

siRNA Therapeutics

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Introduction

Often cancers arise due to overexpression of oncogenes or expression of inappropriate protein products produced by gene translocations, insertions, or rearrangements. For example, some types of chronic myelogenous leukemia, acute myelogenous leukemia, or acute lymphoblastic leukemia are caused by chromosomal translocations that fuse together portions of the BCR serine/threonine kinase and the ABL tyrosine kinase (Perrotti *et al.*, 2010). The phenotypic effect is that the ABL kinase activity is uncontrolled due to the loss of regulatory protein sequences and addition of non-catalytic sequences from BCR. One approach to treating cancers that arise by these types of mechanisms would be to silence the incorrect gene and/or replace it with a normal copy. The later strategy would only be needed in cases of haplo-insufficiency, where one copy of the normal gene would not suffice and an additional copy is needed. A critical barrier, however, for gene silencing or gene replacement is efficient delivery mechanisms. The promise of nanoparticle-mediated delivery is well recognized and early clinical trials have already shown that double-stranded silencing RNAs or “siRNAs” are a feasible strategy for use in humans in the clinic (Davis *et al.*, 2010).

The mechanisms for cellular siRNA processing (as well as for short-hairpin (sh) RNA) have been reviewed elsewhere and will only be briefly addressed here. These RNAs can be taken up by cells “as is” but most efficiently when packaged in either liposomes (siRNAs) or viral vectors (shRNAs). They are processed by the dicer family of enzymes to remove the hairpin sequences (if needed) and then both categories of RNAs are incorporated into the RISC complex which serves to further process them into single-stranded RNAs (Figure 3). According to their sequence homology they bind to endogenous RNAs and either facilitate their degradation or inhibit translation of the RNA into protein, thus effectively silencing gene expression (Morris, 2008). A major advantage to this approach is that once a gene is implicated in cancer initiation, progression, or metastasis, it can be targeted without an intrinsic knowledge of its function, regulation, pathway involvement, etc. In addition, with careful

sequence design and validation, the approach can be very specific with little cross reactivity.

Aside from siRNA efficacy and specificity, two physiological factors loom large, those being stability/pharmacokinetics and cell and tissue targeting. There are a number of ongoing clinical trials addressing various diseases that utilize siRNAs and most of these are simple saline-based formulations for local or topical delivery for the eye, respiratory tract, and skin. Systemically, however, siRNAs injected intravenously are subject to rather rapid degradation and clearance via renal excretion. Despite this, some of these “naked” siRNAs have been shown useful in decreasing tumor growth and metastasis in a number of animal xenograft models (Vaishnav *et al.*, 2010). Modifications of the phosphodiester backbone, bases, or ribose ring have been reported to increase half lives in addition to chemical conjugation to cholesterol and protein moieties and undoubtedly research in this area will continue (Singh *et al.*, 2010). In the area of targeting “naked” siRNAs, researchers have conjugated them to antibodies through a biotin-streptavidin linkage and successfully directed them to glial cells demonstrating the potential to penetrate the blood/brain barrier (Xia *et al.*, 2007).

Delivery strategies for siRNA

In order to increase therapeutic benefit, it would be advantageous to protect the siRNA in “packaging” while specifically delivering the cargo to the intended target cell or tissue (Oh and Park, 2009). This goal in particular is where nanotechnology will shine (Figure 7). Due to the anionic, hydrophilic nature of RNAs, they are especially amenable to packaging within the cationic environment of lipid carriers such as liposomes, micelles, lipid-based nanoparticles, and emulsion formulations. Several examples of siRNA delivery via liposomes are entering phase I trials, including ALN-VSP, which simultaneously targets multiple transcripts of each VEGF and KSP (kinesin spindle protein) for liver tumors (Alnylam Pharmaceuticals website), and ATU027, which targets

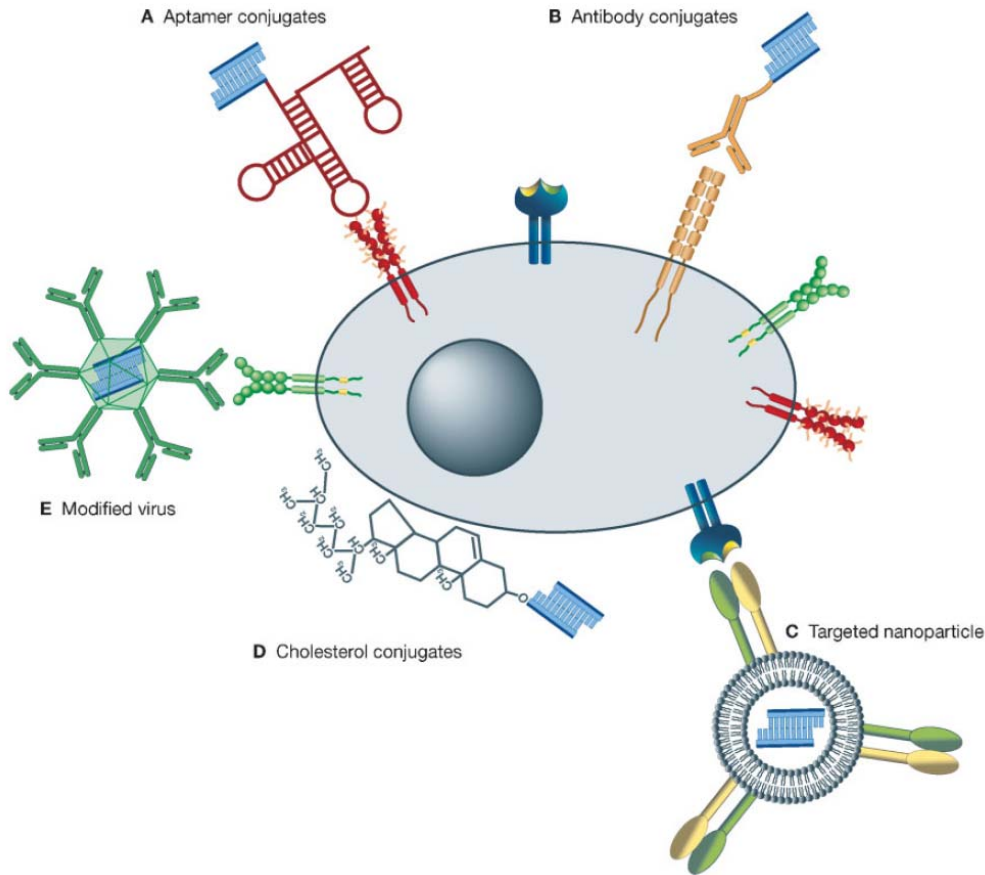


Figure 7 Delivery strategies for RNAi. The cell (grey ellipse) contains a nucleus (dark circle) and a cell membrane (dark ellipse). Cell surface molecules such as receptors are present on the cell surface (shown in color). RNAi therapeutics (mainly siRNA (blue)) can be targeted to the cell surface molecules via different delivery vehicles. They can be conjugated to aptamers (A), which can bind specifically to cell surface molecules and be internalized. siRNAs can also be conjugated to cell specific antibodies (B) and be delivered to the target cells via recognition of cell surface molecules by the specific antibody followed by internalization through endocytosis. Targeted nanoparticles (C) transport RNAi therapeutics to specific cells. The modifications of the nanoparticles (targeting ligand) can interact with receptors on the cell surface and the nanoparticle with its load can be internalized. Cholesterol conjugated siRNAs (D) can be delivered to cells and be internalized by the interaction of the cholesterol with the membrane through hydrophobic interactions, triggering clathrin-dependent endocytosis. Modified viruses (E) can also be used for cell specific delivery of RNAi therapeutics by cell specific cell surface interactions triggering endocytosis (Reprinted with permission from Tiemann and Rossi, 2009, Copyright, Wiley and Sons).

protein kinase N3 (PKN3) and has shown promise in human xenograft tumors of pancreas and prostate in mouse models (Aleku *et al.*, 2008). One study from Germany using one patient with CML resistant to both chemotherapy and the abl tyrosine kinase inhibitor imatinib found that siRNA to *BCR-ABL* packaged within liposomes decreased the fusion transcript and resulted in cellular death without adverse side effects (Koldehoff *et al.*, 2007). All of these siRNA liposomal formulations, however, while showing promise do not appear to be equipped with a cell specific targeting mechanism. Calando Pharmaceuticals, however, is in the process of phase I trials using the first targeted siRNA for human cancers, CALAA-01 (Davis *et al.*, 2010). They silenced the M2 subunit of ribonucleotide reductase (RRM2) by using nanoparticles directed to melanoma cells through a peptide targeting the transferrin receptor. Several lines of evidence indicate that RRM2 mRNA and protein levels are decreased following nanoparticle therapy

and that the mechanism is through cellular action of the siRNA. Given the rapid pace in which the signaling pathways of various tumor types are being dissected as well as biomarkers being identified, we can expect to see an increase in this type of targeted, systemic nanoparticle therapy.

Clinical impact

Currently, 14 siRNA-based clinical trials have been initiated (Vaishnav *et al.*, 2010), four of which are for cancer and three of these are in liposomal formulations. Some remarkable features of nanoparticle delivery are the relatively low amount of immune system response (as discussed in a previous section) and decreased drug induced toxicity. Several clinical trials directed at other

diseases utilizing siRNA therapy that are not nanoparticle based have been terminated due to either no overall improvement of the condition (such as visual acuity), or due to non-specific effects of the treatment such as activation of innate immunity (Kleinman *et al.*, 2008; Vaishnav *et al.*, 2010). The latter clinical outcome might be circumvented by nanoparticle formulations. Since cancer can arise by a vast array of mechanisms, some of which are more specific to tissue type and others that are integral pathways important for the life of all cells, the therapeutic strategy to combat it would be most advantageous if it were targeted to tumor cells and spared normal cells. This approach can be achieved using nanoparticle formulations.

As the research continues to develop siRNA-based nanotherapeutics, we expect an increasing number of diverse packaging systems for siRNAs (Gao *et al.*, 2010). For example, siRNA has recently been incorporated into stimuli-responsive PEGylated nanogels which when subjected to the lower pH of the tumor intracellular environment enhances lysosomal and endosomal release (Oishi and Nagasaki, 2010). In addition, reports have described such concepts as delivering siRNAs via magnetic nanoworms (Agrawal *et al.*, 2009), dendrimers (Ravina *et al.*, 2010), nanocrystals (Namiki *et al.*, 2009), and carbon nanotubes (Menard-Moyon *et al.*, 2010). An alternative approach to siRNA but still targeting RNA degradation to decrease gene expression would be to employ DNAzymes. These are short synthetic DNAs with inherent enzymatic activity capable of cleaving target RNAs (Ravina *et al.*, 2010). Nanoparticles containing DNAzymes could prove to be a valuable therapeutic approach in the future.

Beyond the potential value of siRNAs in therapy they can also be used for *in vitro* and *in vivo* diagnostics. They have already been used to screen for biological regulators as therapeutic targets and validate them for potential clinical applications. In addition, siRNAs can be useful for assay development and can serve as positive and negative controls to establish the relevant signaling pathways involved in cancer progression, angiogenesis, metastasis, etc. Recently, siRNAs have been tagged with fluorescent markers which can, in theory, be used to track which cells have received the siRNA in a living organism (Oishi and Nagasaki, 2010). In the future, we expect that more and more multi-functional nanoparticles will not only deliver siRNAs to the target tumor types but will also enable real-time imaging, thermal ablation, and/or small molecule drug delivery.

Milestones

3 year:

- Expand the repertoire of chemical modifications to the siRNAs themselves as well conjugation to other carbohydrates, lipids, proteins, etc. to increase stability, bioavailability, and intracellular processing.
- Increase research on catalytic oligonucleotides capable of cleaving the target RNAs.

