vivo (Kennedy et al. 2011). In addition, the efficiency of AuNP delivery to tumors in vivo is increased by more than four-fold compared to injection of free PEGylated AuNPs, and the use of the T cell delivery system also dramatically alters the overall nanoparticle biodistribution. Thus, the use of T cell chaperones for AuNP delivery could enhance the efficacy of nanoparticle-based therapies and imaging applications by increasing AuNP tumor accumulation. This could also be used for thermal destruction of tumor by application of NIR laser.

Dendrimers for Anticancer Drug Delivery

Earlier studies of dendrimers in drug-delivery systems focused on their use for encapsulating drug molecules. However, it was difficult to control the release of the drug. One solution to this problem involves the use of dendrimers with pH-sensitive hydrophobic acetal groups on the dendrimer periphery. Loss of acetal group at mildly acidic pH triggers the disruption of micelles and release of the drug. Another approach is to attach the drug to the periphery of the dendrimer so that the release of the drug can be controlled by incorporating a degradable linkage between the drug and the dendrimer. Dendrimers have been used to facilitate boron neutron capture therapy as well as photodynamic therapy of cancer.

Developments in polymer and dendrimer chemistry have provided a new class of molecules called “dendronized polymers,” i.e., linear polymers that bear dendrons at each repeat unit. Their behavior differs from that of linear polymers and provides drug-delivery advantages because of their longer circulation time and numerous possibilities for peripheral attachments of drugs.

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Doxorubicin (DOX) has been conjugated to a biodegradable dendrimer with optimized blood circulation time through size and molecular architecture, drug

loading through multiple attachment sites, solubility through PEGylation, and drug release through the use of pH-sensitive hydrazone linkages (Lee et al. 2006). Dendrimer–DOX is >10 times less toxic than free DOX toward colon carcinoma cells in culture. Upon intravenous administration to tumor-bearing mice, tumor uptake of dendrimer–DOX was ninefold higher than intravenous free DOX and caused complete tumor regression as well as 100% survival of the mice over the 60-day experiment. No cures were achieved in tumor-implanted mice treated with free DOX, drug-free dendrimer, or dendrimer–DOX in which the DOX was attached by means of a stable carbamate bond. The antitumor effect of dendrimer–DOX was similar to that of an equimolar dose of liposomal DOX (Doxil). The remarkable antitumor activity of dendrimer–DOX results from the ability of the dendrimer to favorably modulate the pharmacokinetics of attached DOX.

Application of Dendrimers in Boron Neutron Capture Therapy

Boron neutron capture therapy (BNCT) offers a potential method for localized destruction of tumor cells. The technology is based on the nuclear reaction between thermal neutrons and boron-10 (10B) to yield alpha particles and lithium-7 nuclei. The destructive effect of this reaction is limited to a range of about the diameter of a single cell. In order for BNCT to be effective in cancer therapy, there must be selective delivery of an adequate concentration of 10B to tumors. Various types of antibodies as well as epidermal growth factor have been utilized to investigate receptor-mediated boron delivery; however, in vivo studies have demonstrated only a small percentage of the total administered dose actually accumulates in tumors, while high concentrations end up in the liver.

In normal as well as cancer cells, the low molecular weight vitamin, folic acid, is required for a number of enzymatic pathways. Cell membrane receptors mediating endocytic transport of folic acid into cells are expressed in elevated levels in a variety of human tumors. Folic acid conjugates with macromolecules such as toxins, enzymes, antibodies, genes, and liposomes have been shown to be internalized into tumor cells overexpressing folate receptors. These strategies have been employed to enhance the effect of BNCT. The use of dendrimers as boron carriers for antibody conjugation is based on their well-defined structure and multivalency.

The use of dendrimers as boron carriers for antibody conjugation is based on their well-defined structure and multivalency. Boronated PAMAM dendrimers have been designed to target the epidermal growth factor receptor, a cell surface receptor that is frequently overexpressed in brain tumor cells.

Preclinical evaluation has been described of a multipurpose STARBURST PAMAM (polyamidoamine) dendrimer prototype (Dendritic Nanotechnologies Inc) that exhibits properties suitable for use as (1) targeted, diagnostic MRI/NIR (near-IR) contrast agents, and/or (2) for controlled delivery of cancer therapies (Tomalia et al. 2007). The lead candidate is 1,4-diaminobutane, a dendritic nanostructure ~5 nm diameter, which was selected on the basis of a very favorable biocompatibility profile on in vitro studies, i.e., benign and nonimmunogenic.

The expectation is that it will exhibit desirable mammalian kidney excretion properties and demonstrated targeting features.

Application of Dendrimers in Photodynamic Therapy

Photodynamic therapy (PDT) uses light-activated drugs called photosensitizers to treat a range of diseases characterized by rapidly growing tissue, including the formation of abnormal blood vessels, such as cancer and age-related macular degeneration. The more traditional name for this therapy is photoradiation therapy. Treatment with PDT consists of a two-step process that starts with administration of the drug, or photosensitizer, by intravenous injection. Once the drug enters the bloodstream, it attaches itself to low-density lipoproteins already circulating. As cells undergoing rapid growth require an above-average supply of lipoproteins, the drug reaches these types of cells more quickly and in higher concentrations. Once the necessary level of concentration is attained, the second step is to activate the drug with a specific dose of light of a particular wavelength. This causes the conversion of normal oxygen found in tissue to a highly energized form called singlet oxygen, which in turn disrupts normal cellular functions. Neither the drug nor the light exerts any effect until combined.

Numerous studies have used liposomes, oils, and polymeric micelles as encapsulation methods, with some success. However, all of these techniques suffer from one unpleasant side effect: after controlled release and photosensitization, the drug is free to circulate the body, accumulating in the eyes and skin. This leads to phototoxic side effects, rendering the patient highly sensitive to light. A further disadvantage is that liposomes can be engulfed and destroyed by cells of the reticuloendothelial system. Such problems have limited the emerging field of PDT, but combination of this technique with nanotechnology is promising.

The possibility of improving dendrimers through appropriate functionalization of their periphery makes them promising carriers of PDT. The use of 5-aminolevulinic acid (ALA) is one approach to PDT based on dendrimers. ALA is a natural precursor of the photosensitizer protoporphyrin IX (PIX), and its administration increases the cellular concentrations of PIX. Cellular uptake of the dendrimer occurs through endocytic routes predominantly via a macropinocytosis pathway. A dendrimer conjugate, which incorporated 18 aminolevulinic acid residues attached via ester linkages to a multipodent aromatic core, has been investigated (Battah et al. 2007). The ability of the dendrimer to deliver and release 5-ALA intracellularly for metabolism to the photosensitizer, protoporphyrin IX, was studied in the transformed PAM 212 murine keratinocyte and A431 human epidermoid carcinoma cell lines. The macromolecular dendritic derivatives were shown to be capable of delivering 5-ALA efficiently to cells for sustained porphyrin synthesis.

Another approach to deep tissue penetration is based on two-photon excitation with near-infrared lasers. Multivalent aspects of dendrimer scaffold can be used to conjugate several two-photon-absorbing chromophores to the porphyrin core. Such a system can generate singlet oxygen efficiently on light irradiation at 780-nm wavelength.

Dendrimer-Based Synthetic Vector for Targeted Cancer Gene Therapy

A synthetic vector system based on polypropylenimine dendrimers has the desired properties of a systemic delivery vehicle and mediates efficient transgene expression in tumors after intravenous administration (Dufes et al. 2005). Specifically, the systemic injection of dendrimer nanoparticles containing a TNF-α expression plasmid regulated by telomerase gene promoters (hTR and hTERT) leads to transgene expression, regression of remote xenograft murine tumors, and long-term survival of up to 100% of the animals. The combination of pharmacologically active synthetic transfection agent and transcriptionally targeted antitumor gene creates an efficacious gene medicine for the systemic treatment of experimental solid tumors. The promising results of these experiments could make it possible to treat inaccessible tumors in humans using gene therapy in the future. This new treatment can selectively target cancer cells, without causing damage to surrounding healthy cells.

Poly-L-Lysine Dendrimer as Antiangiogenic Agent

Poly-L-lysine (PLL) sixth-generation (G6) dendrimer molecules exhibit systemic antiangiogenic activity that could lead to arrest of growth of solid tumors. Intravenous administration of the PLL-dendrimer molecules into C57BL/6 mice inhibits vascularization of tumors grown within dorsal skinfold window chambers as demonstrated by intravital microscopy (Al-Jamal et al. 2010). The in vivo toxicological profile of the PLL-dendrimer molecules shows that it is safe at the dose regime studied. The antiangiogenic activity of the PLL dendrimer is further shown to be associated with significant suppression of B16F10 solid tumor volume and delayed tumor growth. Enhanced apoptosis/necrosis within tumors of PLL-dendrimer-treated animals only and reduction in the number of CD31 positive cells are observed in comparison to protamine treatment. This study suggests that PLL-dendrimer molecules can exhibit a systemic antiangiogenic activity that may be used for therapy of solid tumors and, in combination with their capacity to carry other therapeutic or diagnostic agents, may potentially offer capabilities combining diagnosis with therapy.

