NCI Alliance for Nanotechnology in Cancer

BULLETIN

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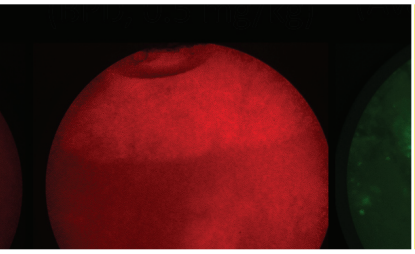

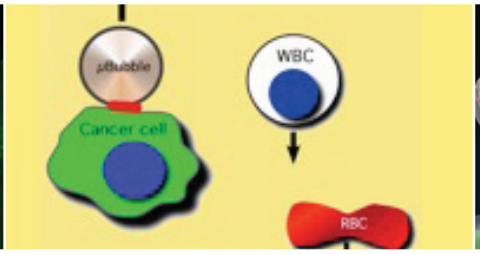

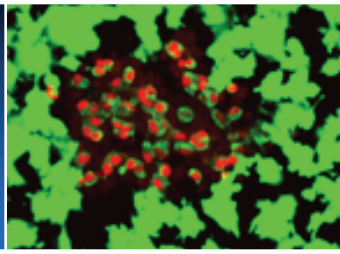

IN THIS ISSUE

Collaborating Across the Alliance

PHOTODYNAMIC THERAPY TO TREAT OVARIAN CANCER

By: Tayyaba Hasan, Ph.D., Prakash Rai, Ph.D., Bryan Spring, Ph.D., Xiang Zheng, Ph.D., Adnan Abu-Yousif, Ph.D., Ramtin Rahmanzadeh, Ph.D., Sarika Verma, Ph.D.

Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School (MGH-CNPP) Boston, MA

Tayyaba Hasan, Ph.D. is an investigator at the Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School. Her primary research focus lies in the development of the photodynamic therapy (PDT) platform to treat malignancies, pathogenic infections and other diseases. PDT is a photochemistry based approach where a light activatable molecule (photosensitizer, PS) is excited with light to generate cytotoxic species. PDT is approved for the treatment of various malignant and non-malignant diseases and is FDA approved as first line treatment for age-related macular degeneration developed in the Hasan laboratory. PDT has had considerable clinical success in treating various cancers and is routinely used for obstructive esophageal cancer and cases of advanced and early lung cancers. PDT has inherent dual selectivity built into it: first from the preferential localization of the PS and second from the spatial confinement of light. The Hasan laboratory focuses on photophysical and biological mechanisms of PDT (such as singlet oxygen formation, cytokine secretion, etc) and exploiting this information for developing new combination strategies. Additional programs include the development of targeted therapeutic and diagnostic online monitoring of biological and chemical processes in the animal models using microendoscopic techniques, and targeted delivery of photoactivatable molecules to specific sites using nanotechnology. Combining nanotechnology with PDT provides a powerful tool for selective destruction of diseased cells and tissues.

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One of the diseases where targeted delivery of PDT is very important is in ovarian cancer (OvCa). PDT can be effective on drug resistant cancer cells and has shown promise in clinical studies of OvCa. It is however dose limited due to the fact that the disseminated nature of the disease requires diffuse light to illuminate the entire peritoneal cavity. This illumination strategy requires the PS to be highly tissue specific in order to limit the phototoxic effects to the tumor sites alone. Developing OvCa specific optical agents would be a significant step forward in the treatment, diagnosis and monitoring of OvCa. The broad goal of the MGH-CNPP is to develop new approaches using targeted nanotechnology and microendoscope-based optical imaging for detection, treatment, and therapy monitoring of cancer. In 80% of OvCa patients the disease is detected at a late stage when the disease has already spread outside of ovaries. In addition, minimal residual disease left behind after surgical debulking and chemotherapy result in recurrent disease and the development of resistance to radiation and chemotherapy. A minimally invasive imaging modality with sensitivity and resolution to detect sub-millimeter OvCa nodules associated with recurrent disease, early in the treatment cycle would allow for timely disease re-assessment and the initiation of additional treatments if needed. Optical microendoscopy is a minimally invasive imaging modality with high sensitivity and micron-scale resolution. The Hasan group has developed such a fluorescence microendoscope and shown that it is capable of detecting small tumor nodules.