5 year:

- Test new nanotechnology-based delivery vehicles for siRNA.
- Develop formulations containing multiple siRNAs to target multiple signal transduction pathways.
- Conduct late stage clinical trials for siRNA delivery.

10 year:

- Increase focus on personalized therapies using tumor sequencing data to direct decisions on nanoformulations using multiple siRNAs specific to the patient's tumor genetic or proteomic profile.
- Gain FDA approval for nanoparticle-based therapies using siRNA delivery.

Multi-pronged strategy to overcome MDR – enhancing delivery efficiency and altering cellular phenotype

As our understanding of cancer deepens, one concept that becomes increasingly evident is that cancer is a heterogeneous disease on both the intra- and inter-patient levels. As such, a therapy that treats only one phenotype is not slated for success. For a cancer therapy to be effective the therapy must be multi-faceted, simultaneously treating multiple aspects of the disease.

Nanocarriers serve as ideal delivery solutions for combination therapy which is required for effectively treating MDR cancer. The benefits of nanocarriers include, (1) they can be engineered to achieve multiple effects using one system; (2) nanocarriers improve the therapeutic index of drugs and can alter the pharmacokinetic profile of agents; (3) they preferentially accumulate in the tumor environment thanks to the EPR effect and their capacity to be conjugated to targeting moieties; and (4) nanocarriers avoid drug efflux by preferentially localizing agents in the peri-nuclear region of a cell, away from membrane localized efflux pumps.

The most effective treatment for MDR should address multiple MDR phenotypes which can be facilitated using the multi-functional platforms available through nanotechnology. As such, combining a traditional cytotoxic chemotherapeutic agent with one or more of the following strategies could prove effective:

1. inhibiting ABC-transporter mediated drug efflux
 - a. small molecule inhibitors such as verapamil
 - b. siRNA/shRNA silencing
2. lowering the apoptotic threshold
 - a. inhibiting the Warburg effect (aerobic glycolysis)
 - b. increasing intracellular ceramide
 - i. exogenous delivery
 - ii. siRNA silencing of glucosylceramide synthase
 - c. stimulating cytochrome c release (mitochondrial permeability transition pore complex)
 - d. increasing pro-apoptotic Bcl2 family members
 - e. decreasing anti-apoptotic Bcl2 family members
3. increasing tumor suppressor activity (such as p53 gene therapy)
4. decreasing oncogene activity
5. decreasing the stem-like properties of MDR cells (*exploratory, e.g. silencing stem cell factor*)

Tumor-targeted multi-functional nano-delivery systems

Although nanocarriers passively target cancer through the EPR effect, using active targeting can increase the specificity of nanocarriers for MDR cells. It is relatively simple to modify the surface of nanocarriers with targeting residues. Common targeting residues include antibodies for cancer antigens, ligands for over-expressed cell-surface proteins, and lectins for carbohydrate targeting.

Active targeting can further improve the therapeutic index of an agent by decreasing off-target accumulation. Common targets include EGFR receptors, transferrin receptors, and folate receptors.

Active targeting also alters the mechanism of uptake of nanocarriers. Non-targeted nanocarriers are taken up by non-specific endocytosis whereas targeted nanocarriers are internalized via their target-specific mechanism. For example, nanocarriers that target the EGFR receptor are internalized via a flip-flop mechanism, a rapid process compared to endocytosis. Active targeting, therefore, not only decreases the residual toxicity of a system, it can further alter the pharmacokinetic profile of a system. Some nanocarrier systems are designed to target more than one MDR phenotype, further increasing their specificity to MDR cells. However, the *in vivo* effects of active targeting are inconclusive and need to be validated and explored.

Milestones

3 year:

- Develop animal models of refractory disease that recapitulate the human disease in terms of location, genotypic and phenotypic heterogeneity, etc.
- Characterize tumor microenvironmental factors (i.e., soluble and insoluble) on the development of clinically-relevant refractory disease.
- Identify and validate drug targets and strategies to overcome resistance through a multi-factorial approach that utilizes efficiency in drug delivery, residence, and intracellular penetration as well approaches to overcome cellular resistance.

5 year:

- Establish robust pre-clinical programs to develop and test multi-functional nanoparticulate drug delivery systems in appropriate models of refractory diseases.
- Evaluate the toxicological properties of nanoparticulate formulations under GLP conditions.

10 year:

- Establish collaborations with pharmaceutical industries and clinical centers to rapidly facilitate the transfer of technologies from academia to cancer patients.
- Establish a clinical development program for multi-functional nanoparticulate systems using the appropriate guidance from regulatory agencies.

New Contrast Agents with Improved Spatial and Temporal Resolution

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Current status

Molecular imaging agents promise new unprecedented opportunities to assess changes in tumor microanatomical and physiological character with greater spatial and temporal resolution in cancer patients. Until recently, the majority of advancements in cancer imaging have favored improved detectability of minute masses. Today, we can detect minute lesions with high resolution CT and MRI, and the challenge has become deciding whether a lesion is a benign fascinoma or an early malignancy. Early categorization of a pathology as benign stable disease, an inflammatory lesion, or a malignancy has dramatic implications in medical management, but commonly minute tissue anomalies cannot be characterized, necessitating a conservative “wait and see” management approach. Re-evaluation in three to six months is common to assess gross morphological changes that would point to cancer but an aggressive tumor may have already disseminated beyond the original primary site.

Delineation of an unknown pathology suspected of cancer requires biopsy for microscopic and biochemical characterization. Although such procedures are routinely performed, the acquisition of tissue specimens by surgical resection or fine-needle aspiration still presents challenges due to lesion accessibility, tissue sample quality and artifacts, and a patient’s willingness to undergo the procedure. Biopsy procedures become particularly troublesome when the lesion is small (< 1 cm) and centrally located. Molecular imaging offers a noninvasive mechanism to assess microanatomical changes, for example the development of a neovasculature, or the expression of important biochemical markers, such as HER-2/neu. These pathological signatures serve not only as an aide in tumor diagnosis and grading, but also as responsive biomarkers to treatment efficacy. Improved noninvasive characterization will lead to definitive diagnoses sooner, and because the lesion is “visualized” *in vivo*, key anatomical and metabolic information destroyed or nonassayable by excising the tissue is retained.

Microanatomical and biochemical measurements of tumors require robust, quantitative techniques with high

spatial and temporal resolution, but what constitutes high resolution is often a matter of perspective and dependent on the medical question posed. For example, nuclear “hot spot” imaging with PET or SPECT tracers are detected with very high sensitivity per tracer concentration but low spatial resolution (millimeters) when compared with MRI. PET has high temporal resolution for kinetic studies given adequate nuclear tracer counts, which allows convenient and rapid assessments of probe “wash-in” or “wash out” of a target tissue. Moreover, in some situations, low spatial resolution may be adequate for noninvasive tissue characterization when a boolean answer based on the presence or lack of radioactivity for a pathognomonic receptor or biochemical pathway is sought. Unfortunately, ¹⁸F-DG is completely nonspecific except for a prevalent accumulation in cells with high metabolic rate and receptor specific ligands are foiled by nature’s utilization of the same receptors and pathways for many cell types. For example, radiolabeled RGD peptides (arginine, glycine, aspartate) and antibodies, particularly directed to the $\alpha_v\beta_3$ -integrin, have been used to target and characterize tumor angiogenesis by PET (Haubner *et al.*, 1999; Beer *et al.*, 2007) and SPECT (Liu *et al.*, 2007). However, these small molecules, despite exquisite chemistry, readily permeate beyond the tumor and bind many cell types, including macrophages and tumor cells, which diminishes the signal specificity for angiogenesis per se (Zitzmann *et al.*, 2002; Liu *et al.*, 2007).

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) can detect changes in tumor microvasculature permeability to MR blood pool contrast agents and some studies have correlated these kinetic estimates with traditional measures like MVD, but initial clinical trials have yielded inconsistent results either due to insufficient standardization of the endpoints or technique issues (Jayson *et al.*, 2002; Liu *et al.*, 2005; Schmieder *et al.*, 2008). However, MR molecular imaging with paramagnetic nanoparticles facilitates high-resolution 3D mapping of angiogenesis (Schmieder *et al.*, 2008; Winter *et al.*, 2008). Such *in vivo* studies clearly indicate that angiogenesis is peripherally distributed nonuniformly around a tumor in a heterogeneous pattern associated with

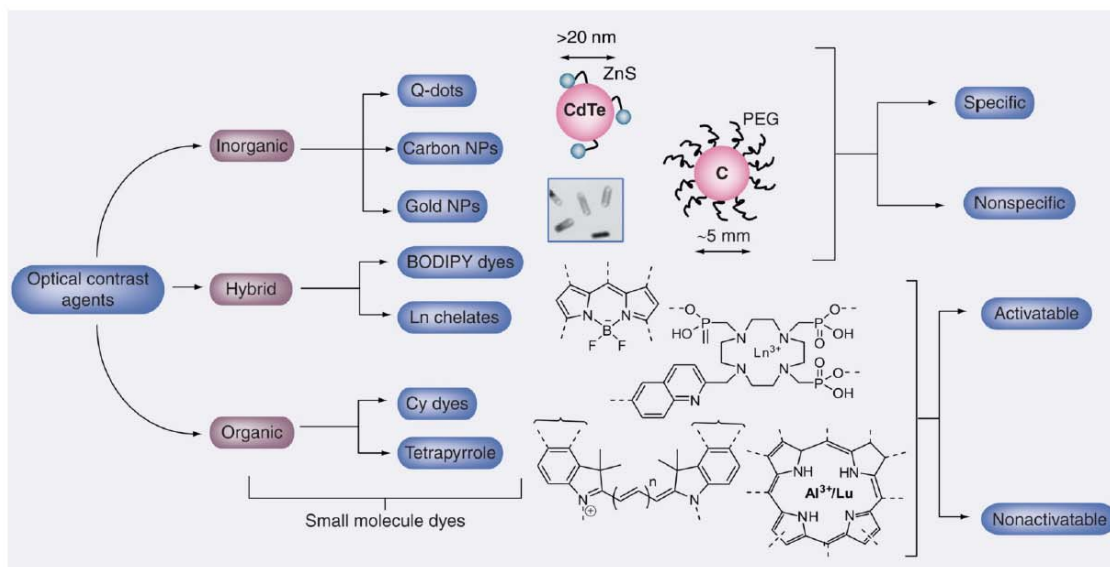


Figure 9 General classification of optical contrast agents (reprinted with permission from Pan *et al.*, 2010, Copyright, Future Science).

rapidly proliferating cancer growth fronts. Clearly, neither fine needle aspiration into a tumor core nor routine histology sections randomly oriented on resected tumors are severely prone to sampling error and cannot provide reasonable quantitative estimates of neovascularity, that could be used to risk-stratify patients for anti-angiogenesis treatment.

Like MRI, CT offers tomographic imaging with very high spatial and temporal resolution, which overcomes the issues of motion in many tissues including pulmonary and gut. However, the inherent tissue x-ray contrast is low, necessitating the use of iodinated low molecular weight contrast agents. Although like gadolinium-based DCE, CT can be used for kinetic modeling, the data provide no biochemical and limited pathological prognostic information. New nanoparticle based homing agents have been reported that overcome the marked insensitivity of CT to contrast, but the majority of pre-clinical applications studied to date have been directed toward targets with high epitope density or to passive accumulation in macrophages, liver, or spleen.