RNA Nanotechnology for Delivery of Cancer Therapeutics

RNA has immense promise as a therapeutic agent against cancer, but the problem has been to have an efficient system to bring multiple therapeutic agents directly into specific cancer cells where they can perform different tasks. The 25-nm RNA nanoparticles enable repeated long-term administration and avoid the problems of short retention time of small molecules and the difficulties in the delivery of particles larger than 100 nm. Nanoparticles, which are assembled from three short pieces of RNA and resemble miniature triangles, possess both the right size to gain entry into cells and also the right structure to carry other therapeutic strands of RNA inside with them, where they are able to halt viral growth or cancer's progress.

RNA molecules come in many variant forms, and the one mimicked from the phi29 virus – called pRNA – also can be linked to other types of RNA to form longer hybrid strands with properties that could be assigned. Incubation of cancer with the pRNA dimer, one subunit of which harbored the receptor-binding moiety and the other harboring the gene-silencing molecule, resulted in their binding and entry into the cells and subsequent silencing of anti-/proapoptotic genes. The chimeric pRNA complex was found to be processed into functional double-stranded siRNA by Dicer (RNA-specific endonuclease). Animal studies have confirmed the suppression of tumorigenicity of cancer cells by ex vivo delivery.

RNA nanotechnology has been used to engineer both therapeutic siRNA and a receptor-binding RNA aptamer into individual pRNAs of phi29's motor. The RNA building block harboring siRNA or other therapeutic molecules is subsequently incorporated in a trimer through the interaction of engineered right and left interlocking RNA loops. The incubation of the protein-free nanoscale particles containing the receptor-binding aptamer or other ligands results in the binding and co-entry of the trivalent therapeutic particles into cells, which can modulate the apoptosis of cancer cells as shown in animal studies. The use of such antigenicity-free 20–40-nm particles holds promise for the repeated long-term treatment of cancer and other chronic diseases.

Delivery of siRNAs for Cancer

Targeted delivery of siRNAs is considered to be safer and more effective therapeutics for oncology applications. Although macromolecules accumulate nonspecifically in tumors through the enhanced permeability and retention (EPR) effect, previous studies using nanoparticles to deliver siRNA demonstrated that attachment of cell-specific targeting ligands to the surface of nanoparticles leads to enhanced potency relative to nontargeted formulations. Although both nontargeted and transferrin-targeted siRNA nanoparticles exhibit similar biodistribution and tumor localization by PET, transferrin-targeted siRNA nanoparticles reduce tumor luciferase activity by ~50% relative to nontargeted siRNA nanoparticles one day after injection (Bartlett et al. 2007). Compartmental modeling is used to show that the primary advantage of targeted nanoparticles is associated with processes involved in cellular uptake in tumor cells rather than overall tumor localization. Optimization of internalization may, therefore, be a key to the development of effective nanoparticle-based targeted siRNA therapeutics.

Tumor Priming for Improving Delivery of Nanomedicines to Solid Tumors

Effectiveness of nanomedicines in cancer therapy is limited in part by inadequate delivery and transport in tumor interstitium. Tumor priming to overcome these limitations includes measures for extravasation and interstitial transport (Wang et al. 2011). Experimental approaches to improve delivery and transport of nanomedicines

in solid tumors include tumor vasculature normalization, interstitial fluid pressure modulation, enzymatic extracellular matrix degradation, and apoptosis-inducing tumor priming technology, which is exemplified by enhancement of delivery and efficacy of liposomal doxorubicin by paclitaxel.

Nanotechnology-Based Cancer Therapy

Devices for Nanotechnology-Based Cancer Therapy

Convection-Enhanced Delivery with Nanoliposomal CPT-11

Combination of convection-enhanced delivery (CED) with a novel, highly stable nanoparticle/liposome containing CPT-11 (nanoliposomal CPT-11) is a potential dual-drug-delivery strategy for brain tumor treatment. Following CED in rat brains, tissue retention of nanoliposomal CPT-11 was shown to be greatly prolonged, with >20% injected dose remaining at 12 days (Noble et al. 2006). In contrast, CED of free CPT-11 resulted in rapid drug clearance. At equivalent CED doses, nanoliposomal CPT-11 increased area under the time-concentration curve by 25-fold and tissue t1/2 by 22-fold over free CPT-11; CED in intracranial U87 glioma xenografts showed even longer tumor retention. Plasma levels were undetectable following CED of nanoliposomal CPT-11. Importantly, prolonged exposure to nanoliposomal CPT-11 resulted in no measurable CNS toxicity at any dose tested, whereas CED of free CPT-11 induced severe CNS toxicity. In the intracranial U87 glioma xenograft model, a single CED infusion of nanoliposomal CPT-11 resulted in significantly improved median survival compared with CED of control liposomes. The study concluded that CED of nanoliposomal CPT-11 greatly prolonged tissue residence while also substantially reducing toxicity, resulting in a highly effective treatment strategy in preclinical brain tumor models.

Nanoengineered Silicon for Brachytherapy

BrachySil™ (^{32}P BioSilicon, pSivida Corporation) is a nanoparticle in which the isotope 32-phosphorus is immobilized. It demonstrates a very high degree of isotope retention following injection into the liver, thus reducing the risk of soluble radioactive material affecting healthy hepatic tissue, or entering the circulation and causing systemic toxicity. Unlike titanium seeds, which remain forever in the body, phosphorus seeds degrade over time and enable repetition of treatment if necessary. Other treatments for primary liver cancer include a variety of embolization and radiofrequency ablation techniques. BrachySil offers a more versatile and safer product for the treatment of such tumors. The procedure is undertaken without surgery under local anesthetic, and patients can be discharged the following day. A phase IIa trial in primary liver cancer has shown that it is safe and effective in tumor regression with increased efficacy. An efficacy/safety study for the treatment of pancreatic

cancer was completed in 2008 and showed that the treatment was well tolerated with disease control in 82% of patients and an overall median survival of 309 days.

Anticancer Effect of Nanoparticles

Antiangiogenic Therapy Using Nanoparticles

Integrin-targeted nanoparticles can be used for site-specific delivery of a therapeutic payload. Selective targeting of upregulated $\alpha_v\beta_3$ and Flk-1 on the neovasculature of tumors is a novel antiangiogenesis strategy for treating a wide variety of solid tumors. A study provides proof of principle that targeted radiotherapy works using different targeting agents on a NP, to target both the integrin $\alpha_v\beta_3$ and the vascular endothelial growth factor receptor (Li et al. 2004). These encouraging results demonstrate the potential therapeutic efficacy of the IA-NP-90Y and anti-Flk-1 MAb-NP-90Y complexes as novel therapeutic agents for the treatment of a variety of tumor types.

The mechanism of inhibition of the function of pro-angiogenic heparin-binding growth factors (HB-GFs), such as vascular endothelial growth factor 165 (VEGF165) and basic fibroblast growth factor (bFGF) by gold nanoparticles (GNPs), has been investigated (Arvizo et al. 2011). It was shown that a naked GNP surface is required and core size plays an important role to inhibit the function of HB-GFs and subsequent intracellular signaling events. The authors also demonstrated that the inhibitory effect of GNPs is due to the change in HB-GFs conformation/configuration (denaturation) by the NPs, whereas the conformations of non-HB-GFs remain unaffected. This study will help structure-based design of therapeutic NPs.

Cytotoxic Effects of Cancer Nanoparticles

Nanoparticles may have a direct cytotoxic effect on cancer cells by various mechanisms. DNA degradation and anticancer activity of copper nanoparticles of 4–5 nm size have been reported, e.g., dose-dependent degradation of isolated DNA molecules by copper nanoparticles through generation of singlet oxygen. Singlet oxygen scavengers such as sodium azide and tris[hydroxylmethyl]aminomethane were able to prevent the DNA degradation (Jose et al. 2011). Additionally, it was observed that the copper nanoparticles are able to exert cytotoxic effect toward U937 and Hela cells of human histiocytic lymphoma and human cervical cancer origins, respectively, by inducing apoptosis.

Nanoshell-Based Cancer Therapy

Nanoshells may be combined with targeting proteins and used to ablate target cells. This procedure can result in the destruction of solid tumors or possibly metastases

not otherwise observable by the oncologist. In addition, Nanoshells can be utilized to reduce angiogenesis present in cancer. Experiments in animals, in vitro and in tissue, demonstrate that specific cells (e.g., cancer cells) can be targeted and destroyed by an amount of infrared light that is otherwise not harmful to surrounding tissue. This procedure may be performed using an external (outside the body) infrared laser. Prior research has indicated the ability to deliver the appropriate levels of infrared light at depths of up to 15 cm, depending upon the tissue. Photothermal tumor ablation in mice has been achieved by using near-infrared-absorbing nanoparticles. The advantages of nanoshell-based tumor cell ablation include:

- Targeting to specific cells and tissues to avoid damage to surrounding tissue.
- Superior side-effect profile than targeted chemotherapeutic agents or photodynamic therapy.
- Repeatability because of:
  - No "tissue memory" as in radiation therapy
  - Biocompatibility
  - Ability to treat metastases and inoperable tumors
- Nanoshells enable a seamless integration of cancer detection and therapy.