While the main effort of the MGH-CNPP at Wellman has been for the targeted delivery of photosensitizers using nanoparticles, the use of nanomaterials has now expanded to also include the encapsulation and delivery of cytokine inhibitors. Nanotechnology provides an exciting opportunity to develop multifunctional constructs capable of combining multiple therapies. As the expression levels of cytokines in cancer cells are known to vary in response to treatments including PDT, one new approach is to use nanomaterials as a platform for the delivery of both PDT agents and inhibitors of such proteins. This strategy is part of a greater goal outlined in the initial project which is the need to approach cancer treatment

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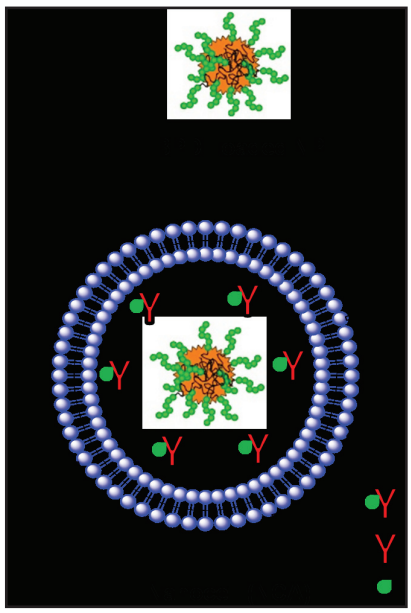

FIGURE 1A. *Multiplexing capabilities of the nanocells are demonstrated in ovarian cancer models where the delivery and localization of BPD and Avastin-Alexa 488 is imaged* in vitro. *(A) Design of nano-constructs. Cartoons showing a BPD loaded nanoparticle (BPD-NP) and a nanocell encapsulating BPD-NP and Avastin (NCA). The Avastin is labeled with a fluorophore Alexa Fluor 488.*

using not one but multiple, mechanistically distinct pathways. Encapsulating multiple therapies within the nano-constructs, specifically tailored to the molecular mechanisms being targeted would enhance the treatment outcome significantly. In order to potentiate mechanism-based combination treatments with PDT using nanotechnology, the Hasan laboratory has developed a nano-construct termed nanocell in which the PS is non-covalently trapped inside a polymer nanoparticle (NP) and these NPs, along with additional therapeutic and imaging agents, are encapsulated inside liposomes. One such therapeutic agent is Avastin which is a monoclonal antibody against vascular endothelial growth factor (VEGF) and has been approved for a variety of cancers. PDT is known to sensitize cancers to anti-VEGF therapies. Here, the effect of neutralizing intracellular VEGF using nanotechnology for the delivery of Avastin in combination with PDT is investigated. Synthesis and characterization of polymeric NP encapsulating a PS called benzoporphyrin derivative monoacid (BPD) has been done. These BPD loaded NP have then been encapsulated along with Avastin inside a liposome to give a nanocell (NCA, **Figure 1A**). The ability of these nanocells to deliver Avastin and BPD intracellularly in OVCAR-5 cells which is a human ovarian cancer cell line has been visualized using confocal microscopy (**Figure 1B**). Avastin is cell-impermeant and blocks secreted VEGF. Encapsulation of Avastin in NCA allows for intracellular delivery of Avastin. However, Avastin was not delivered into the cytoplasm if it was not encapsulated within the liposomes (NC+A). The nanocells also enhance BPD delivery over a commercially available liposomal formulation of BPD referred to as Verteporfin (L-BPD). BPD fluorescence intensity was significantly enhanced in cells delivered through NC vehicle (NC, NCA and NC+A) compared to L-BPD (**Figure 1B**).