Ultrasound is another important clinical imaging modality with moderately high spatial (mm to micron, dependent on frequency) and very high temporal resolution (real-time). Once a planar technique, the advent of 3D ultrasound provides improved spatial registration. Ultrasound is the clear favorite with regard to cost, portability, and ease of use, but it has significant limitations. The most common problems are derived from the limited “acoustic windows” available where bone, gas (bowel or lung), or depth of tissue do not preclude or compromise imaging results. Moreover, achieving high imaging resolution is dependent upon increasing the transducer insonification frequency. While high frequency transducers, 25 MHz and up, offer the best temporal –

spatial resolution, but sound penetration decreases with increasing frequency, requiring these targets to be near the skin or accessible with intravascular ultrasound catheters. Ultrasound molecular imaging with microbubbles (Klibanov *et al.*, 1999), echogenic liposomes (Alkan-Onyuksel *et al.*, 1996), and PFC nanoparticles (Lanza *et al.*, 1996) have been demonstrated *in vivo*, but the microbubbles due to their highly amplified ultrasound reflectance, offer the greatest contrast and the greatest noise, even a single bubble, targeted or random, is detectable.

Temporal resolution is becoming an important factor in the clinical use of ligand-targeted molecular imaging agents, particularly with respect to drug delivery with theranostic agents. Initially, molecular imaging will play a role in stratifying patients into optimal treatment plans, but soon thereafter, the effectiveness of treatment, particularly for small tumors in asymptomatic patients, will utilize molecular imaging follow response and manage the pharmacologic strategy. With the advent of theranostic agents (as discussed previously), now demonstrated repeatedly in pre-clinical models, imaging will be used not only to stratify patients to best treatment regimen, but also to confirm dosing of targeted therapy using the coupled imaging feature. While repeat imaging with ultrasound and MRI will pose no known health threats, recurrent use of ionizing radiation (PET and CT) may predispose to unwarranted health side effects. This issue gains significance as younger patients with cancer identified and treated earlier.

A concern of temporal resolution will be inherent in the contrast agent used. For instance, with MRI paramagnetic nanoparticles (and the like), imaging occurs with one to three hours after injection and there is no residual contrast signal at the target site 24 hours after

treatment. Repeat imaging can easily occur within two days. In contradistinction, most targeted iron oxide contrast agents cannot be imaged until 24 or 48 hours after treatment due to blood pool induced magnetic artifacts and the persistence of the iron oxide nanoparticles at the target site can last variably from weeks to months, limiting timely reinterrogation. Ultrasound microbubbles have very short blood half-life and tissue persistence, making them an excellent choice for serial imaging, but the acoustic rupture of microbubbles for perfusion-reperfusion targeting techniques or for acoustically enhanced drug delivery, may alter the presentation of bioepitopes for homing and confound serial imaging results. Both CT and MRI agents dependent upon heavy elements and repeat dosing must address the possibility of toxic accumulation. Metal administered for contrast must be chemically stable *in vivo* and predominantly eliminated from the body in a few days with virtually all of the remaining metal excreted in a few weeks.

Future challenges

The clinical utility of molecular imaging with high spatial and temporal resolution depends on the quantitative reproducibility of signal estimates derived within an individual patient. Contrast imaging must be quantitatively correlated with target expression and be repeatable. To date, the depiction of a tumor hot-spot PET or angiogenic map with MRI are dependent on thresholding techniques, which must be optimized for pathologic correlation and normalized for serial within patient comparison over time. Today's clinical imaging techniques present have 20 to 30% variability related to performance technical issues (e.g., MR coil or nuclear detector placement) and manufacturer provided internal hardware recalibration routines. The current drive to quantitative, reproducible imaging must continue with the institution of more stringent operational standards, development of National Institute of Standards and Technology (NIST) calibration phantoms, and rigorously validated imaging software and hardware capable of absolute measurements. Without meeting this essential challenge, molecular imaging with or without drug delivery will not achieve its potential and could fail to become a proven, clinically relevant and reimbursable procedure to improve cancer management. Fortunately, these goals are more or less engineering accomplishments that can be achieved with determined effort.

Milestones

3 year:

- As nanotechnologies reach the clinic, the potential for early application for molecular imaging will become known as will the challenges of signal detection, reconstruction, and calibration within the human body. This information will be critical as clinical trials proceed. We expect each new agent reaching the clinic will elucidate new problems and uncover unexpected

opportunities which will enhance formulation of the global and specific issues governing efficacy, safety, and clinical use compatibility.

5 year:

- The information achieved in clinical trials must drive hardware-software vendors to implement improved validated software to optimize image acquisition and presentation to physicians for clinical interpretation. Molecular imaging literally means detecting, presenting and characterizing nascent cancers, which is akin to finding the proverbial "needle in a haystack" with robust quantitative rigor.
- Concurrently, basic and clinical scientists must work together to devise guidelines for utilizing the imaging information alone and with drug delivery in an effective, cost-responsible manner leading to the improved health care management of cancer patients.
- Because the information developed over the first five years of the clinical molecular imaging revolution will be "first of its kind data in man", dogmatic views and perceptions of the past will need to be revisited, revised and often discarded. Willingness to accept new molecular imaging data and to discard our preconceived notions will be the greatest achievement of this period.

10 year:

- Expanded use of first generation molecular imaging technologies combined with new generation systems, which must robustly overcome the transendothelial barrier to nanoparticle delivery and expand opportunities for direct to cancer cell theranostic medicine. Insight into these pathways and mechanisms to utilize nature's machinery has already been achieved and our understanding is rapidly increasing.
- Next generation product candidates created over the next five years will reach the IND stage for clinical testing in five to eight years with the homing and size specificities needed overcome this targeting obstacle.
- During the last two years of this decade, these new generation nanomedicines should clear phase I safety and proof of concept hurdles and begin focused clinical study toward efficacious cancer applications considered intractable today.

Multi-modal Imaging

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Introduction

Currently a number of advanced imaging modalities are available in the pre-clinical and clinical setting, including magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT), and optical imaging (OI) (Willmann *et al.*, 2008). However, all these modalities vary in their limits of sensitivity, resolution, and depth profiling. Therefore, it is unlikely that a single imaging modality will provide conclusive evidence of a biological process or therapeutic response. In this regard, a synergistic combination of multiple non-invasive imaging technologies will play a critical role in the early detection of cancer and other diseases. The choice of these imaging techniques is driven by their ability to provide complimentary (structural and functional) information and enable cross-validation of imaging signals along with differences in resolution, sensitivity and clinical application. For example, OI approaches are valuable for *in vitro* and *in vivo* evaluation in pre-clinical model systems but are not ready for ‘prime-time’ in the surgical setting. In contrast, advanced imaging techniques such as MRI are widely used in clinical diagnosis and monitoring the response of patients to therapy. Therefore, the combined OI-MRI approach is likely to provide valuable information on the diagnostic/staging potential of our nanoplatform. In addition, combined with a therapeutic modality, such a system will facilitate the monitoring of therapy in real time. Such real-time monitoring would allow patients with ‘non-responsive’ tumors to avoid the side effects of ineffective treatment by enabling them to be switched in a timely manner to more appropriate therapies that are likely to offer better survival benefit (Prasad, 2004). However, successful realization of these objectives will require the development of novel multi-modal and biocompatible agents, along with multi-imaging instrumentation and software capable of co-registering the signals obtained from the various imaging modalities.

Nanoparticle-based probes have several advantages over traditional molecular agents because: (1) they provide a tunable, optically traceable (fluorescence, NIR and/or bioluminescence) chassis upon which targeting agents (antibodies, peptides, small molecules, etc.) can be

added or changed to suit a specific need; (2) they enable multi-modality (e.g., optical, MR and radionuclide) imaging thus permitting concurrent evaluation for the same nanoparticle across different imaging platforms; (3) they enable targeted and sustained delivery of potent chemotherapeutic agents specifically to diseased sites, avoiding normal organs; (4) they can be functionalized with both imaging and therapeutic abilities (i.e., “theranostic” nanoparticles); (5) they are of sufficient size to permit multi-valency and therefore the potential for higher affinity binding than standard molecular agents; and (6) they enable imaging from the molecular level, to single cells, and to the entire, intact organism. This attribute further enables validation of the imaging marker by correlating results obtained *in vitro*, e.g., relying on the optical (fluorescence/near-infrared [NIR]) aspects of the probe, with those obtained *in vivo*, which may also rely on optical, radionuclide or MR imaging. Therefore, targeted multi-modal nanoparticles are expected to play a pivotal role in the development of the “next generation” of clinical agents for cancer diagnosis and treatment, as they will facilitate detection of both structural and functional anomalies which are characteristic of the early stages of cancer. Furthermore, the ability to simultaneously deliver chemotherapeutic agents specifically to tumor sites would greatly improve patient survival and post-treatment quality of life.

Current status

The rapid growth of *in vivo* multi-modal imaging arises from the convergence of established fields of *in vivo* imaging technologies, along with nanotechnology, as well as molecular and cell biology (Caruthers *et al.*, 2007). The major hallmark of nanomedicine is the fabrication of multi-modal nanoprobables, which would not only incorporate multiple image-contrast agents, but also therapeutic probes and targeting molecules for site-specific delivery. Multi-modal nanoprobables can provide both structural and metabolic information specifically from diseased sites, thus leading to significantly improved imaging techniques for the detection of a variety of human cancers (e.g. breast,

Nanotechnology for Image-Guided Interventions

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Overview

There are major opportunities and challenges in developing nanotechnology and advanced instrumentation for image-guided cancer surgery and biopsies (Singhal *et al.*, 2010). The ability to visualize tumors in real-time will help the surgeon to delineate tumor margins, to identify residual tumor cells and micrometastases, and to determine if the tumor has been completely removed. This would apply to tumors of many organ sites, especially aggressive lung, pancreatic, ovarian, and metastatic breast cancers. Nanometer-sized particles such as quantum dots, colloidal gold, and biodegradable nanoparticles have functional and structural properties that make them appealing for tumor imaging. When conjugated with targeting ligands such as monoclonal antibodies, peptides or small molecules, these nanoparticles can be used to target malignant tumor cells and the tumor microenvironments (such as tumor stroma and tumor vasculature) with high specificity and affinity. In the “mesoscopic” size range of 10-100 nm diameter, nanoparticles also have large surface areas available for conjugating multiple diagnostic and therapeutic agents, opening up new possibilities for integrated cancer imaging and therapy (Nie *et al.*, 2007). Similarly, advanced optical instrumentation provides unique advantages for intraoperative cancer detection that are not available from other imaging modalities. In the visible spectrum, optically labeled tumors are visible to the human eye, and can be seen and resected by the surgeon without any visual aid. In the near-infrared spectrum, standard fiber optics and silicon-based CCD cameras can be used for tumor visualization at high sensitivity and low costs (De Grand and Frangioni, 2003).

Nanotechnology is well suited for image-guided interventions because several problems that are often associated with nanoparticles and optical instrumentation are circumvented under surgical or biopsy conditions. For example, optical methods have relatively limited penetration depths due to tissue scattering and blood absorption, but this is no longer a major limitation during intraoperative cancer detection because the tumors are surgically exposed and are accessible to optical illumination and detection. Another common problem in using nanoparticles and macromolecules for cancer therapy

is that they are unable to deeply penetrate solid tumors. This is not an issue as defining the tumor’s external margin is the actual goal for surgical resection and internal staining is inconsequential. For intraoperative detection of small and residual tumors, deep penetration is also not required because the small tumors do not have high intra-tumoral pressures or hypoxic/necrotic cores, two main factors in limiting tumor penetration of nanoparticle imaging and therapeutic agents (Lunt *et al.*, 2009). Thus, the combined use of nanoparticle contrast agents and imaging technologies is expected to improve the sensitivity and specificity of detecting microscopic tumors and residual tumor cells after resection, with important applications in both image-guided surgery and image-guided biopsy.