Nanobody-Based Cancer Therapy

A nanobody with subnanomolar affinity for the human tumor-associated carcinoembryonic antigen (CEA) has been identified (Cortez-Retamozo et al. 2004). This nanobody was conjugated to *Enterobacter cloacae* beta-lactamase, and its site-selective anticancer prodrug activation capacity was evaluated. The conjugate was readily purified in high yields without aggregation or loss of functionality of the constituents. In vitro experiments showed that the nanobody–enzyme conjugate effectively activated the release of phenylenediamine mustard from the cephalosporin nitrogen mustard prodrug 7-(4-carboxybutanamido) cephalosporin mustard at the surface of CEA-expressing LS174T cancer cells. In vivo studies demonstrated that the conjugate had an excellent biodistribution profile and induced regressions and cures of established tumor xenografts. The easy generation and manufacturing yield of nanobody-based conjugates together with their potent antitumor activity makes nanobodies promising vehicles for new-generation cancer therapeutics.

Nanoparticles Combined with Physical Agents for Tumor Ablation

Several physical agents have been used for ablation of tumors. Nanoparticles can be combined with these techniques, and some examples are shown here.

Boron Neutron Capture Therapy Using Nanoparticles

Boron carbide nanoparticles are proposed as a system for T cell-guided boron neutron capture therapy (Mortensen et al. 2006). Nanoparticles were produced by ball milling in various atmospheres of commercially available boron carbide. The physical and chemical properties of the particles were investigated using transmission electron microscopy, photon correlation spectroscopy, X-ray photoelectron spectroscopy, X-ray diffraction, vibrational spectroscopy, gel electrophoresis, and chemical assays and revealed profound changes in surface chemistry and structural characteristics. In vitro thermal neutron irradiation of B16 melanoma cells incubated with sub-100-nm nanoparticles induced complete cell death. The nanoparticles alone induced no toxicity.

A cancer therapeutic plus diagnostic has been developed that is a variation of BNCT using radioactivate boron-nitride (BN) nanotubes. BNs are covalently bound to tumor-cloned antibodies or immunoglobulins (IgGs) to deliver intense, short-lived, therapeutic doses of radiation specifically to active tumor sites. The therapy involves activation of the BN nanotubes with a neutron beam (as in BNCT) once the IgG carrier molecules reach their target tissue. In contrast to conventional BNCT, instant BN nanotubes can deliver significant numbers of boron atoms (100–1,000 s) specifically to the tumor site while avoiding exposures to surrounding tissue. BNCT is a technique that relies on (nonradioactive) 10B delivery specifically to a tumor site and then activating it using an accurate beam of epithermal neutrons (low-energy neutrons with velocities adjusted to penetrate tissue to the specific tumor depth where the 10B has lodged). BN nanotube structure is similar to the "rolled-up-graphite" structure of a CNT, six member rings but with boron atoms bound to three surrounding nitrogen atoms, and the nitrogen atoms bound to surrounding boron atoms (no conjugation). Thus, each BN nanotube is composed of a substantial number of boron atoms, e.g., 50%, meaning hundreds to thousands for each nanotube. Boron has a relatively large radioactive cross section and can be easily made radioactive in a neutron flux.

Gold Nanoparticles Combined with Radiation Therapy

High-atomic number metals, such as gold, preferentially absorb much more X-ray energy than soft tissues and thus augment the effect of ionizing radiation when delivered to cells. Proteins that regulate poly-SUMO (small ubiquitin-like modifier)-chain conjugates play important roles in cellular response to DNA damage, such as those caused by cancer radiation therapy. A study has demonstrated that conjugation of a weak SUMO-2/3 ligand to gold nanoparticles (AuNPs) facilitates selective multivalent interactions with poly-SUMO-2/3 chains leading to efficient inhibition of poly-SUMO-chain-mediated protein–protein interactions (Li et al. 2012). The ligand–gold particle conjugate significantly sensitized cancer cells to radiation but was not toxic to normal cells. This study demonstrates a viable approach for selective targeting of poly-Ubl chains through multivalent interactions created by nanoparticles that can be chosen based on their properties, such as abilities to augment radiation effects.

Laser-Induced Cancer Destruction Using Nanoparticles

Biological systems are known to be highly transparent to 700–1,100-nm NIR light. It is shown here that the strong optical absorbance of SWCNT in this special spectral window, an intrinsic property of carbon nanotubes, can be used for optical stimulation of nanotubes inside living cells to afford multifunctional nanotube biological transporters. For oligonucleotides transported inside living cells by nanotubes, the oligos can translocate into cell nucleus upon endosomal rupture triggered by NIR laser pulses. Continuous NIR radiation can cause cell death because of excessive local heating of carbon nanotubes in vitro. Selective cancer cell destruction can be achieved by functionalization of carbon nanotubes with a folate moiety, selective internalization of carbon nanotubes inside cells labeled with folate receptor tumor markers, and NIR-triggered cell death, without harming receptor-free normal cells. Thus, the transporting capabilities of carbon nanotubes combined with suitable functionalization chemistry and their intrinsic optical properties can lead to new classes of novel nanomaterials for drug delivery and cancer therapy (Kam et al. 2005). One example for application is lymphoma as lymphoma cells have well-defined surface receptors that recognize unique antibodies. When attached to a carbon nanotube, the antibody would play the role of a Trojan horse. This approach is being tested in laboratory mice with lymphoma. The researchers want to determine if shining NIR on the animal's skin will destroy lymphatic tumors while leaving normal cells intact. Carbon nanotubes also can be delivered to diseased cells by direct injection. The idea is to use the nanotube to deliver therapeutic molecules of DNA, RNA, or protein directly into the cell nucleus to fight various infections and diseases.

Plasmon-resonant gold nanorods, which have large absorption cross sections at near-infrared frequencies, are excellent candidates as multifunctional agents for image-guided therapies based on localized hyperthermia. The controlled modification of the surface chemistry of the nanorods is of critical importance, as issues of cell-specific targeting and nonspecific uptake must be addressed prior to clinical evaluation. Nanorods coated with cetyltrimethylammonium bromide (a cationic surfactant used in nanorod synthesis) are internalized within hours into cancer cells by a nonspecific uptake pathway, whereas the careful removal of cetyltrimethylammonium bromide from nanorods functionalized with folate results in their accumulation on the cell surface over the same time interval. Thus, the nanorods render the tumor cells highly susceptible to photothermal damage when irradiated at the nanorods' longitudinal plasmon resonance, generating extensive blebbing of the cell membrane at laser fluences as low as 30 J/cm^2 (Huff et al. 2007).

A light-controlled delivery system that can be tailored to release nonbiological molecules into living cells can be remotely controlled and can release quantifiable amounts on demand. The technique utilizes gold nanoparticles, in the form of nanoshells, to transport the target molecule into the cell, where it can subsequently be released. dsDNA nanoshells can be loaded with molecules, which are associated with the DNA; these molecules can be released inside the cell when triggered by light (Huschka et al. 2010). The research describes how the nanoshell complexes were loaded with 4′,6-diamino-2-phenylindole (DAPI), a fluorescent blue dye that

is able to reversibly bind to DNA. The nanoshells were then introduced to cancer cells, and once uptake of the nanoparticles by the cells was confirmed, the cells were illuminated using a continuous wave laser at a specified wavelength. The wavelength of the laser excitation is tailored to the specific DNA, and the plasmon resonance wavelength dehybridizes the DNA, causing the release of the DAPI molecule. The DAPI molecule is released from the nanoshell and diffuses through the cytoplasm into the cell nucleus. The diffusion of the DAPI molecule into the cell nucleus was confirmed by the staining of the nuclear DNA. The ability of DAPI to reversibly stain DNA fluorescent blue allowed the intracellular release process to be easily visualized. The research concluded that the light-triggered release of DAPI using a laser did not have an adverse effect on the cells, due to the low power of the laser and the minimal irradiation times required to stimulate the release of the molecule. The researchers also discerned that the uptake of the nanoshells had no adverse effects on the living cells.

Poly(lactic-co-glycolic acid) nanoparticles have been produced, which encapsulate the photosensitizer meso-tetraphenylporpholactol and are stable and nonphototoxic upon systemic administration (McCarthy et al. 2005). Upon cellular internalization, the photosensitizer is released from the nanoparticle and becomes highly phototoxic. Irradiation with visible light results in cell-specific killing of several cancer cell lines. In vivo experiments have shown complete eradication of cancers in mouse models. The concept of photosensitizers with selective phototoxicity should have widespread applications in cancer therapy.

A nanocarrier consisting of polymeric micelles of diacylphospholipid-poly(ethylene glycol) (PE-PEG) coloaded with the photosensitizer drug 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH) and magnetic Fe3O4 nanoparticles has been used for guided drug delivery together with light-activated photodynamic therapy for cancer (Cinteza et al. 2006). The nanocarrier shows excellent stability and activity over several weeks. The loading efficiency of HPPH is practically unaffected upon coloading with the magnetic nanoparticles, and its phototoxicity is retained. The magnetic response of the nanocarriers was demonstrated by their magnetically directed delivery to tumor cells in vitro. The magnetophoretic control on the cellular uptake provides enhanced imaging and phototoxicity. These multifunctional nanocarriers demonstrate the exciting prospect offered by nanochemistry for targeting photodynamic therapy.