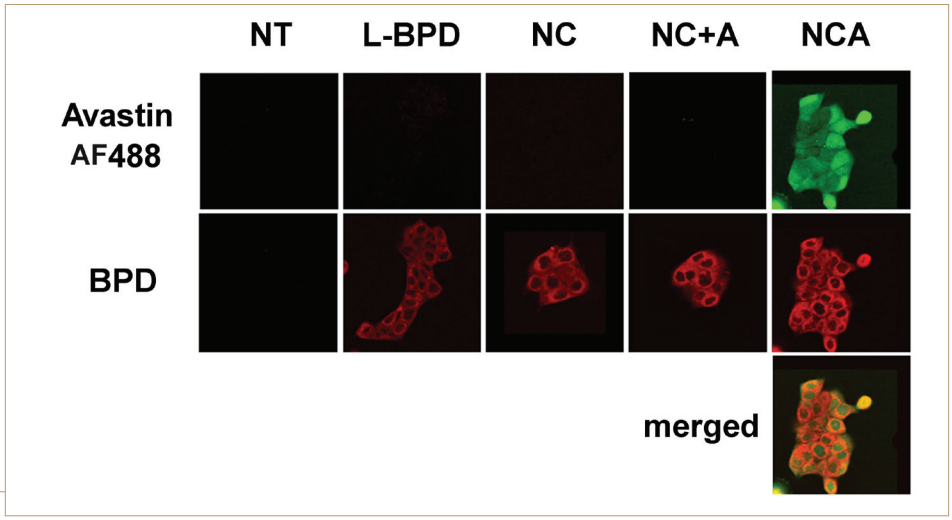

FIGURE 1B. *OVCAR-5 cells were incubated with various constructs for 90 minutes and imaged using confocal microscopy. Avastin-Alexa 488, as shown in green, is only delivered intracellularly when encapsulated in a nanocell (NCA). Free Avastin-Alexa 488 (NC+A) is cell-impermeant and is not delivered intracellularly.*

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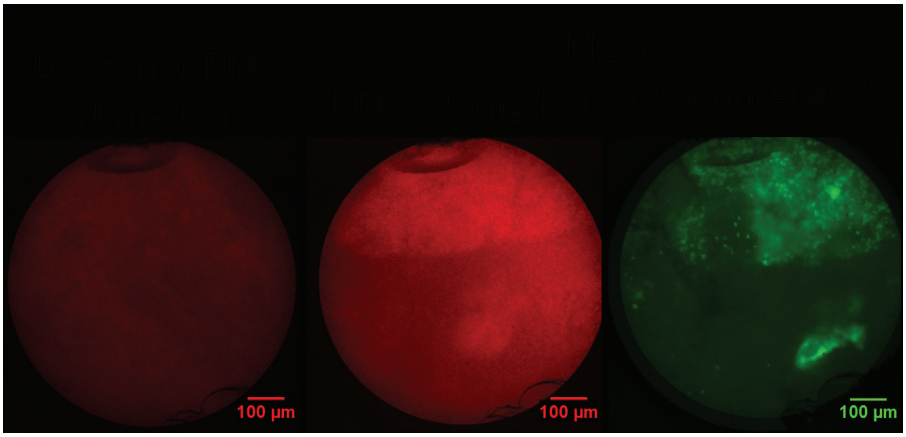

FIGURE 2. *In vivo fluorescence images of nanocell-delivered BPD and Avastin tissue uptake in a mouse xenograft model of disseminated ovarian cancer. Following intraperitoneal injection of nanocells co-encapsulating BPD nanoparticles and Avastin-Alexa Fluor 488 conjugates, a fiber-optic fluorescence microendoscope probe was inserted through a catheter into a tumored mouse to capture images of BPD and fluorescently labeled Avastin. Note that the fluorescence signal and image contrast are enhanced for nanocell-injected mice (middle and right images) relative to a mouse imaged using liposomal BPD (left image). The left and middle images of BPD fluorescence are displayed using the same intensity scale for direct comparison. The images were collected using excitation light provided by a blue LED. BPD fluorescence emission was collected using a 700 nm bandpass filter and the Alexa Fluor 488 emission through a 530 nm bandpass filter.*

The ability of these nanocells as contrast agents has been tested *in vivo* to detect ovarian cancer in an orthotopic mouse model using a fluorescence microendoscope. Using this non-targeted nanocell construct and the endoscope the ability to detect micron size tumor nodules has been demonstrated. Further, the microendoscope has also been used to establish nanocell-based drug delivery to the tumor tissue. The nanocells improve delivery of BPD as compared to Verteporfin (L-BPD) and can deliver higher payloads of Avastin *in situ* to the tumor (**Figure 2**).