Clinical significance

Most human cancers are treated by surgical resection, chemotherapy and/or radiation. Surgery cures approximately 45% of all patients with cancer, and provides a dramatic survival advantage (<http://seer.cancer.gov/>). To cure a patient with surgery, the surgeon must remove the entire tumor at the time of surgery. A complete resection is the single most important predictor of patient survival for almost all solid tumors. This includes removing the primary tumor and draining lymph nodes that may contain tumor cells and small adjacent satellite nodules. In lung, breast, prostate, colon, and pancreatic cancers, a complete resection has a three to five fold improvement in survival compared to a partial or incomplete resection. Clearly, it is important to maximize the efficacy of surgical procedures because it is the most important method that exists to cure people of their cancer.

Minimally invasive cancer surgery

One of the most important changes in surgical oncology has been the development of minimally invasive surgery, which promises to alter the delivery of cancer care in the U.S. and in the world. Historically, one challenge of cancer surgery has been the loss of six to eight weeks that

occurs following an open procedure. After surgery, there can be a lengthy recovery time during which no adjuvant therapies can be given. Many common set backs including a urinary tract infection, pneumonia or arrhythmia, can delay the start of chemotherapy or radiation an additional month, during which the disease can still progress. Another problem is that many patients do not qualify for open procedures due to frail health and advanced age. The development of minimally invasive surgery has solved these challenges. Lung cancers are now removed by thoracoscopic lobectomy, colon cancers by a laparoscopic colectomy, and prostate cancers by robotic surgical instruments. Consequently, recovery time has dramatically decreased. These surgical techniques have translated well into other realms making rapid diagnoses and specimen retrieval possible with minimal patient duress.

Furthermore, minimally invasive surgery has largely replaced open surgery as an important tool to obtain rapid diagnostic information and specimens. For example, laparoscopic examination of the abdomen is used to evaluate and obtain diagnostic material for ovarian cancer, gastric cancer, and pancreatic cancer. Similarly, thoracoscopic (chest) surgery is used to obtain pleural biopsies in metastatic breast cancer, lymphomas, and mesothelioma. These procedures require only three to four small ports on a patient's chest, and can take place as an outpatient with costs under \$5000 (vs. \$30,000 for open surgery).

Nanoparticle contrast agents

As advancements in the field of nanoparticle imaging science are made, one of the first theatres for their use will be open and endoscopic conditions. There is considerable evidence indicating that the use of injected contrast agents can improve the detection of tumor margins and small metastases (Sajja *et al.*, 2009). New and innovative targeting and contrast agents including small molecules, antibodies, and nanoparticles should be developed for a broad range of tumor types such as breast, brain, pancreatic, and ovarian cancers. At present, a number of organic dye molecules have been approved for human use including (1) indocyanine green (ICG), a near-infrared fluorescent dye; (2) fluorescein, a green fluorescent dye; (3) photofrin, a mixture of fluorescent protoporphyrin oligomers approved for photodynamic therapy, and (4) 5-aminolevulinic acid (ALA), a small molecule that is preferentially taken up by tumor cells leading to biosynthesis and accumulation of protoporphyrin IX, a natural fluorophore with red fluorescence emission. On the other hand, nanoparticles have not received FDA approval for clinical tumor imaging.

A major task is, therefore, to develop biocompatible and nontoxic nanoparticle contrast agents with the potential for FDA approval and human use. Such agents need to show improved sensitivity and specificity for tumor imaging in comparison with small-molecule dyes. In this regard, it is highly promising to develop "smart" or activatable nanoparticles with improved pharmacokinetic, tumor-targeting, and organ clearance properties, based on the use of natural, biodegradable

polymers (dextran and heparin). Dextran-based particles are sensitive to pH, and can be rapidly broken down under acidic conditions. Under neutral or slightly basic conditions, on the other hand, the dextran nanoparticles are stable and are able to circulate systemically in blood for 14-15 hours (Gaur *et al.*, 2000). In contrast, self-assembled heparin nanoparticles have much shorter blood circulation half lives (about 60-80 min) (Chen *et al.*, 2009). For intraoperative use, this short circulation time could be beneficial because the probes will be cleared from the blood quickly, so that surgical operations can start without much delay or waiting. For near-term clinical applications, it is important that both the dextran and heparin particles are able to trap an FDA-approved dye (such as indocyanine green), leading to a new class of imaging contrast agents with improved biodistribution and photophysical properties. Figure 13 shows a class of "nano-ICG" contrast agents that are quenched in their initial state but are activated under *in vivo* conditions (Mohs *et al.*, 2010). This class of nanoparticle contrast agents could also be conjugated with tumor targeting ligands such as folate, EGF, or RGD for improved sensitivity and specificity.

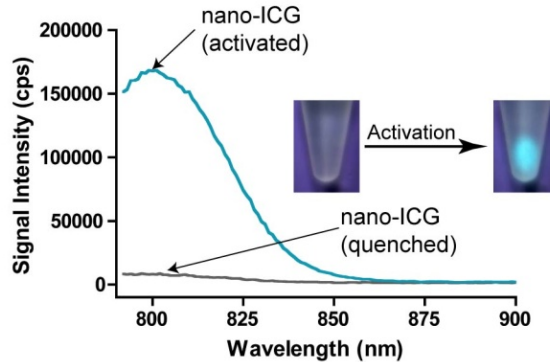


Figure 13 Optical properties of nano-ICG, a new class of biodegradable and self-assembled particles with physically trapped indocyanine green (ICG) molecules. In this type of "nano-ICG" imaging agent, the ICG fluorescence is quenched in the trapped state, and is activated when the dye is released under *in vivo* conditions (reprinted with permission from Mohs *et al.*, 2010, Copyright, American Chemical Society).

Milestones

3 year:

- Generate polymer-coated nanoparticles using current FDA-approved fluorescent dyes for residual tumor and metastases labeling. Incorporate tumor-targeting ligands for increased sensitivity and specificity.
- Develop novel nanoparticle imaging dyes that are not subject to photobleaching with targeting moieties to differentiate tumor from normal tissues and precisely delineate tumor margins.

5 year:

- Study *in vivo* toxicity in model organisms.
- Begin clinical trial evaluation of the most successful nanoparticles, coupled with minimally invasive delivery procedures.

10 year:

- Commercialize several targeted nano-imaging particles.

“Real-time” tomosynthesis image guidance for radiation therapy

Utilizing the distributed x-ray source array technology, Siemens and XinRay Systems developed a high-speed tomosynthesis scanner to provide real-time image guidance for radiation therapy (Maltz *et al.*, 2009). The development won the team the 2010 Sorokin Award from the American Association of Physicists in Medicine. The technology will enable the oncologists to “see” tumors in real time during treatment and will allow more accurate radiation delivery. The scanner has been integrated with the Siemens Artiste treatment system. It is currently under testing at the UNC Cancer Hospital. Clinical tests are scheduled for this year and Institutional Review Board approval has already been obtained.

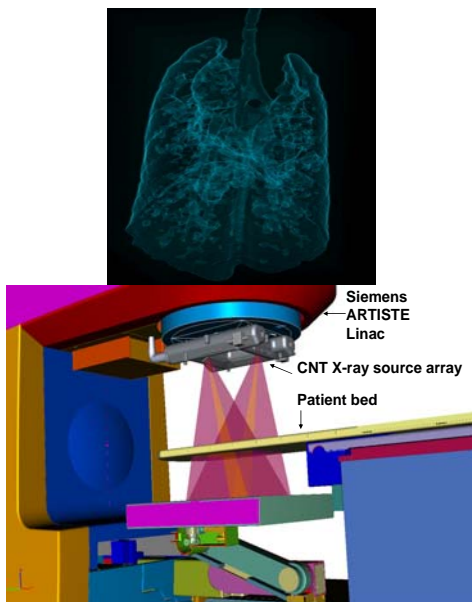


Figure 15 Prospective-gated micro-CT image of a mouse lung tumor model (top; UNC data. Mouse model from Dr. B. Kim). An illustration of CNT x-ray source array mounted on a radiotherapy machine (bottom; image courtesy of J. Maltz of Siemens and P. Lagani of XinRay).

Digital tomosynthesis for early stage detection of human breast tumors

Digital breast tomosynthesis (DBT) has the potential to become the next generation screening tool for breast cancer, replacing the current two-view mammography scanners. This limited-angle tomography technique provides quasi 3D views of the breasts which help radiologists differentiate breast tumor from the surrounding tissues. Utilizing the spatially distributed nanotube x-ray source array technology, a proof-of-concept stationary DBT scanner increases the imaging resolution,

improves the detectability of micro-calcification, and reduces the imaging time which reduces the patient discomfort from breast compression, compared to the rotating DBT scanners from commercial vendors that are currently under clinical trials for FDA approval (Qian *et al.*, 2009). Encouraged by the initial results a second generation, clinical test ready, scanner is currently under development which will integrate the nanotube x-ray source with a commercial mammography scanner.

Future challenges

From the engineering perspective, the reliability, consistency, and lifetime durability of the devices need to be demonstrated to be comparable or even better than the existing systems before they can be adapted in the clinics. Since imaging and radiotherapy devices are complicated, new device development requires a large multi-disciplinary team with complementary expertise in a wide range of fields as well as close collaborations with industry. The question as to how to organize and finance the research and development effort is always a challenging one.

Clinical potential

Recent research has clearly demonstrated the potentials of the nanotube x-ray based systems for clinical *in vivo* cancer imaging and radiation therapy applications. Some examples include early detection of breast cancer, image guidance for radiation therapy, and novel radiotherapy techniques.

Milestones

3 year:

- Develop stationary tomosynthesis scanners for applications such as breast imaging and image-guided radiation therapy and conduct clinical tests.
- Commercialize imaging systems for small animal models.

5 year:

- Develop microbeam radiation therapy using the nanotube x-ray source array technology for small animal models.
- Conduct studies of their therapeutic effects on small animal brain tumor models.
- Commercialize tomosynthesis imaging systems.

10 year:

- Develop a new generation of CT scanners based on this technology and utilize it in radiotherapy for human patients (for example, microbeam radiation therapy).

Nanotechnology and Cancer Prevention

Sara S. Hook

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Patient prevention strategies

There are several possible approaches to cancer prevention. Patients can decrease behaviors that put them at risk, be more vigilant in screening and surveillance, opt for surgical pre-intervention, and/or utilize “medicinal” approaches. The latter three areas in particular can benefit from the advances that nanotechnology can offer.

It is well recognized that several factors contribute to and enhance cancer prevention including dietary and lifestyle changes. The field of epidemiology has long been examining what types of risk factors are correlated with certain types of cancers. For instance, probably one of the best documented and most studied behavioral risk factors is that smoking increases the incidence of lung cancer. In fact, smoking also greatly increases the risk of many types of cancers as well as heart attacks (Khan *et al.*, 2010). A second well documented example is increased exposure to UVB rays from sunlight clearly damages DNA and can result in an increased risk of various types of skin cancer including the most deadly, melanoma (Cooper and Bowden, 2007).

Patients themselves can also implement mechanisms of surveillance. This would include performing breast self-exams to detect lumps and nodules, monitoring the skin for changes in moles, and seeing a doctor for routine physical exams. For those with a family pre-disposition to cancer, additional monitoring may be in order. For instance, patients who have a primary relative such as a mother or sister with breast cancer might want to undergo genetic testing to determine whether they are carriers of the familial breast cancer susceptibility genes, *BRCA1* and *BRCA2*. Additionally, imaging such as mammography has played an important role in screening at risk women and those over 40 for breast cancer. The areas of diagnostic imaging and molecular *in vitro* screening are areas in which nanotechnology can play a significant role. For example, Dr. Otto Zhou’s group at UNC-Chapel Hill is developing a stationary digital breast tomosynthesis scanner using carbon nanotube (CNT) multi-pixel field emission x-ray (MBFEX) technology. This approach will increase image resolution and decrease both patient discomfort and radiation exposure times (Qian *et al.*,

2009). The advances and future challenges in cancer imaging have been outlined in several previous sections. Likewise, *in vitro* genomic and proteomic testing strategies based on nanotechnology, such as those outlined earlier in this document, can be more sensitive, more cost effective, more rapid, and possibly more accurate than technologies currently in clinical use. Surgical intervention for “pre-cancerous” lesions detected during routine colonoscopies, or prophylactic breast, ovary or complete hysterectomies for patients at high risk for reproductive cancers likely also play a role in primary and secondary cancer prevention. As previously discussed in other sections, nanotechnology offers the physician increasing ability for image-guided surgical resection of tumors and possibly also pre-cancerous lesions. In fact, one example of this is from Dr. Sanjiv Sam Gambhir’s research group where they have used single-walled carbon nanotubes (SWCNTs) combined with Raman imaging to visualize tumors in live small animal models (Keren *et al.*, 2008). They are pursuing applications for this technology such as clinical colonoscopy and have already built a flexible endoscope capable of Raman imaging.