In a novel nanoformulation of for PDT of cancer, the photosensitizer molecules are covalently incorporated into organically modified silica (ORMOSIL) nanoparticles (Ohulchanskyy et al. 2007). The incorporated photosensitizer molecules retain their spectroscopic and functional properties and can robustly generate cytotoxic singlet oxygen molecules upon photoirradiation. The synthesized nanoparticles are of ultralow size (approximately 20 nm) and are highly monodispersed and stable in aqueous suspension. The advantage offered by this covalently linked nanofabrication is that the drug is not released during systemic circulation, which is often a problem with physical encapsulation. These nanoparticles are also avidly taken up by tumor cells and demonstrate phototoxic action, thereby improving the diagnosis as well as PDT of cancer.

Magnetic Nanoparticles for Thermal Ablation of Cancer

An experimental procedure for the treatment of breast cancer is called magnetic thermal ablation. Magnetic nanoparticles are promising tools for the minimal invasive elimination of small tumors in the breast using magnetically induced heating. The approach complies with the increasing demand for breast conserving therapies and has the advantage of offering a selective and refined tuning of the degree of energy deposition allowing an adequate temperature control at the target.

Anti-HER2 antibody can induce antitumor responses and can be used in delivering drugs to HER2-overexpressing cancer. Anti-HER2 immunoliposomes containing magnetite nanoparticles, which act as tumor-targeting vehicles, have been used to combine anti-HER2 antibody therapy with hyperthermia in experimental studies. SWCNTs emit heat when they absorb energy from NIR light. Tissue is relatively transparent to NIR, which suggests that targeting SWCNTs to tumor cells, followed by noninvasive exposure to NIR light, will ablate tumors within the range of NIR. One study has demonstrated the specific binding of MAb-coupled SWCNTs to tumor cells in vitro, followed by their highly specific ablation with NIR light (Chakravarty et al. 2008). Only the specifically targeted cells were killed after exposure to NIR light.

Targeted nanotherapeutics (TNT) system is an innovation of thermal ablation of cancer that bonds iron nanoparticles and MAbs into bioprobes. The magnetic field energy is converted to lethal heat by the particles causing a rapid temperature increase to more than 170 C at the surface of the cancer cells, killing them and their blood supply with negligible damage to surrounding healthy tissues. To evaluate the potential of TNT for in vivo tumor targeting, efficacy and predictive radionuclide-based heat dosimetry were studied using ^{111}In-ChL6 bioprobes (ChL6 is chimeric L6) in a human breast cancer xenograft model (Denardo et al. 2007). Mice in the study received a series of alternating magnetic field (AMF) bursts in a single 20-min treatment. Dosing was calculated using an equation that included tumor concentration of bioprobes, heating rate of particles at different amplitudes, and the spacing of AMF bursts. MAb-guided bioprobes (iron oxide nanoparticles) effectively targeted the tumors without causing particle-related toxicity. Tumor total heat dose, calculated using empirically observed ^{111}In-bioprobe tumor concentration and in vitro nanoparticle heat induction by AMF, correlated with tumor growth delay. The biggest problem of thermotherapy of cancer has been how to apply it to the tumor alone, how to predict the amount needed, and how to determine its effectiveness. By combining nanotechnology, focused AMF therapy, and quantitative molecular-imaging techniques, a safe technique has been developed that could be considered for clinical use as a treatment for breast and other cancers.

Nanoshells for Thermal Ablation of Cancer

Metal nanoshells belong to a class of nanoparticles with tunable optical resonances that have been used for thermal ablative therapy for cancer. Nanoshells can be tuned to strongly absorb light in the NIR, where optical transmission through tissue is

optimal. Nanoshells placed at depth in tissues can be used to deliver a therapeutic dose of heat by using moderately low exposures of extracorporeally applied NIR. In vivo studies under MRI guidance have revealed that exposure to low doses of NIR in solid tumors treated with metal nanoshells reach temperatures capable of inducing irreversible tumor destruction within minutes. Gold nanoshells are ~120 nm in diameter and a cancer cell is 170 times bigger. Therefore, nanoshells can penetrate the tumor capillaries and lodge in the tumor. Application of NIR light, which passes through the skin harmlessly, heats the nanoshells and kills the tumor cells. Since no drug is used, the cancer cells are unlikely to develop drug resistance.

The ability to control both wavelength-dependent scattering and absorption of nanoshells offers the opportunity to design nanoshells which provide both diagnostic and therapeutic capabilities in a single nanoparticle. A nanoshell-based all-optical platform technology can integrate cancer imaging and therapy applications. Immunotargeted nanoshells, engineered to both scatter light in the near-infrared range enabling optical molecular cancer imaging and to absorb light, enable selective destruction of targeted carcinoma cells through photothermal therapy. In a proof of principle experiment, dual-imaging/therapy-immunotargeted nanoshells were used to detect and destroy breast carcinoma cells that overexpress HER2, a clinically relevant cancer biomarker. This approach has some significant advantages over alternatives that are under development. For example, optical imaging is much faster and less expensive than other medical imaging techniques. Gold nanoparticles are also more biocompatible than other types of optically active nanoparticles, such as QDs.

Nanospectra Biosciences Inc is already developing nanoshells for the targeted destruction of various cancers using nanoshells (AuroShell™). AuroLase™ cancer therapy combines the unique physical and optical properties of AuroShell™ microparticles with a near-infrared laser source to thermally destroy cancer cells without significant damage to surrounding tissue. AuroShell™ microparticles are injected intravenously and specifically collect in the tumor through the associated leaky vasculature (the Enhanced Permeability and Retention effect, or EPR). After the particles accumulate in a tumor, the area is illuminated with a near-infrared laser at wavelengths chosen to allow the maximum penetration of light through tissue. Unlike solid metals and other materials, AuroShell™ microparticles are designed to specifically absorb this wavelength, converting the laser light into heat. This results in the rapid destruction of the tumor along its irregular boundaries. The basics of this approach have been tested experimentally.

The blood vessels inside tumors develop poorly, allowing small particles like nanoshells to leak out and accumulate inside tumors. An animal trial involved 25 mice with tumors ranging in size from 3 to 5.5 mm. The mice were divided into three groups. The first group was given no treatment. The second received saline injections, followed by three-minute exposure to near-infrared laser light. The final group received nanoshell injections and laser treatments. In the test, researchers injected nanoshells into the mice, waited 6 h to give the nanoshells time to accumulate in the tumors, and then applied a 5-mm laser beam on the skin above each tumor. Surface temperature measurements taken during the laser treatments showed

a marked increase that averaged about 46 °F (7.7 °C) for the nanoshells group. There was no measurable temperature increase at the site of laser treatments in the saline group. Likewise, sections of laser-treated skin located apart from the tumor sites in the nanoshells group also showed no increase in temperature, indicating that the nanoshells had accumulated as expected within the tumors. All signs of tumors disappeared in the nanoshells group within 10 days. These mice remained cancer-free after treatment. Tumors in the other two test groups continued to grow rapidly. All mice in these groups were euthanized when the tumors reached 10 mm in size. The mean survival time of the mice receiving no treatment was 10.1 days; the mean survival time for the group receiving saline injections and laser treatments was 12.5 days. The advantages of nanoshell-based tumor cell ablation include:

- Targeting to specific cells and tissues to avoid damage to surrounding tissue
- Less adverse effects than targeted chemotherapeutic agents or photodynamic therapy
- Repeatability because of lack of "tissue memory" as in radiation therapy and biocompatibility
- Ability to treat malignancies such as glioblastoma multiforme, metastases, and inoperable tumors

Thermosensitive Affibody-Conjugated Liposomes

Thermosensitive liposomes have been used as vehicles for the delivery and release of drugs to tumors. To improve the targeting efficacy for breast cancer treatment, a HER2-specific affibody molecule was conjugated to the surface of thermosensitive small unilamellar liposomes measuring 80–100 nm, referred to as "affisomes," to study effects of this modification on physical characteristics and stability of the resulting preparation (Puri et al. 2008). Affisomes released calcein, a water-soluble fluorescent probe, in a temperature-dependent manner, with optimal leakage (90–100%) at 41 °C. Affisomes, when stored at room temperature, retained >90% entrapped calcein up to 7 days. Affisomes are promising candidates for targeted thermotherapy of breast cancer.

Ultrasound Radiation of Tumors Combined with Nanoparticles

Nanoparticles have been introduced in tumors followed by ultrasound-induced cavitation for safe and efficient drug and gene delivery. In a study on athymic nude mice-bearing human colon KM20 tumors, polystyrene nanoparticles (100 and 280 nm in diameter) were injected intravenously in combination with ultrasound to enhance delivery of chemotherapeutic agent 5-fluorouracil (Larina et al. 2005). This combination significantly decreased tumor volume and resulted in complete tumor regression at optimal irradiation conditions.

Impact of Nanotechnology-Based Imaging in Management of Cancer

The role of nanotechnology in diagnostic imaging of cancer, particularly MRI, has already been described earlier in this chapter. Nanotechnology-based cancer imaging will lead to sensitive and accurate detection of early-stage cancer. Nanoparticle-enabled imaging can help accurate delivery of cancer therapy.