“Medicinal” prevention strategies

Many might hope that one day cancer could be prevented using some type of vaccine or pill to ward off the disease. The etiology, however, makes this a huge task due to the myriad of mechanisms by which the disease arises, the ability of cancer cells to escape immune system detection (due to recognition as “self”), the tissue specificity of some tumor types, the altered cellular growth and metabolism pathways, etc. Thus the concept of medical prevention in terms of vaccines and drugs is extremely challenging.

There are strong indications that avenues of medical prevention of cancers may be successful. One approach that is very promising is in the area of human papillomavirus (HPV) vaccines to prevent genital warts and hopefully also cervical, vulvar, and vaginal cancers. Two FDA approved vaccines, Cervarix (GlaxoSmithKline) and Gardasil (Merck), are recombinant versions of virus

has shown that part of the cancer progression phenotype is chronic inflammation (Grivennikov and Karin, 2010). Quite a number of natural products have been shown to decrease inflammation but in almost all cases, the bioavailability of these compounds is limited. Thus, nanoparticle delivery of such agents as curcumin, green tea polyphenols, coenzyme Q, etc. could be very useful. For example, a catechin, epigallocatechin-3-gallate (EGCG) found in green tea, has chemopreventive potential for human breast, pancreatic, colon, esophageal, and lung cancers, but its oral absorption rate is only 1% (Nair *et al.*, 2010). Consequently, more than 5 cups of green tea would need to be consumed for a health benefit (Johnson *et al.*, 2010). Nanoparticle delivery of EGCG then would be beneficial. In fact, the formulation of EGCG into PLA-PEG nanoparticles offered a more than 10 fold decrease in the IC50 over free EGCG when monitoring tumor cell viability (Siddiqui *et al.*, 2009). EGCG can inhibit tumor cell growth and decrease angiogenesis in mouse xenograft models (Siddiqui *et al.*, 2009) on its own but it can also sensitize tumors to growth inhibition by other agents such as interferon- α 2b (Nihal *et al.*, 2009). In addition to this compound's anti-inflammatory properties, it also decreases signaling of several kinase pathways, insulin-like growth factor, and androgen receptor signaling. In fact, clinical studies in men with prostatic intraepithelial neoplasia (PIN), a pre-cancerous lesion of the prostate, revealed a 90% reduction in the progression to prostate cancer when taking EGCG containing supplements (Bettuzzi *et al.*, 2006). Additional studies have, however, indicated that the controlled formulation of nutritional supplements is quite important for biologically efficacious effects (Johnson *et al.*, 2010). Green tea catechins are just one example within many that are being evaluated for their chemopreventative potential in similar research studies (Nair *et al.*, 2010). Although a great deal of discussion was devoted to natural products, researchers could also build upon these chemical structures using rational drug design approaches to improve upon what nature has given us.

The main focus here has been on prevention meaning before malignant growth has started. Confirmation of whether a compound has this potential is usually through prospective studies where patient cohorts are followed over a long period of time to correlate behavioral risks with cancer development. Neutraceuticals can, however, have an impact with other chemotherapeutic agents even after malignancy has been diagnosed to enhance the effectiveness of these treatment regimes (Mehta *et al.*, 2010). As depicted in Figure 16, neutraceuticals can by a vast variety of mechanisms feed into the processes of apoptosis, cell cycle arrest, DNA repair, protection against free radicals, etc., all processes known to be important in preventing cancer formation. Thus, future research will undoubtedly include an increasing focus not only on neutraceutical effects by themselves but also in combination with other therapeutic strategies.

Milestones

3 year:

- Publish more studies on characterizing natural products and their chemopreventive potential.
- Develop nanotechnology delivery systems for neutraceuticals and other chemopreventive agents.
- Carry out more prospective studies to identify genetic, behavioral, and environmental risks for various types of cancers.

5 year:

- Incorporate natural products with more standard therapeutic approaches in an increasing number of clinical trials.
- Conduct rational design experiments to improve on the potential therapeutic effects of existing neutraceuticals.
- Identify other potential targets for cancer vaccine development.

10 year:

- Follow-up studies with patients vaccinated with HPV vaccines will reveal whether they actually decrease the development of cervical, vulvar, and vaginal cancers.
- Develop nanotechnology mechanisms to limit exposure to environmental toxins.

NCI's Nanotechnology Characterization Laboratory

Scott E. McNeil

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Mission

The NCI's Nanotechnology Characterization Laboratory (NCL) provides infrastructure support to NCI's Alliance for Nanotechnology in Cancer. The lab's mission is to provide pre-clinical characterization to its sponsors, and to accelerate the translation of promising nanotechnology-derived cancer treatments into clinical applications. The NCL was founded in 2004 as a formal interagency collaboration among NCI, NIST, and the FDA and is operated through the NCI's Federally Funded Research and Development Center (FFRDC) at SAIC/NCI-Frederick.

NCL has a number of key objectives which include characterizing nanoparticles using standardized methods and conducting structure activity relationship (SAR) studies to identify and delineate critical parameters related to nanomaterial pharmacological properties and toxicology. Additionally they facilitate the regulatory review of nanotechnological constructs and engage in educational and knowledge sharing efforts.

The NCL's services are available for free to researchers developing a nanotechnology cancer therapy or diagnostic. Nanomaterials accepted by NCL are subjected to a three-tiered Assay Cascade of scientific tests, including physico-chemical characterization, *in vitro* assessment, and *in vivo* evaluation for safety and efficacy. The data generated from the NCL characterization are intended for use in support of IND or Investigational Device Exemption (IDE) applications to the FDA. As such, the NCL serves as a bridge to take promising cancer nanotechnology research to regulatory approval.

Achievements

In just six years of operation, the NCL has become a recognized authority in nanotechnology for biomedical applications. The Lab has over 50 collaborations with researchers from academia, industry, and government and has characterized almost 200 different nanomaterial samples – including liposomes, metal colloids, dendrimers, polymers, quantum dots, metal

oxides, and fullerene derivatives. Multiple NCL collaborators have now submitted an IND or IDE application and one collaborator has begun Phase II clinical trials.

Lessons learned

One of the ways that the NCL contributes to the Alliance and to the nanotechnology research community in general is by sharing the observations made in its Assay Cascade. Investigators benefit from these “Lessons Learned” thus accelerating the progress of the entire community.

Stability and Scalability. The Lab now has several examples where stability issues negatively impact the rapid development of nanoparticle-based therapies. Particles that release their payload within seconds to minutes of administration offer minimal advantage over traditional small molecule drugs. On the other end of the stability spectrum are nanoparticle formulations that are *too* stable – that is, the drug is not released from the nanopatform and is generally ineffective. In the case where drugs are covalently linked to the carrier, it is essential that this linkage is cleavable or otherwise degradable by the intracellular environment. Scale-up is also a common hurdle in the development process. In the case of nanoparticle formulations, early-stage planning can easily circumvent obstacles in this path to commercialization. An obvious example of this pitfall is found in the misunderstanding that academic studies are simply smaller versions of large-scale production.

Sterility. Another problem common to small-scale synthesis is contamination. Academic labs often use glassware not dedicated to aseptic procedures, and generally do not utilize “best practices” to prevent endotoxin contamination. On numerous occasions, investigators have submitted material to the NCL that is rife with endotoxin or other microbial contamination. This type of contamination severely impedes *in vitro* and *in vivo* studies, as it perturbs cell signaling pathways and may induce an immune response.

allow the NCL to leverage such resources without great expense.

- Establish collaborations with industry to reformulate discontinued cancer drugs using nanotechnology.

5 Year:

- Increased interaction with Contract Research Organizations (CROs) will facilitate the scale-up process and transition to GMP manufacturing. NCL will endeavor to make contacts at the CROs that have experience with nanoparticle formulations and to increase our visibility to these organizations.
- Devise analytical methods to differentiate nanoparticle-bound vs. free drug. To support regulatory review of nanoformulations, analytical methods that can determine the free, and therefore “active” drug component of a nanoparticle drug profile are needed.

10 year:

- As NCI’s Alliance moves into its second iteration and the nanotech concepts submitted to the NCL continue to mature, the NCL’s relationship with the FDA will necessarily evolve as more NCL-characterized concepts enter the regulatory process. Specifically, we expect increased interaction with FDA reviewers. NCL will continue to seek input from the FDA on its assays and to collaborate with the FDA on the regulatory aspects of nanotechnology and SAR studies. In 10 years, NCL aims to facilitate three to five IND filings.

Safety Issues in Pre-clinical and Clinical Evaluation of Nanotechnology-based Products

Subhas Malghan and Carlos Pena

U.S. Food and Drug Administration

Nanotechnology allows scientists to create, explore, and manipulate materials in the nanoscale range. Behavior of such materials in terms of chemical, physical, and biological properties may differ from those of their larger counterparts. A general finding of the “FDA Nanotechnology Task Force Report 2007” is that nanoscale materials present regulatory challenges similar to those posed by products using other emerging technologies. However, distinct challenges may also arise because at the nanoscale, properties of a material might change in ways that could affect the performance, quality, safety and/or effectiveness. While applications of nanoscale materials in cancer treatment are continuing to evolve, one needs to consider the potential unintended health impact of these materials. One reason for this potential is that some of these materials will eventually come into contact with biological structures and processes that frequently occur at the nanoscale.

Understanding interactions of nanoscale materials with biological systems

To assess the interaction of nanoscale material with biological surfaces, reliable and reproducible screening methods are needed. Achieving this goal has become a challenge because of the large variety of new nanoscale materials that are under development, their unique set of novel physico-chemical properties, and uncertainty of how those properties relate to biological outcomes. There is a possibility of a vast number of physico-chemical interactions with biological surfaces when nanoscale materials of different size, composition, shape, surface area, aggregation, crystallinity, surface coating and functionality, and hydrophilic/hydrophobic interactions come in contact with biological fluids,

proteins, lipids, DNA, cell membranes, lysosomes, mitochondria, and biological processes (Nel *et al.*, 2009). Therefore, a comprehensive physico-chemical characterization as well as pharmacokinetic and biodistribution studies are required to evaluate safety as well as efficacy. Currently, there is considerable discussion on nanomaterial toxicity testing, with the major discussion centering around which toxicological end points to screen for, the adequacy of the screening effort, and the correct balance of *in vitro* (cellular and molecular) versus *in vivo* (animal or whole organism) testing (Oberdorster *et al.*, 2005; Borm and Berube, 2008; Nel *et al.*, 2009). Attempts to use traditional toxicological assays and models have resulted in conflicting and sometimes irreproducible results.

Additional important questions exist concerning the transport of nanoscale particles in the human body and mechanisms of interaction at the sub-cellular and molecular levels. The unique and diverse physico-chemical properties of engineered nanoscale materials suggest that their toxicological properties may differ from materials of similar composition but larger size. Studies also suggest that particle size, surface area, and surface chemistry of engineered nanoscale materials can impact toxicity equally, if not more so, than chemical composition (Nel *et al.*, 2009). Research is in progress to evaluate toxicity of nanoscale materials that represent a cross-section of composition, size, surface coatings, and physico-chemical properties. Many of these studies are designed to investigate fundamental questions concerning how nanoscale materials are absorbed and distributed *in vivo* and whether they can adversely impact biological systems. More studies are needed to detect and quantify nanoscale particles in tissues, mechanisms of nanoscale material absorption, distribution in the body, and subsequent up take by cells. These studies have the potential to develop a better understanding of biological and toxicological interactions.

Different uses may have different requirements with regard to nanoscale material

While biocompatibility and toxicity would be important for devices, absorption, distribution, metabolism, and excretion are relevant in the evaluation of safety of nanoscale materials contained in drugs. Concepts that have been applied in the micron size range may be usefully applied to the nanoscale range, but new challenges are presented based on the small size and possible change in the dissolution-translocation relationship (Nel *et al.*, 2009). Solute concentration, surface area, surface morphology, surface energy, dissolution layer properties, adsorbing species, and aggregation are some relevant parameters when considering dissolution at the nanoscale. With regard to the etiopathology caused by nanoscale particles, the metrics of dose (particle number, surface area, mass or shape) is not yet well defined. Analytical procedures for assessing dissolution and translocation include chemical assay and particle characterization. Leaching of components from particle surfaces as well as compartmentalization within the respiratory tract may add another dimension of complexity. Dissolution may be a critical step for some nanoscale materials in determining their fate within the body. An integrated approach combining particle toxicology, material science, and analytical chemistry is required to provide a useful basis for developing relevant dissolution assay(s) for nanoscale particles.

Studies have indicated that various attributes of a particular nanoscale material, including increased specific surface area, morphology, surface features, and charge, can affect the distribution of that material in the body, that material's toxicity, and/or its biocompatibility. In addition, current testing approaches may need to be evaluated and new approaches developed to assess safety, effectiveness, and quality of a product that uses a nanoscale material.

A conclusion of some studies in this area is that current risk assessment methodologies require some modification to address hazards associated with nanoscale materials and in particular that existing toxicological and biocompatibility methods may not be sufficient to address all issues related to nanoscale particles. For exposure evaluation, dose determination requires information on the number of nanoscale particles and/or their surface area in addition to the traditional mass concentration characterization. Equipment for routine measurements in various media for representative exposure to free nanoscale particles is inadequate. In addition, existing assessment methods may not be appropriate to determine the fate of nanoscale particles. While an understanding of general risks of products using nanoscale materials is continuing to evolve, there is greater need for understanding the risks of free or "unconjugated" nanoscale materials because they are likely to behave differently from the same material/compound in a complex nanoparticle which may result in altered biological and toxicological behavior. Nanoscale materials may exhibit unique physico-chemical

properties due to surface coatings or other nanotopographical features.

Summary

Inclusion of a nanoscale material in an FDA-regulated product or a change in the nanoscale material(s) used may affect the quality, safety, and effectiveness of that product and may raise questions regarding appropriate testing methods. Accordingly, additional data and testing methods may be needed for assessing the effects of a nanoscale material on a product, whether subject to premarket authorization or not (FDA Nanotechnology Task Force Report 2007). In some cases, the presence of a nanoscale material may also affect the regulatory requirements applicable to a product.

The FDA is available to assist manufacturers and sponsors in identifying and addressing regulatory issues raised by specific uses of particular nanoscale materials, including issues with regard to safety, effectiveness, good manufacturing practices, and possible changes in the regulatory classification or pathway for product approval. Both research and development groups are encouraged to contact the FDA to discuss the proposed use of specific nanoscale materials in an FDA-regulated product even if no legal requirement to notify the Agency applies.

Regulatory Aspects Related to Products Containing Nanoscale Materials

Subhas Malghan and Carlos Pena

U.S. Food and Drug Administration

The FDA regulates a broad range of products under the Federal Food, Drug, and Cosmetic Act (FFDCA) and the Public Health Service Act (PHS Act). The Agency's statutory authorities subject some types of products to premarket authorization requirements, either individually or by category, while permitting other products to be marketed without prior Agency authorization (FDA Nanotechnology Task Force Report 2007). The term "premarket authorization" refers to a number of regulatory actions that the FFDCA, the PHS Act, and agency regulations may refer to by other names, including "approval," "clearance," "licensing," and "listing." Most, if not all, laws and regulations under which the FDA operates are by design general in nature. Therefore, the agency's authorities usually are able to accommodate products made with the use of emerging science, new technologies, or containing new kinds of materials. The use of nanoscale materials in an FDA-regulated product may raise questions regarding which regulatory requirements apply and how they can be satisfied. Nanoscale materials are of particular interest to the FDA, since there is significant potential for their application to a large number of products regulated by the FDA. Nanoscale materials can have physical or biological properties that are different from those of their larger counterparts because of their small size and high specific surface area. Such differences may include altered magnetic properties, altered electrical or optical activity, increased structural integrity, or increased chemical or biological activity. Because of some of their special properties, these materials may present different safety and efficacy issues than their larger counterparts.

Medical products

Drug products (FDA Nanotechnology Task Force Report 2007): New drugs for humans, as well as new

animal drugs, are subject to premarket authorization on a product-by-product basis. Information on the identity of products such as the type of product, the size of the components, and the manufacturing protocol is required as part of marketing applications if it is relevant to safety or effectiveness. In the case of replacing a current drug substance or excipient with a nanoscale version, the resulting product may be considered a new product for which a new approval would be needed.

Biological products (FDA Nanotechnology Task Force Report 2007): With regard to human cell and tissue products that might otherwise be subject to regulation only under section 361 of the PHS Act and, therefore, not subject to premarket authorization, we encourage manufacturers to contact the FDA before marketing any version that incorporates nanoscale materials or is otherwise modified at the nanoscale, to confirm whether these features trigger premarket authorization requirements.

Devices (FDA Nanotechnology Task Force Report 2007): Medical devices are regulated according to a tiered classification system that is largely based on the degree of risk posed by the product. Devices that are low risk, for which safety and effectiveness are generally well-established, are designated as Class I devices. These device types are subject to general controls, such as labeling, good manufacturing practices and adverse event reporting. Class II devices are more complex and carry a higher risk than Class I devices. For certain Class I devices and most Class II devices, manufacturers must submit to the FDA a premarket notification to demonstrate that their device is as safe and effective as another legally marketed device in order to obtain FDA clearance before marketing. Class III devices are the most complex, high risk devices and are reviewed under a premarket approval application (PMA). In a PMA, pre-clinical and clinical data, in addition to manufacturing information, are typically used to support the agency's determination that the device provides a reasonable assurance of safety and effectiveness.

Nanoscale material manufacturing issues

Products regulated under the FD&C and PHS Acts must be manufactured to conform with applicable requirements concerning, for example, safety, quality, and purity, and so as to avoid being adulterated. Some are additionally subject to current good manufacturing practice requirements (FDA Nanotechnology Task Force Report 2007). In some cases, the use of nanoscale materials in the development of an FDA regulated product may raise new safety issues that require new or different testing methods. Since there may be some uncertainty in the use of nanoscale materials and its impact upon such products, questions regarding safety may not be specifically addressed in existing guidance. Accordingly, manufacturers may have questions regarding how to ensure sound manufacturing practices for products that use nanoscale materials and they are encouraged to consult with the relevant FDA product center to ensure that new technologies do not present any new safety issues.

Contact FDA

There is a possibility that the presence of certain nanoscale materials used in the manufacture of medical products may affect the safety or effectiveness. Therefore, we encourage applicants to clearly indicate in regulatory submissions the presence of nanoscale materials.

If you are considering using a nanoscale material in your product, contact the FDA to confirm whether the product contains nanoscale material by FDA standards. In addition, the FDA should be contacted to discuss appropriate manufacturing practices and developing testing methods for assessment of product safety, effectiveness, and quality. Communications with the FDA regarding new nano-products will help ensure compliance with all legal obligations and will help the FDA to regulate products effectively and to address regulatory and patient safety issues proactively and efficiently. Following these recommendations will minimize delays to market entry and avoid evoking enforcement authorities to protect the public health.

Clinical Translation of Nanotechnologies: From Academic Laboratory to Start-up Company

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Developing a successful model of translation

At the highest level, the key elements to successfully translating a technology from academic research into clinical development are: technology, team, innovation and financing. These basic elements hold true for any start-up company, but even more so for the field of cancer nanomedicine given the challenges, complexities, and consequences of optimizing nano-scale technology for the treatment of people suffering from cancer.

When a start-up company is founded based upon academic research, the initial scientific efforts focus on the transfer of the technology from the academic labs into the hands of the company to develop an in-depth understanding of the technology's strengths, weaknesses, and potential when viewed from the very different lens of drug development. From the outset, the regulatory requirements dictated by the FDA for pharmaceutical development of the drug product candidate (as discussed previously) must be taken into consideration along with the pharmaceutical development considerations of product candidate optimization through rigorous pre-clinical evaluation, development of appropriate and robust analytical characterization methods, and of critical importance, manufacturing process development and scale up. The optimization approach requires evaluation of nanoparticle performance using *in vitro* cell-based assays (particle binding interactions, uptake and toxicity, drug activity), *in vivo* pre-clinical evaluation (PK, biodistribution, targeting, tolerability/toxicity, efficacy) as well as several CMC (Chemistry, Manufacturing, and Controls) requirements mandated by current good manufacturing practices (cGMP) and the FDA. These requirements assure among other things batch to batch reproducibility and shelf-life stability based on testing a variety of properties (particle size, drug content and purity, drug release rates, targeting ligand content and activity [if applicable], stability of nanoparticles and drug under storage and in-use conditions). Through the course of pharmaceutical

development, the CMC requirements become more stringent; however, it is at this early stage where the company first begins testing these critical parameters.

Innovation and financing are the remaining key elements for successful clinical translation. Not all technologies are created equal, so matching your technology to the right drug and indication and the required technical and clinical innovation to make it happen are critical. A start-up company cannot afford to get it wrong with their first product candidate, as second chances are very difficult to come by. Financing is extremely challenging, with venture capital being the most common funding mechanism for start-up companies. Economic climate has strong impact and over the last few years venture funding has been extremely competitive and sparse making it very challenging to raise the capital required to fund the significant early development costs for pre-clinical testing, GLP pharm/tox studies, process scale-up and GMP clinical drug product manufacturing. Unfortunately, government funding of start-up companies is also quite limited and extremely competitive, often with grant opportunities pitting academic research and start-up early development as competitors in what can be difficult projects to fairly assess against one another given their potentially very different scope and goals. Ironically, venture and government funding are sometimes at odds with each other. If one assumes that venture firms will often fund the most promising companies, then these companies are typically ineligible for SBIR funding, which limits the government from providing additional key funding to reach the clinic.

The two most notable nanotechnology-based drugs are DOXIL[®] (PEGylated-liposomal doxorubicin, approved in 1995, developed by SEQUUS) for the treatment of ovarian cancer and ABRAXANE[®] (albumin-bound paclitaxel, approved in 2005) for the treatment of metastatic breast cancer. DOXIL is more potent than doxorubicin and decreases cardiac-related side effects whereas ABRAXANE eliminates the use of the toxic excipient cremophor, allowing a higher dose of paclitaxel. Despite these successes, several nanotechnology start-up

companies have struggled to navigate the clinical translation of their technologies with process scalability and lack of robust analytical characterization leading to some failures, while other companies have appeared to match either the wrong drug or cancer indication with their technology resulting in disappointing clinical outcomes.