Cornell Dots for Cancer Imaging

Cornell dots (C dots) are ultrasmall, cancer-targeted, multimodal silica nanoparticle <7 nm in diameter, which has been surface functionalized with cyclic arginine–glycine–aspartic acid peptide ligands and radioiodine. C dots exhibit high-affinity binding, favorable tumor-to-blood residence time ratios, and enhanced tumor-selective accumulation in αvβ3 integrin-expressing melanoma xenografts in mice (Benezra et al. 2011). The silica shell, essentially glass, is chemically inert and small enough to pass through the body and out in the urine. Coating the dots by PEGylation further protects them from being recognized by the body as foreign substances, giving them more time to find targeted tumors. The outside of the shell can also be coated with organic molecules that can attach to desired targets on tumor surfaces or within tumors. The cluster of dye molecules in a single dot fluoresces under near-infrared light much more brightly than single-dye molecules, and the fluorescence identifies malignant cells, showing a surgeon exactly what needs to be cut out and helping ensure that all malignant cells are found. C dots can reveal the extent of a tumor's blood vessels, cell death, treatment response, and invasive or metastatic spread to lymph nodes and distant organs. The FDA has approved the first clinical trial in humans of C dots that can light up cancer cells in PET–optical imaging. The technology aims to safely show surgeons extent of tumors in human organs. The trial is being conducted at Memorial Sloan-Kettering Center, New York. Commercial development will be in collaboration with Hybrid Silica Technologies Inc.

Nanoparticle MRI for Tracking Dendritic Cells in Cancer Therapy

Several techniques have been developed that allow an effective cellular internalization of clinical SPIO formulations without affecting cell proliferation, differentiation, and function, with "magnetoelectroporation" being the most recent labeling paradigm. Animal studies have shown that the MR distribution pattern is reliable when cells have limited cell division, as validated by conventional histological techniques. Magnetically labeled stem cells are not yet in clinical use due to safety concerns about the in vivo behavior of stem cells. A phase I trial has shown the

feasibility and safety of imaging autologous dendritic cells that were labeled with a clinical superparamagnetic iron oxide formulation or ^{111}In-oxine and were co-injected intranodally in melanoma patients under ultrasound guidance. In contrast to scintigraphic imaging, MRI allowed assessment of the accuracy of dendritic cell delivery and of inter- and intranodal cell migration patterns of MRI cell tracking using iron oxides that appear clinically safe and well suited to monitor cellular therapy in humans. It is believed that MRI cell tracking will become an important technique that someday may become routine in standard radiological practice once stem cell therapy enters clinical practice.

Nanoparticle CT Scan

Use of nanomaterials for one of the most common imaging techniques, computed tomography (CT), has remained unexplored. Current CT contrast agents are based on small iodinated molecules. They are effective in absorbing X-rays, but nonspecific distribution and rapid pharmacokinetics have rather limited their microvascular and targeting performance. While most of the nanoparticles are designed to be used in conjunction with MRI, bismuth sulfide (Bi_2S_3) nanoparticles naturally accumulate in lymph nodes containing metastases and show up as bright white spots in CT images (Rabin et al. 2006). A polymer-coated Bi_2S_3 nanoparticle preparation has been proposed as an injectable CT imaging agent. This preparation demonstrates excellent stability at high concentrations, high X-ray absorption (fivefold better than iodine), very long circulation times (>2 h) in vivo, and an efficacy/safety profile comparable to or better than iodinated imaging agents. The utility of these polymer-coated Bi_2S_3 nanoparticles for enhanced in vivo imaging of the vasculature, the liver, and lymph nodes has been demonstrated in mice. These nanoparticles and their bioconjugates are expected to become an important adjunct to in vivo imaging of molecular targets and pathological conditions. Tumor-targeting agents are now being added to the surfaces of these polymer-coated Bi_2S_3 nanoparticles.

QDs Aid Lymph Node Mapping in Cancer

An improved method for performing sentinel lymph node (SLN) biopsy, which depends on illuminating lymph nodes during cancer surgery, has been developed using QDs that emit NIR light, a part of the spectrum that is transmitted through biological tissue with minimal scattering. SLN mapping is a common procedure used to identify the presence of cancer in a single “sentinel” lymph node, thus avoiding the removal of a patient’s entire lymph system. SLN mapping relies on a combination of radioactivity and organic dyes, but the technique is inexact during surgery, often leading to removal of much more of the lymph system than necessary, causing unwanted trauma. QD technique is a significant improvement over the dye/radioactivity method currently used to perform SLN mapping. The imaging system and QDs allowed the pathologist to focus on specific parts of the SLN that would be most likely to contain malignant cells, if cancer were present.

Different varieties of PEG-coated QDs have been injected directly into tumors in mouse models of human cancer and their course tracked through the skin using NIR fluorescence microscopy to image and map SLNs (Ballou et al. 2007). In tumors that drained almost immediately to the SLNs, the QDs were confined to the lymphatic system, mapping out the connected string of lymph nodes. This provided easy tagging of the SLNs for pathology, and there was little difference in results among the different QD types used. Examination of the SLNs identified by QD localization showed that at least some contained metastatic tumor foci. The animals used in this study were followed for >2 years, with no evidence of toxicity, even though QDs could still be observed within the animals. SLN mapping has already revolutionized cancer surgery. NIR QDs have the potential to improve this important technique even further. Because the QDs in the study are composed of heavy metals, which can be toxic, they have not yet been approved for clinical use until safety has been established.

Nanosensor Device as an Aid to Cancer Surgery

Scientists at the University of Nebraska-Lincoln have developed a high-resolution touch sensor, one that uses a self-assembling nanoparticle device and acts much like a human finger. The self-assembly process developed by the research team involves no complex lithography, thus proving to be cost-effective and would be relatively easy to reproduce. This device has the ability to sense texture by touch, which is vital for surgeons who need the "touch sensation" in order to operate with precision and accuracy, such as when it comes to detecting and removing cancer cells from the body. One of the most important applications of this newly created sensor is the potential it holds for cancer surgeons, who are faced with the difficult task of knowing where to stop cutting when removing cancer cells in the body. In the development of artificial skin, the nanodevice structure can attain resolution of ~20 μm or even less. As this dimension is comparable to single-cell dimension, one can hope to "see" a single cancer cell in a tissue. The next goal is to make a high-resolution thermal imaging device and develop an ultrasound detector with a much better image resolution to enable detection of malignant tumors at early stages.

Role of Nanoparticle-Based Imaging in Oncology Clinical Trials

Currently, CT scans are used as surrogate end points in cancer clinical trials. The size of the tumor gives only limited information about the effectiveness of therapy. New imaging agents could speed the clinical trials process in two ways: (1) better imaging data could help oncologists better select which therapies to use on a particular patient and (2) and increasingly sensitive and specific imaging agents will be able to provide real-time information about whether a therapy is working. Currently, oncologists and their patients must wait months to determine if a given therapy is working. Shorter clinical trials would mean that effective new drugs would reach patients quicker and ineffective drugs would be dropped from clinical trials sooner, allowing drug discoverers to better focus their efforts on more promising therapies.

Nanoparticle-Based Anticancer Drug Delivery to Overcome MDR

Although multidrug resistance (MDR) is known to develop through a variety of molecular mechanisms within the tumor cell, many tend to converge toward the alteration of apoptotic signaling. The enzyme glucosylceramide synthase (GCS), responsible for bioactivation of the proapoptotic mediator ceramide to a nonfunctional moiety glucosylceramide, is overexpressed in many MDR tumor types and has been implicated in cell survival in the presence of chemotherapy.

A study has to investigate the therapeutic strategy of coadministering ceramide with paclitaxel in an attempt to restore apoptotic signaling and overcome MDR in the human ovarian cancer cell line using modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticles to encapsulate and deliver the therapeutic agents for enhanced efficacy (van Vlerken et al. 2007). Results show that indeed the complete population of MDR cancer cells can be eradicated by this approach. Moreover, with nanoparticle drug delivery, the MDR cells can be resensitized to a dose of paclitaxel near the IC50 of non-MDR (drug sensitive) cells, indicating a 100-fold increase in chemosensitization via this approach. Molecular analysis of activity verified the hypothesis that the efficacy of this therapeutic approach is due to a restoration in apoptotic signaling, although the beneficial properties of PEO-PCL nanoparticle delivery enhanced the therapeutic success even further, showing the promising potential for the clinical use of this therapeutic strategy to overcome MDR. Besides MDR, this novel paclitaxel–ceramide nanoparticle therapy also shows great potential for use in the treatment of non-MDR cancer types, in which therapeutic efficacy of paclitaxel is also enhanced.