Future steps

An exciting opportunity for the future of nanomedicine is the targeting of nanoparticle drugs to specific disease cells through specific binding interactions between ligands on the nanoparticle surface and cell surface receptors present only on or at highly upregulated levels on cancer cells or tumor neovasculature. As is the case with DOXIL, this approach will also require optimization of particle characteristics to take advantage of the enhanced permeability and retention effect to allow for particle circulation in the bloodstream and extravasation through the irregular tumor neovasculature. It is the added impact of the specific nanoparticle binding as well as potential nanoparticle and drug uptake to provide intracellular delivery that offers very exciting possibilities. Early leaders in this area are Calando, which has recently reported early clinical data for their transferrin-receptor targeted siRNA demonstrating dose-dependent accumulation of drug in the melanoma cancer target tissues as well as BIND Biosciences which intends to initiate clinical studies for their prostate specific antigen-targeted docetaxel in multiple solid tumor indications in 2010.

In order to drive these promising nanomedicine technologies and others into clinical development it is essential to build start-up teams that possess the right dynamics. Having the appropriate skills is an obvious requirement, so that the team of scientists, engineers, clinicians and management are equipped to do the job. Early stage drug development presents many obstacles, so recruiting people who have experienced the challenges, failures and successes puts the company in an excellent position. From a culture perspective, individually and collectively, there must be a tremendous work ethic and enthusiasm, a willingness to put the team goals as top priority knowing that if the team wins individuals will win. There also needs to be an understanding that they are facing a marathon and not a sprint with respect to the number of achievements and time required to accomplish the ultimate goal of treating patients with cancer.

Training Programs in Cancer Nanotechnology: Preparing the Next Generation of Researchers and Clinicians

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Introduction

An important consideration when contemplating the potential that nanotechnology holds to treat cancer and other diseases is how we can best train and educate our young people to meet the challenges of doing research, establishing start-up companies, translating knowledge to the clinic and the like. Harnessing the power of nanomedicine will require scientists and clinicians with inter- and multi-disciplinary training in key aspects of chemistry, physics, biology, medicine, computer science, engineering, and clinical sciences. Interdisciplinary science requires a departure from a parallel-processing model in which individual investigators worked alone. The best scientists in nanomedicine will not be experts in all fields of research, but they will comprehend the role each discipline plays and will competently communicate across fields to achieve better solutions. As most scientists are not trained in an interdisciplinary fashion, it is imperative to develop training programs in nanoscience that fulfill the goals of offering interdisciplinary nanoscience courses and research experiences where trainees will learn many aspects of nanoscience, with a focus on one particular area in this discipline.

The worldwide workforce necessary to support the field of nanotechnology is estimated at two million by 2015 (http://www.nano.gov/html/edu/home_edu.html). Questions arise as to how the U.S. educational system can train technicians, scientists, and clinicians, and how to assure that the students choose the appropriate educational path. Raising awareness and educating K-12 school children hopefully prompts students to study nanoscience at the undergraduate and graduate levels. Formal, didactic degree programs for undergraduate students, as well as strong graduate education and research in nanomedicine are also essential. There are currently many educational programs in nanotechnology at all levels of training, from K-12 to postgraduate experiences. However, the vast

majority of the educational programs in place focus on the materials science and engineering aspects of the field. We should encourage programs that combine the physical sciences/engineering aspects with biology and/or medicine to foster the groundbreaking discoveries in the chemistry and materials fields that can be applied towards life-saving cancer treatments.

Current status

The field of nanotechnology has grown exponentially over the past 10 years, in part through government initiatives. The National Nanotechnology Initiative (NNI) was established in 2001 to coordinate Federal nanotechnology research and development. Today the NNI consists of the individual and cooperative nanotechnology-related activities of 25 Federal agencies with a range of research, regulatory roles, and responsibilities

(http://www.nano.gov/html/about/home_about.html). The NNI does not fund research; however, it informs and influences the Federal budget and planning processes. One of the key goals of the NNI is to “Develop and sustain educational resources, a skilled workforce, and the supporting infrastructure and tools to advance nanotechnology.” The Education Center on the NNI website provides information on K-12 activities as well as listings of undergraduate and graduate programs in nanotechnology.

Resources for teaching nanotechnology to K-12 children

Several websites have nanoscience resources for classroom teachers and students’ families including the The

National Science Foundation (NSF) (<http://www.nsf.gov/news/classroom/nano.jsp>), The Nanobiotechnology Center (<http://www.nbt.cornell.edu/>), Rice University (<http://nanokids.rice.edu/>), and the University of Albany (SUNY) College of Nanoscale Science and Engineering (http://cnse.albany.edu/Nano_for_Kids/K_12_links.html).

In addition to web-based resources several other resources for hands-on experience for youth are also available. The Nanobiotechnology Center sponsors such things as field trips for middle school children to learn about scanning electron microscopy and visits to the Strong Museum in Rochester, NY. Likewise the Nanoscale Informal Science Education (NISE) Network has sponsored NanoDays since 2008. NanoDays combine simple hands-on activities for young people with exploration of current research for adults at over 200 science museums, research centers and universities across the country. Through the Program of Excellence in Nanotechnology (PEN) and the Siteman Center of Cancer Nanotechnology Excellence (CCNE) at Washington University, researchers are participating at NanoDays at the St. Louis Science Center by hosting two booths with hands-on activities.

Undergraduate training

Currently, several community colleges working with larger universities offers Associate degrees in Nanotechnology. For instance, the University of Pennsylvania collaborates with Pennsylvania community colleges to offer an Associate degree in Nanobiotechnology. Dakota County Technical College (Rosemount, MN) in conjunction with the University of Minnesota offers an Associate degree in Applied Science in Nanoscience Technology. The North Seattle Community College offers an Associate of Applied Science-T degree in nanotechnology.

At this time, there are no advertised bachelor's degree programs in nanoscience. However, there are several institutions that offer either a minor or a concentration in nanoscience or related discipline. At the University of Texas at Dallas, undergraduates can minor in nanoscience by taking three core NANO-designated courses, the content of which is exclusively related to nanoscience and nanotechnology. Yale University has an undergraduate minor in nanotechnology, where students are required to take an Introduction to Nanotechnology course and five other courses from a selection of engineering and biotechnology electives. Neither of these undergraduate minors require courses related to biology or medicine.

At the University of Wisconsin-Stout, students can obtain a B.S. in Applied Science with a Nanoscience concentration, and a B.S. in Engineering Technology with a concentration in Nanotechnology. Michigan Technological University offers an interdisciplinary minor in Nanotechnology. Several institutions have courses on nanotechnology, targeted towards either undergraduates or graduate students, including Cornell, Florida Institute of Technology, George Mason University, Rice University,

University of Central Florida, University of Maryland, University of Texas at Austin, University of Washington, Washington University, and University of Wisconsin.

The majority of these programs emphasize the area of the physical sciences and engineering. There is definitely a need to see more education in nanoscience that incorporates biology and medicine, which will provide a larger pool of trainees for graduate programs, as well as provide a background for students studying medicine to have knowledge of how nanotechnology can be used to treat diseases such as cancer.

Graduate training

There are numerous institutions in the U.S. that train graduate students to do research in the area of nanotechnology, nanoscience, or nanomedicine. There are fewer universities that have formal programs that offer coursework and either a degree, certificate, or specialization. The majority of these programs are focused in the physical sciences and engineering, and there are few that combine the physical sciences and engineering with biology and medicine. One of the more innovative and interdisciplinary programs is at Northeastern University, where they have a Nanomedicine program funded by the NSF IGERT (Integrative Graduate Education and Research Traineeship) initiative and the NCI. There are over 20 faculty involved from Northeastern University, with collaborations with other Boston-area researchers and scientists from neighboring hospitals and industries. Students are enrolled in a Ph.D. program in Biology, Chemistry, Physics, or one of their Engineering programs, and then graduate with a specialization in Nanomedicine Science and Technology. This is one of the best examples of a graduate program that allows students to obtain an interdisciplinary education, learning the science and/or engineering, as well as the biomedical applications.

The University of Michigan has the Michigan Nanotechnology Institute for Medicine and Biological Sciences (<http://nano.med.umich.edu/>). This program has several talented scientists with expertise in fields ranging from chemistry, biology, medicine, and engineering. Students can earn a Ph.D. in a typical field of study and obtain a certificate in NanoBiology. Coursework is selected from biology, physical sciences, and engineering. The nanoscience courses appear to be explicitly in the areas of the physical sciences and engineering rather than incorporating biology and/or medicine. The University of Texas Health Science Center at Houston opened a Department of NanoMedicine and Biomedical Engineering in 2009 whose mission is "to introduce students to the field of Nanomedicine and the vast opportunities it provides for enhanced therapeutics, personalized medicine, medical diagnostics, imaging, screening, prevention, and regenerative medicine." This program is unique in that it is probably the only one that educates and prepares *medical* students to learn emerging new technologies in biomedical nanotechnology and engineering. Students are required to complete a scholarly research project and present the data at a scientific meeting, as well as prepare a manuscript to

obtain the certificate of completion. There are also journal clubs and other meetings, but at the time of this writing, there were no formal courses described on the website.

Clinical potential

For nanomedicine to reach its full potential, there needs to be more training centers like the ones at Northeastern, University of Michigan, and University of Texas Health Science Center at Houston. Having top-notch researchers in nanomedicine at institutions is obviously important for training the future scientists in the field. However, combining the research with didactic training will provide another level of skill for these future scientists and clinicians. Incorporating nanomedicine into medical student training will also ensure that these students understand how nanomaterials and nanodevices can be applied in medicine, particularly cancer treatments and diagnosis. Additionally, post-graduate training of research residents would also fulfill this role.

Obtaining the support of the NCI cancer centers in promoting nanotechnology education will also be key for future success. Of the Centers for Cancer Nanotechnology Excellence (CCNE) that were funded in 2005, the Siteman at Washington University had outreach and education cores that promoted education to medical specialists, the general public, as well as students at the K-12 through graduate levels. A course in Nanomedicine was offered yearly to graduate and undergraduate students. Outreach events to promote nanomedicine to the public at the St. Louis Science Center were also sponsored by the CCNE.

Future challenges

Federal grants have provided resources for the infrastructure of several educational programs in nanotechnology and have sustained them for the past decade or more. One of the challenges will be to maintain these programs when the funding expires, in particular the K-12 outreach programs. Many of these initiatives are for a limited time, are not renewable, and it is apparent that many programs have ceased over the past few years. Novel ways to maintain K-12 education in nanoscience, possibly through school teachers themselves, as well as alternative funding sources, such as private donors or foundations should be investigated. Encouraging universities and institutions that have strong nanotechnology research and education programs to engage in outreach activities to K-12 school children and the general public would be an inexpensive way to expand the awareness of nanotechnology and nanomedicine and increase the pool of future trainees.

One of the major concerns in undergraduate and graduate education in nanomedicine is that aside from the few programs described above, the vast majority of existing programs offering minors, certificates and/or specializations in nanotechnology are highly focused in the areas of materials science and engineering, with little or no emphasis on combining this with biology and/or medicine.

Some of the programs that are focused in the physical sciences and/or engineering are affiliated with strong medical schools and/or cancer centers, and these institutions should be encouraged to collaborate with the cancer biologists and oncologists in educating nano-scientists regarding these medical applications.

As the NNI funding initiatives phase out, funding of research in nanomedicine will likely continue and hopefully expand as the nano grants are submitted to NIH through the traditional mechanisms (e.g. R01, P01, etc.). Unfortunately, requesting funds for educational initiatives through these mechanisms is not allowed. Finding the resources to develop new educational programs in nanomedicine, or even maintenance of existing programs will be a significant challenge. For example, currently only the University of Texas Health Science Center at Houston has a program to train medical students in nanomedicine. Mechanisms for funding the development of similar programs at other institutions should be investigated.