Nanotechnology, used in conjunction with existing therapies, such as gene therapy and P-glycoprotein inhibition, has been shown to improve the reversal of drug resistance. The mechanisms involved include specific targeting of drugs, enhanced cellular uptake of drugs, and improved bioavailability of drugs. Important strategies in the reversal of drug resistance include (Palakurthi et al. 2012):

- A multifunctional nanoparticulate system
- Therapeutics to kill resistant cancer cells and cancer stem cells
- Release of encapsulated cytotoxic therapeutics in a stimuli-responsive tumor microenvironment

Nanoparticles for Targeting Tumors

Nanoparticles can deliver chemotherapy drugs directly to tumor cells and then give off a signal after the cells are destroyed. Drugs delivered this way are 100 times more potent than standard therapies. Gold nanoparticles can help X-rays kill cancerous cells more effectively in experiments on mice. Combination of nanoparticles followed by X-ray treatment reduced the size of the tumors, or completely eradicated them, whereas tumors that had received only X-ray therapy continued to grow. The gold nanoparticles had no therapeutic effect on their own. The technique

works because gold, which strongly absorbs X-rays, selectively accumulates in tumors. This increases the amount of energy that is deposited in the tumor compared with nearby normal tissue.

Efficient conversion of strongly absorbed light by plasmonic gold nanoparticles to heat energy and their easy bioconjugation suggest their use as selective photothermal agents in molecular cancer cell targeting (El-Sayed et al. 2006). Two oral squamous carcinoma cell lines and one benign epithelial cell line were incubated with anti-epithelial growth factor receptor (EGFR) antibody-conjugated gold nanoparticles and then exposed to continuous visible argon ion laser at 514 nm. Malignant cells required less than half the laser energy to be killed than the benign cells after incubation with anti-EGFR antibody-conjugated Au nanoparticles. No photothermal destruction was observed for all types of cells in the absence of nanoparticles at four times energy required to kill the malignant cells with anti-EGFR/Au conjugates bonded. Au nanoparticles thus offer a novel class of selective photothermal agents using a CW laser at low powers. The ability of gold nanoparticles to detect cancer was demonstrated previously. Now, it will be possible to design an "all-in-one" active agent that can be used to noninvasively find the cancer and then destroy it. This selective technique has a potential in molecularly targeted photothermal therapy in vivo.

Nanocarriers with TGF-β Inhibitors for Targeting Cancer

TGF-β inhibitors can prevent the growth and metastasis of certain cancers. However, there may be adverse effects caused by TGF-β signaling inhibition, including the induction of cancers by the repression of TGF-β-mediated growth inhibition. Application of a short-acting, small-molecule TGF-β type I receptor (TR-I) inhibitor at a low dose has been shown to be effective in treating several experimental intractable solid tumors, including pancreatic adenocarcinoma and diffuse-type gastric cancer, characterized by hypovascularity and thick fibrosis in tumor microenvironments. Low-dose TR-I inhibitor alters neither TGF-β signaling in cancer cells nor the amount of fibrotic components (Kano et al. 2007). However, it decreases pericyte coverage of the endothelium without reducing endothelial area specifically in tumor neovasculature and promotes accumulation of macromolecules, including anticancer nanocarriers, in the tumors. Compared with the absence of TR-I inhibitor, anticancer nanocarriers exhibit potent growth-inhibitory effects on these cancers in the presence of TR-I inhibitor. The use of TR-I inhibitor combined with nanocarriers may thus be of significant clinical and practical importance in treating intractable solid cancers.

Nanobombs for Cancer

Nanobombs are nanoscale bombs, which infiltrate into tumors in a minimally invasive manner and then explode on exposure to physical or chemical triggers. Various nanomaterials have been used for the construction of nanobombs including gold

and silica nanoparticles as well as carbon nanotubes. Nanobombs are effective anticancer agents as the shock waves that are generated after local explosion inside the tumor kill cancer cells and also disrupt cancer pathways so that the effect spreads beyond the area of explosion.

Temperature change can be used to trigger explosion. Nanogels fabricated by light cross-linking exhibit abrupt volume expansion upon exposure to sudden temperature change, causing cell death (Lee et al. 2009). In another approach, nanoclusters (gold nanobombs) can be activated in cancer cells only by confining near-infrared laser pulse energy within the critical mass of the nanoparticles in the nanocluster (Zharov et al. 2005). Once the nanobombs are exploded and kill cancer cells, macrophages can effectively clear the cell debris and the exploded nanotube along with it.

Blending of supramolecular chemistry and mechanostereochemistry with mesoporous silica nanoparticles has led to a new class of materials that are biological nanoscale bombs with the potential to infiltrate cells and explode upon the pulling of a chemical trigger (Cotí et al. 2009). The triggers are initiated by changes in pH, light, and redox potentials, in addition to enzymatic catalysis. This approach has been tried in in vitro experiments where loaded mechanized silica nanoparticles are endocytosed selectively by cancer cells and an intracellular trigger causes release of a cytotoxin, effectively leading to apoptosis.

Combination of Diagnostics and Therapeutics for Cancer

Aptamer-Conjugated Magnetic Nanoparticles

Magnetic nanoparticles have shown promise for targeted drug delivery, hyperthermia, and MRI imaging in cancer. Aptamer-conjugated magnetic nanoparticles controlled by an externally applied 3-D rotational magnetic field have been developed as a nanosurgical approach for the removal of cancerous cells selectively from the interior of an organ or tissue without any collateral damage (Nair et al. 2010). This system could be upgraded for the selective removal of complex cancers from diverse tissues by incorporating various target-specific ligands on magnetic nanoparticles.

Biomimetic Nanoparticles Targeted to Tumors

Nanoparticle-based diagnostics and therapeutics hold great promise because multiple functions can be built into the particles. One such function is an ability to home to specific sites in the body. Biomimetic particles that not only home to tumors but also amplify their own homing have been described (Simberg et al. 2007). The system is based on a peptide that recognizes clotted plasma proteins and selectively homes to tumors, where it binds to vessel walls and tumor stroma. Iron oxide nanoparticles and liposomes coated with this tumor-homing peptide accumulate in tumor vessels, where they induce additional local clotting, thereby producing

new binding sites for more particles. The system mimics platelets, which also circulate freely but accumulate at a diseased site and amplify their own accumulation at that site. The self-amplifying homing is a novel function for nanoparticles. The clotting-based amplification greatly enhances tumor imaging, and the addition of a drug carrier function to the particles is envisioned.

Dendrimer Nanoparticles for Targeting and Imaging Tumors

Dendrimer nanoparticles have been used to entrap metal nanoparticles, a combination that could serve as a potent imaging and thermal therapy agent for tumors if it were not for associated toxicity issues. To eliminate the toxicity associated with dendrimer–metal nanoparticle combinations, methods have been developed for modifying the surface of dendrimers laden with gold nanoparticles. This chemical treatment greatly reduces the toxicity of the hybrid nanoparticle without changing its size. Construction of novel dendrimers with biocompatible components and the surface modification of commercially available dendrimers by PEGylation, acetylation, glycosylation, and amino acid functionalization have been proposed to solve the safety problem of dendrimer-based nanotherapeutics (Cheng et al. 2011). There are several opportunities and challenges for the development of dendrimer-based nanoplatforms for targeted cancer diagnosis and therapy.

Gold Nanoparticle Plus Bombesin for Imaging and Therapy of Cancer

Bombesin (BBN) peptides have demonstrated high affinity toward gastrin-releasing peptide (GRP) receptors in vivo that are overexpressed in prostate, breast, and small-cell lung carcinoma. In vivo studies using gold nanoparticles (AuNPs)–BBN and its radiolabeled surrogate ^{198}AuNP–BBN constructs are GRP-receptor specific showing accumulation with high selectivity in GRP-receptor-rich prostate tumors implanted in severe combined immunodeficient mice (Chanda et al. 2010). The intraperitoneal mode of delivery was found to be efficient as AuNP–BBN conjugates showed reduced RES organ uptake with concomitant increase in uptake at tumor targets. The selective uptake of this new generation of GRP-receptor-specific AuNP–BBN peptide analogues have clinical potential in molecular imaging using CT techniques as the contrast numbers in prostate tumor sites are severalfold higher as compared to the pretreatment group. They also provide synergistic advantages by combining molecular imaging with therapy of cancer.

Gold Nanorods for Diagnosis Plus Photothermal Therapy of Cancer

Photothermal therapy is based on the enhancement of electromagnetic radiation by noble metal nanoparticles due to strong electric fields at the surface. The nanoparticles also absorb laser light more easily, so that the coated malignant cells only require half the laser energy to be killed compared to the benign cells. This makes it relatively easy to ensure that only the malignant cells are being destroyed. These unique

properties provide the potential of designing novel optically active reagents for simultaneous molecular imaging and photothermal cancer therapy. Gold nanorods with suitable aspect ratios (length divided by width) can absorb and scatter strongly in the NIR region (650–900 nm). Changing the spheres into rods lowers the frequency to which the nanoparticles respond from the visible light spectrum used by the nanospheres to the NIR spectrum. Since these lasers can penetrate deeper under the skin than lasers in the visible spectrum, they can reach tumors that are inaccessible to visible lasers.

In vitro studies have demonstrated that gold nanorods are novel contrast agents for both molecular imaging and photothermal cancer therapy (Huang et al. 2006). Nanorods are synthesized and conjugated to anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies (MAbs) and incubated in cancer cell cultures. The anti-EGFR antibody-conjugated nanorods bind specifically to the surface of the malignant-type cells with a much higher affinity due to the overexpressed EGFR on the cytoplasmic membrane of the malignant cells. As a result of the strongly scattered red light from gold nanorods in dark field, observed using a laboratory microscope, the malignant cells are clearly visualized and diagnosed from the nonmalignant cells. It is found that, after exposure to continuous red laser at 800 nm, malignant cells require about half the laser energy to be photothermally destroyed than the nonmalignant cells. Thus, both efficient cancer cell diagnostics and selective photothermal therapy are realized at the same time.

Magnetic Nanoparticles for Imaging as well as Therapy of Cancer

Several multifunctional nanoparticles are being developed for simultaneous imaging and therapeutic applications in cancer. Tumor-targeting dendrimers can contain an imaging as well as a delivery agent for drugs and genetic materials. A dendrimer linked to a fluorescent-imaging agent and paclitaxel can identify tumor cells and kill them simultaneously.

In ovarian cancer, metastasis occurs when cells slough off the primary tumor and float free in the abdominal cavity. If one could use the magnetic nanoparticles to trap drifting cancer cells and pull them out of the abdominal fluid, it may be possible to predict and perhaps prevent metastasis. With this aim, magnetic cobalt-spinel ferrite nanoparticles, which have cobalt-spiked magnetite at their core, were coated with biocompatible polygalacturonic acid and functionalized with ligands specific for targeting expressed EphA2 receptors on ovarian cancer cells (Scarberry et al. 2008). By using such magnetic nanoparticle-peptide conjugates, targeting and extraction of malignant cells were achieved with a magnetic field. The particles, which are just 10 nm or less in diameter, are not magnetic most of the time, but when a magnet is present, they become strongly attracted to it. Targeting ovarian cancer cells with receptor-specific peptide-modified magnetic nanoparticles resulted in cell capture from a flow stream in vitro and from the peritoneal cavity of mice in vivo. Successful removal of metastatic cancer cells from the abdominal cavity and from circulation using magnetic nanoparticle conjugates indicate the feasibility of a dialysis-like treatment and may improve long-term survival rates of ovarian

cancer patients. This approach can be applied for treating other cancers, such as leukemia, once the receptors on malignant cells are identified and the efficacy of targeting ligands is established. This technique will provide a way to test for and even treat metastatic ovarian cancer. Although the nanoparticles were tested inside the bodies of mice, it is possible to construct an external device that would remove a patient's abdominal fluid, magnetically filter out the cancer cells, and then return the fluid to the body. After surgery for removal of the primary tumor, a patient would undergo such a treatment to remove any residual cancer cells. The researchers are currently developing such a filter and testing it on abdominal fluid from human ovarian cancer patients.

Micelles for Targeted Drug Delivery and PET Imaging in Cancer

H40-DOX-cRGD, a multifunctional unimolecular micelle made of a hyperbranched amphiphilic block copolymer with attached doxorubicin (DOX), was tested for targeted anticancer drug delivery and PET imaging in tumor-bearing mice (Xiao et al. 2012). A uniform size distribution and pH-sensitive drug release behavior was observed. There was a much higher cellular uptake in U87MG human glioblastoma cells due to integrin $\alpha v\beta 3$-mediated endocytosis than nontargeted unimolecular micelles (i.e., H40-DOX), thereby leading to a significantly higher cytotoxicity. Thus, unimolecular micelles formed by hyperbranched amphiphilic block copolymers integrate passive and active tumor-targeting abilities with pH-controlled drug release. Simultaneous PET imaging for diagnosis provides the basis for personalized cancer therapy.

Nanobialys for Combining MRI with Delivery of Anticancer Agents

Although gadolinium has been the dominant paramagnetic metal for MRI contrast, the recent association of this lanthanide with nephrogenic systemic fibrosis, an untreatable disease, has spawned renewed interest in alternative metals for molecular MRI. Manganese was one of the first examples of a paramagnetic contrast material studied in cardiac and hepatic MRI because of efficient site-specific MR T1-weighted molecular imaging. Similar to Ca^{2+} and unlike the lanthanides, manganese is a natural cellular constituent and often a cofactor for enzymes and receptors. Mangafodipir trisodium, a manganese blood pool agent, has been approved as a hepatocyte-specific contrast agent with transient side effects due to dechelation of manganese from the linear chelate. A self-assembled, manganese(III)-labeled nanobialys MRI nanoparticle has been developed for combined diagnosis and delivery of a chemotherapeutic agent (Pan et al. 2008). The "bialy" shape affords increased stability. Nanobialys nanoparticles have been characterized for targeted detection of fibrin, a major biochemical feature of thrombus. A complementary ability of nanobialys to incorporate anticancer compounds with greater than 98% efficiency and to retain more than 80% of these drugs after infinite sink dissolution point to the potential of this platform technology to combine a therapeutic agent with a diagnostic agent.

Nanoparticles: MRI and Thermal Ablation of Tumors

Nanostructures with surface-bound ligands can be used for the targeted delivery and ablation of colorectal cancer (CRC), the third most common malignancy and the second most common cause of cancer-related mortality in the USA. Normal colonic epithelial cells as well as primary CRC and metastatic tumors all express a unique surface-bound guanylyl cyclase C (GCC), which binds the bacterial heat-stable enterotoxin (ST) – a peptide. This makes GCC a potential target for metastatic tumor ablation using ST-bound nanoparticles in combination with thermal ablation with near-infrared or radio-frequency energy absorption (Fortina et al. 2007). Furthermore, the incorporation of iron or iron oxide nanoparticles into such structures would provide advantages for MRI.

Gold nanoshell-based, targeted, multimodal contrast agents in the near-IR are fabricated and utilized as a diagnostic and therapeutic probe for MRI, fluorescence optical imaging, and photothermal cancer therapy of breast carcinoma cells in vitro (Bardhan et al. 2009). This may enable diagnosis as well as treatment of cancer during one hospital visit.

In the future, it may be possible for a patient to be screened for breast cancer using MRI techniques with engineered enhanced ferrites as the MRI contrast agent. Enhanced ferrites are a class of ferrites that are specially engineered to have enhanced magnetic or electrical properties and are created through the use of core-shell morphology. Magnetic nanoparticles are coupled to the radio frequency of the MRI, which converts the radio frequency into heat. If a tumor is detected, the physician could increase the power to the MRI coils and localized heating would destroy the tumor without damage to the surrounding healthy cells. The only hindrance to the development of enhanced ferrites for 100-MHz applications is a lack of understanding of the growth mechanisms and synthesis–property relationships of these nanoparticles. By studying the mechanism for the growth of the enhanced ferrites, it will be possible to create shells that help protect the metallic core from oxidation in biologically capable media.

pHLIP Nanotechnology for Detection and Targeted Therapy of Cancer

The pH-selective insertion and folding of a membrane peptide, pHLIP (pH low insertion peptide), can be used to target acidic tissue in vivo, including acidic foci in tumors. pHLIP nanotechnology is considered to be a promising approach for mapping areas of elevated acidity in the body. The peptide has three states: soluble in water, bound to the surface of a membrane, and inserted across the membrane. At physiological pH, the equilibrium is toward water, which explains its low affinity for cells in healthy tissue; at acidic pH, the equilibrium shifts toward membrane insertion and tissue accumulation. This peptide acts like a nanosyringe to deliver tags or therapy to cells. Tumors can be detected by labeling pHLIP peptide with Cy5.5 and imaging by use of NIR fluorescence with wavelengths in the range of 700–900 nm. In a mouse breast adenocarcinoma model, fluorescently

labeled pHLIP detects solid acidic tumors with high accuracy and accumulates in them even at a very early stage of tumor development (Andreev et al. 2007). The fluorescence signal is stable and is approximately five times higher in tumors than in healthy counterpart tissue. Tumor targeting is based on the fact that most tumors, even very small ones, are acidic as a result of the way they grow, known as the Warburg effect (Nobel Prize 1931). Tumors may be treated by attaching and delivering anticancer agents with pHLIP.

QD Conjugates Combine Cancer Imaging, Therapy, and Sensing

The specificity and sensitivity of a QD–aptamer–doxorubicin (QD-Apt(Dox)) conjugate as a targeted cancer imaging, therapy, and sensing system has been demonstrated in vitro (Bagalkot et al. 2007). By functionalizing the surface of fluorescent QD with a RNA aptamer, which recognizes the extracellular domain of the prostate-specific membrane antigen (PSMA), the system is capable of differential uptake and imaging of prostate cancer cells that express the PSMA. The intercalation of Dox, an anticancer drug with fluorescent properties, in the double-stranded stem of the aptamer results in a targeted conjugate with reversible self-quenching properties based on a Bi-FRET mechanism. A donor–acceptor model FRET between QD and Dox and a donor–quencher model FRET between Dox and aptamer result when Dox is intercalated within the aptamer. This simple multifunctional nanoparticle system can deliver Dox to the targeted prostate cancer cells and sense the delivery of Dox by activating the fluorescence of QD, which concurrently images the cancer cells.