Milestones

3-year:

- Encourage more universities with strong nanotechnology/nanomedicine programs to reach out to the general public and/or K-12 school children and/or their teachers.
- Sponsor a workshop on nanomedicine education, with sessions and panel discussions on education at all levels (general public, K-12 school children, school teachers, undergraduates, graduate students, medical students, and post-graduate education).

5-year:

- Three to five of the existing undergraduate minors/specialties in nanoscience will incorporate biology and medicine into their curriculum.
- An additional two to four graduate programs in nanoscience will add a focus on nanobiology and/or nanomedicine.
- Using the University of Texas Health Science Center at Houston's program for training medical students as a model, there will be one to two more of these programs offered at major universities.

10-year:

- There will be more medical students and graduate students graduating from existing and recently developed programs in nanomedicine, thus increasing the number of qualified scientists working in academia, industry and possibly even private medical practices.
- Due to advances in research and education in nanomedicine, there will be more nano-based agents approved for the diagnosis and/or treatment of cancer as well as other diseases.

Maximizing Research and Technology Development Effectiveness Through a Team Approach

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In order to develop effective devices and treatments for cancer using nanotechnology, the NCI recognizes that it is imperative for diverse professionals to unite toward this common goal. "Team science is about developing new ideas, forging new partnerships, and collaboratively using new tools to understand cancer as a disease process and a highly complex system," explained Dr. Anna D. Barker, the former deputy director of NCI. "The model includes teams of experts who can not only view the many elements of the cancer process, but can integrate that knowledge and design an innovative and targeted strategy of drugs, biologics, and even devices that can be used at all phases of the cancer process in an integrated fashion. Although the individual investigator will continue to drive innovation, the old model of cancer research taking place in isolated silos is fading away." As an illustration, chemists and engineers have the expertise to design and synthesize the best types of nanoparticles with physical properties that will solubilize drugs, RNAs, and proteins and ensure transport across the blood/brain barrier if needed. Meanwhile, the expertise of biologists and clinicians is imperative to know what tumor type to target, through which molecular mechanism, and which biological read-out to use to monitor the effectiveness of treatment. In 2004 the NCI established the Alliance for Nanotechnology in Cancer to foster this type of interdisciplinary collaboration. One of the avenues they used was to establish CCNEs through an open competition. These centers were lead by multiple program directors (PD) and primary investigators (PI) coming from the areas of medicine, biology, chemistry, physics, and engineering. The power of team science can best be illustrated by stories of the program participants themselves.

Dr. Dennis Carson, the director of the University of California, San Diego's (UCSD) Moores Cancer Center, had a vision for incorporating aspects of engineering into cancer research, and he knew there was significant talent and interested faculty at UCSD to carry out the large scale multi-disciplinary effort necessary to establish a CCNE. He

needed to identify a director at UCSD, however, who could lead this diverse talent to success. In consultation with Dr. Roger Tsien, UCSD's leading biochemist in the field of nanotechnology, and Dr. Andrew Kummel, a chemist and materials scientist very familiar with the engineering faculty, they quickly reached a bold and unusual decision. Their choice to lead the effort was Dr. Sadik Esener, a professor of Electrical and Computer Engineering and of Materials Sciences at the Jacobs School of Engineering with a strong expertise in electronics and photonics but surprisingly little involvement with cancer or nanoparticle research at UCSD. However, Dr. Esener had the key attributes required for successful leadership of the new center: (1) respect of his colleagues and proven success in running large scientific projects, (2) multiple successes in commercializing medically related chip-based technologies, (3) the ability to work with scientists of different backgrounds and personalities on their ideas, and (4) speed in learning new fields of science.

When Dr. Carson contacted Dr. Esener to ask him if he would agree to serve as the PI, Dr. Esener's first reaction was there must be a mistake. Dr. Esener was eventually won over and concludes, "Nothing comes close to the fulfillment one feels as a researcher to know that you are wrestling with a problem that if resolved would eliminate so much pain and suffering in the world. Although, I had some doubts before I accepted this position that entails tremendous responsibility, I am now so grateful to have been given this remarkable opportunity to bring a new perspective to this disease as a result of NCI's bold undertaking and Dennis' courageous decision. I cannot imagine how I could have been involved with leading edge cancer research without this center and the team science approach."

Since its inception, the Alliance program has demonstrated that multi-disciplinary teams can synergize to develop clinically translatable technologies and therapies for cancer. The research groups involved in the Alliance have published over 1000 research articles, generated 250

patent applications and disclosures, and started more than 30 companies by which the technologies will be developed and marketed. Currently, 10 clinical trials are ongoing using therapies that have been developed using funds from the program. These innovative technologies and therapies would not have been possible had it not been for the willingness of scientists from divergent fields coming together to lend their expertise, ideas, vision, and passion. With the recent renewal of the Alliance for Nanotechnology in Cancer program, there should be even more outstanding contributions to cancer diagnosis, imaging, treatment, and management in the years to come.

Interdisciplinary collaboration is critical to effectively train young scientists in the area of nanotechnology (as discussed in the previous section). Program efforts to foster a collaborative spirit in the first phase of the Alliance resulted not only in research projects and publications, but in exchanges of personnel and materials. This personnel exchange was particularly important for the program's training components, as numerous graduate students and postdoctoral researchers were able to use network connections formed at investigator meetings to establish their next positions. The next five years of the program, Phase II, has increased research training funding to include Cancer Nanotechnology Training Centers (CNTCs) and Pathway to Independence Awards in Cancer Nanotechnology Research (K99/R00). The funded CNTCs will target graduate student and post-doctoral researchers of broad background (in medicine, biology, and other health sciences as well as in the physical sciences, chemistry, and engineering). The program of multi-disciplinary research education in cancer nanotechnology will primarily focus on mentored training, usually from multiple investigators in different disciplines, through laboratory-based research projects. In addition, centers will offer both short courses and workshops as well as outreach experiences. Given the challenges more senior post-doctoral fellows face in finishing projects and establishing themselves as independent investigators, the program has invested in funding several Pathway to Independence Awardees. These trainees will benefit not only from their direct mentors but from the more informal mentoring and interaction at PI meetings across the Alliance.

The bread and butter of the program remain the CCNEs and CNPPs. The CCNEs of this new program edition will have a greater focus on clinically-worthy technologies as compared to Phase I. The new program will emphasize more heavily cancers having particularly poor outcomes, including brain, lung, pancreatic, and ovarian cancers. The science will continue to pursue basic discovery and innovation, but will also explore the clinical utility and translation development of the technologies. The collaborative effort then between the physical and basic scientists will be driven by those pressing questions facing clinicians. Collaborations benefit from complimentary skills, experience, perspective, and the use of diverse methodologies, as such the right mix of expertise is crucial for a highly effective interdisciplinary research team. When basic and physical scientists realize, for instance, that one of the important aspects of pancreatic tumorigenesis is the microenvironment, they can begin to address how to

develop interventions and therapies to intercede in relevant pathways. The "begin with the end in mind" approach can save valuable time and resources by honing in on the most profitable research direction, foreseeing possible roadblocks, and planning for alternate avenues. Likewise, it is important to consider what data is needed for pre-clinical testing and characterization of various nanoparticles and devices so that the proper experiments can be done early and the process of clearing institutional, legal, and regulatory hurdles may be initiated. It may be wise to seek the advice and guidance of institutional and federal regulatory bodies such as the FDA so that applications for INDs, IDEs, and patents will progress unhampered.

As part of NCI's commitment to clinical translation the NCL will continue to work with investigators as a hub for the pre-clinical characterization of nanomaterials and to assist in the process of bringing nanotechnologies to the stage of IND or IDE submission. The NCL has established protocols for bio-nanoparticle characterization and is currently expanding these protocols as well as working on others pertaining to GMPs such as scale-up process, purity, and batch-to-batch consistency. The lab will continue basic discovery and innovation, but it will also take great care in the evaluation of clinical utility of the technology and put strong emphasis on the translation.

The cross Alliance activity of the investigators can be enhanced by using the Alliance's Cancer Nanotechnology Laboratory (caNanoLab) where researchers and NCL are able to deposit, store, and retrieve nanoparticle characterization data. To date it has primarily been used to house *in vitro* data (physico-chemical properties and biological assays) and protocols but it is expanding to include *in vivo* characterizations of nanoparticles and their functional components. Data relating to the toxicity, pharmacokinetics, and ADME (absorption, distribution, metabolism, and excretion) in vertebrate animals will be collected. Another important aspect of caNanoLab is its contribution to nanotechnology ontology through standardizing vocabulary terms relating to the physical, chemical, and functional characteristics of nanotechnology.

The idea of data sharing usually makes scientific researchers uneasy. After all they have invested huge amounts of time and resources to generating this data. In addition, graduate students and post-doctoral fellows realize the importance to their graduate committees and careers of making an intellectual contribution to a project that results in several high quality, first author publications. However, it is important for trainees to recognize that they can obtain a significant benefit from working with a group of individuals to produce co-authored publications, promote idea exchange, and develop a network of colleagues within their field.

In order for effective data sharing to become a reality, there needs to be trust between all parties involved. First of all, there needs to be trust within each CCNE. Strong committed leadership breeds trust as well as motivation. "Within our own consortium, trusting relationships between people have already been established," noted Dr. Sanjiv Sam Gambhir of the Stanford CCNE. "Indeed, the whole process of building

and applying for the CCNE grant built a great deal of trust between members, and between the university and companies involved.” As the leadership development website, <http://www.thelearningcenter.net/>, states “There are two parts to trust: a feeling part that indicates trust and a performance track record that confirms trust.” Many of the investigators within established CCNEs have collaborated and published together thus “confirming” their trust with a previous track record. Trust within new CCNEs and across the Alliance program could be more difficult to establish. Through various programmatic mechanisms, not the least of which is the annual PI meeting, a large number of cross Alliance collaborations have been built. A key to building trust is effective communication. Physical scientists, for instance, know the language and acronyms of their field. Oncologists, however, do not know that specialized language. As Phase II of the Alliance takes shape, sensitivity to communication style, scientific “language,” and effective listening strategies becomes crucial for building productive teams and collaborative efforts.

The Alliance has demonstrated that a multi-disciplinary approach to research can catalyze scientific developments and enable clinical translation. Alliance investigators have advanced diagnostic technology, using both *in vitro* assays and novel imaging methods, and offered improved therapies and therapeutic efficacy measures. Many of the technologies developed and clinically translated have applied novel engineering to existing cancer biology strategies. The next stage of cancer nanotechnology research should enable new avenues of cancer care through revolutionary diagnostic tools, imaging techniques, treatment options, and *in situ* tumor characterization.

The scientific strategy for the 2010-2015 segment of the program was formulated based on the lessons learned from Phase I, the evolving strategy of the NNI, and, most importantly, the input of the extramural community. Phase II of the program will promote early diagnosis and better monitoring of therapeutic efficacy using emerging *in vitro* diagnostic techniques and novel imaging technologies such as multiplexed, multi-modal molecular contrast agents. It will be important to correlate outcomes from both approaches. On the therapeutic front, an increasing number of treatments will exploit tumor targeting via cell surface ligands and enhanced formulations for chemotherapeutics that reduce systemic toxicity and improve therapeutic index. Cooperative treatment regimes in which drug delivery is combined with tumor microenvironment engineering to improve treatment response will emerge. In addition, despite early hopes that gene therapy approaches would change the face of medicine, virtually no success has been garnered to date. There are glimpses that silencing genes and hopefully also replacing mutated genes will become routine modalities of treatment due to nanoparticle delivery options. In conclusion, while we do not want to over speculate or promise what we cannot achieve, we feel confident that patients facing this disease will have many more options in their arsenal due to the concerted effort, commitment, dedication, and ingenuity of those in the cancer nanotechnology research field.

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