Squalene-Based Nanocomposites for Tumor Imaging and Therapy

Nanocomposites, constructed of magnetite nanocrystals into NPs by self-assembling molecules of the squalenoyl gemcitabine (SQgem) bioconjugated, are characterized by an unusually high drug loading, a significant magnetic susceptibility, and a low burst release. When injected into a subcutaneous mice tumor model, these magnetite/SQgem NPs were magnetically guided and displayed considerably greater anticancer activity than other anticancer treatments including nonmagnetically guided magnetite/SQgem NPs (Arias et al. 2011). The histology and immunohistochemistry investigation of the tumor biopsies clearly evidenced the therapeutic superiority of the magnetically guided nanocomposites, while Prussian blue staining confirmed their accumulation at the tumor periphery. The superior therapeutic activity and enhanced tumor accumulation has been successfully visualized using T2-weighted MRI imaging. This concept was further enlarged by (1) the design of squalene-based NPs containing the T1 Gd3+ contrast agent instead of magnetite and (2) the application to other anticancer squalenoyls, such as, cisplatin, doxorubicin, and paclitaxel. This nanotechnology platform is expected to have important applications in imaging-guided cancer therapy.

Radiolabeled Carbon Nanotubes for Tumor Imaging and Targeting

Single-walled carbon nanotubes (SWCNTs) with covalently attached multiple copies of tumor-specific MAbs, radiometal-ion chelates, and fluorescent probes can target lymphomas and deliver both imaging and therapeutic molecules to these tumors (McDevitt et al. 2007). Each nanotube, which contained approximately six antibody molecules and 114 radioactive atoms, proved to be stable in human plasma for at least 96 h and was able to bind to targeted tumor cells. Most importantly, the chemical linkages binding the radioactive element indium-111 were completely stable in human plasma for the entire 4-day experiment. Tests using a mouse model of human lymphoma showed that the nanotube construct successfully targeted tumors while avoiding healthy cells. The ability to specifically target tumor with prototype-radiolabeled or fluorescent-labeled, antibody-appended SWCNT constructs was encouraging and suggested further investigation of these as diagnostic combined with drug delivery for cancer.

Ultrasonic Tumor Imaging and Targeted Chemotherapy by Nanobubbles

Drug delivery in polymeric micelles combined with tumor irradiation by ultrasound results in effective drug targeting, but this technique requires prior tumor imaging. A new targeted drug-delivery method uses ultrasound to image tumors, while also releasing the drug from nanobubbles into the tumor (Rapoport et al. 2007). Mixtures of drug-loaded polymeric micelles and perfluoropentane (PFP) nanobubbles stabilized by the same biodegradable block copolymer were prepared. Size distribution of nanoparticles was measured by dynamic light scattering. Cavitation activity (oscillation, growth, and collapse of microbubbles) under ultrasound was assessed based on the changes in micelle-to-nanobubble volume ratios. The effect of the nanobubbles on the ultrasound-mediated cellular uptake of doxorubicin (Dox) in MDA MB231 breast tumors in vitro and in vivo (in mice-bearing xenograft tumors) was determined by flow cytometry. Phase state and nanoparticle sizes were sensitive to the copolymer-to-perfluorocarbon volume ratio. At physiologic temperatures, nanodroplets converted into nanobubbles. Doxorubicin was localized in the nanobubble walls formed by the block copolymer. Upon intravenous injection into mice, Dox-loaded micelles and nanobubbles extravasated selectively into the tumor interstitium, where the nanobubbles coalesced to produce microbubbles. When exposed to ultrasound, the bubbles generated echoes, which made it possible to image the tumor. The sound energy from the ultrasound popped the bubbles, releasing Dox, which enhanced intracellular uptake by tumor cells in vitro to a statistically significant extent relative to that observed with unsonicated nanobubbles and unsonicated micelles and resulted in tumor regression in the mouse model. In conclusion, multifunctional nanoparticles that are tumor-targeted drug carriers, long-lasting ultrasound contrast agents, and enhancers of ultrasound-mediated drug delivery have been developed and deserve further exploration as cancer therapeutics.

Nanorobotics for Management of Cancer

It is within the realm of possibility to use molecular tools to design a miniature device, e.g., a nanobot that can be introduced in the body, locate and identify cancer cells, and finally destroy them. The device would have a biosensor to identify cancer cells and a supply of anticancer substance that could be released on encountering cancer cells. A small computer could be incorporated to program and integrate the combination of diagnosis and therapy and provide the possibility to monitor the in vivo activities by an external device. Since there is no universal anticancer agent, the computer program could match the type of cancer to the most appropriate agent. Such a device could be implanted as a prophylactic measure in persons who do not have any obvious manifestations of cancer. It would circulate freely and could detect and treat cancer at the earliest stage. Such a device could be reprogrammed through remote control and enable change of strategy if the lesion encountered is other than cancer.

Bacterial Nanorobots for Targeting Cancer

Flagellated nanomotors combined with the nanometersized magnetosomes of a single magnetotactic bacterium (MTB) can be used as an effective integrated propulsion and steering system for devices such as nanorobots designed for targeting locations only accessible through the smallest capillaries in humans while being visible for tracking and monitoring purposes using modern medical imaging modalities such as MRI (Martel et al. 2009). Through directional and magnetic field intensities, the displacement speeds, directions, and behaviors of swarms of these bacterial actuators can be controlled from an external computer. Such a device can be used for diagnosis as well as therapy of cancer.

DNA Robots for Targeting Cancer

DNA nanotechnology is widely investigated for its potential to deliver drugs and molecular signals to cells in the body because DNA is a biocompatible and biodegradable material. However, opinions differ as about the best nanorobot design, i.e., the ideal structure to load, transport, and deliver molecules. Various designs include a spiderlike robot that moves along a chemical track, a nanofactory with mobile robotic walkers and molecular forklifts, and DNA tweezers that open and close to grasp and release molecules.

An autonomous DNA nanorobot has been described that is capable of transporting molecular payloads to cells, sensing cell surface inputs for conditional, triggered activation, and reconfiguring its structure for payload delivery (Douglas et al. 2012). The nanorobot, constructed using a computer-aided design tool called DNA origami, is a hexagonal barrel, 35 nm in diameter, and opens like a clam shell. The device can be loaded with a variety of materials and is controlled by an

aptamer-encoded logic gate, enabling it to respond to a wide array of cues that have demonstrated their efficacy in selective regulation of nanorobot function. This barrel-shaped DNA nanorobot seeks out cancer cells and delivers self-destruct instructions. It can successfully deliver antibody fragments to surfaces of cancer cells to kill them and bacterial proteins to activate T cells.

Fullerenes for Protection Against Chemotherapy-Induced Cardiotoxicity

Therapeutic use doxorubicin as an anticancer drug is limited due to its cardiotoxicity. Generation of free radicals plays an important role in the mechanism of doxorubicin-induced cardiotoxicity. There is significant evidence indicating that mitochondria are the principal targets in this pathological process. Efficacy of fullerenol ($C_{60}OH_{24}$) in preventing single, high-dose doxorubicin-induced cardiotoxicity has been investigated in rats with malignant neoplasm (Injac et al. 2008). Study was performed on adult female Sprague Dawley rats with chemically induced mammary carcinomas. The animals were sacrificed 2 days after the application of doxorubicin and/or fullerenol, and the serum activities of cardiac enzymes were determined. The results obtained showed that the administration of a single dose of 8 mg/kg in all treated groups induces statistically significant cardiotoxicity. There were significant changes in the enzymes' lactate dehydrogenase and creatine kinase and increase in level of tissue malondialdehyde (MDA), a product of lipid peroxidation, after intraperitoneal administration of doxorubicin. The results revealed that doxorubicin-induced oxidative damage and that the fullerenol antioxidant effect caused significant changes in the levels of biomarker MDA in the heart. Thus, fullerenol may have an important role as for cardioprotection in doxorubicin-treated individuals.

Concluding Remarks and Future Prospects of Nanooncology

The rationale for using nanobiotechnology in oncology is that nanoparticles have optical, magnetic, or structural properties that are not available from larger molecules or bulk solids. When linked with tumor-targeting ligands such as MAbs, peptides, or small molecules, nanoparticles can be used to target tumor antigens (biomarkers) as well as tumor vasculatures with high affinity and specificity. In the size range of 5–100 nm diameter, nanoparticles have large surface areas and functional groups for conjugating to multiple diagnostic and therapeutic anticancer agents. Recent advances have led to bioaffinity nanoparticle probes for molecular and cellular imaging, targeted nanoparticle drugs for cancer therapy, and integrated nanodevices for early cancer detection and screening. These developments have provided opportunities for personalized oncology in which biomarkers are used to diagnose and treat cancer based on the molecular profiles of individual patients.

Nanoparticles have shown promise for incorporating multiple functions including diagnosis and therapy of cancer. Most of the work done in this area is still experimental, and some challenges need to be resolved before clinical applications. These include the following:

- Preventing capture/removal by the reticuloendothelial system
- Difficulties in selective targeting as well as penetration of tumor by systemic administration of anticancer nanostructures, which requires identification of receptors unique to a particular cancer
- Investigation of long-term fate and toxicity concerns of nanoparticles

Efforts are being made to use nanostructures to develop anticancer treatment strategies based on various mitochondrial targets that play vital roles in cancer development and progression. Cancer mitochondria-targeted multifunctional compounds have been identified that could provide an alternative strategy for the development of novel solutions for cancer diagnosis and therapy (Zhang et al. 2011).