The non-targeted nanocells show excellent contrast between diseased and healthy tissue. But for the treatment of complex sites such as the peritoneal cavity it is likely that enhanced selectivity will be required in order to prevent collateral damage to adjacent organs. Therefore in the future, the plan is to covalently attach targeting ligands on these multifunctional nanocell constructs to further improve their selectivity and thereby enhance the treatment outcome while also improving their disease imaging capabilities. The primary molecular target selected for this study is an epidermal growth factor receptor (EGFR) and the targeting vector is an aptamer which recognizes this trans-membrane receptor. The aptamer is developed in a new collaboration with Dr. Andrew Ellington's group at the Institute for Cellular and Molecular Biology, the University of Texas CNPP. The collaboration was initiated as a result of the annual nano-alliance meeting held in Chicago in September, 2008 and was sparked by a poster describing EGFR targeting aptamers presented by Dr. Na Li, a postdoctoral research fellow in Dr. Ellington's laboratory. The nano-alliance focuses on the collaborative development of translational technologies that can impact practical applications in the clinic. The annual nano-alliance meeting is helpful in its approach to delivering on these goals — it brings

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together a multidisciplinary community of oncologists, biologists, physical scientists, and engineers and helps build multi-disciplinary collaborations within this alliance network. The collaboration between Dr. Hasan's group and Dr. Ellington's group is an excellent example of this. Aptamers have rapidly emerged as a novel class of ligands that are capable of binding to target molecules with high affinity and specificity. Dr. Ellington is a leader in the field of aptamer design and one of the originators of aptamer selection technologies. Dr. Ellington's group has developed a 2'F-Py modified extended anti-EGFR RNA aptamer with demonstrated specificity for EGFR expressing cell lines. This is a stable RNA aptamer on which a complimentary oligonucleotide is hybridized to provide a fluorescence probe for tracking and an amino terminal group for conjugation to the nano-constructs. Despite the collaboration being fairly recent (January 2009), in the short span of 9 months, the groups have established the aptamers for EGFR specificity by using flow cytometer, where competitive binding assay of aptamers was performed with clinically approved anti-EGFR antibody (Cetuximab, C225) in cell lines with varying levels of EGFR expression. Confocal microscopy experiments shown in **Figure 3** establish the distinct localization of BPD-NPs and the aptamers. In the future, nano-constructs will be combined to give the high payload of therapeutic and imaging agents and are expected to localize at EGFR rich sites.

The Hasan and the Ellington groups plan to continue the collaboration and to use these aptamers for conjugation to the nanocells to target ovarian cancer. Using both in vitro and in vivo models of disease their aim is to study this targeted nanocell construct through the custom designed microendoscope to improve early detection of microscopic malignancies *in vivo*, online monitoring of treatment effectiveness, and initiation of secondary treatments, where necessary, to enhance overall survival.

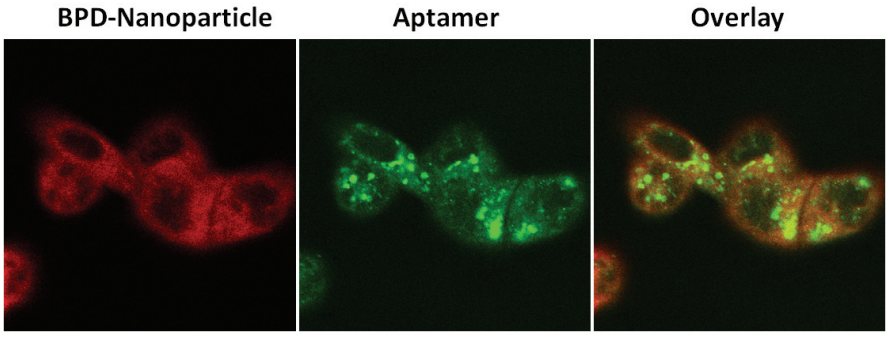

FIGURE 3. *Both aptamers and nanoparticle constructs were taken by OVCAR-5 cells after 1 hour of incubation. BPD-NP is shown in red and FITC-labeled anti-EGFR aptamer is shown in green. Combining of the aptamers with nanoparticles is expected to localize them to EGFR-rich sites.*

Collaborating Across the Alliance

DIVERSITY TRAINING OPPORTUNITIES AT THE UNIVERSITY OF CALIFORNIA, SAN DIEGO

By: LeeAnn Bailey, Ph.D. and Robert C. Rivers, Ph.D.

NCI Center to Reduce Cancer Health Disparities (CRCHD) & NCI Clinical Proteomic Technologies for Cancer (CPTC)

The NCI Alliance for Nanotechnology in Cancer and the Center to Reduce to Cancer Health Disparities (CRCHD) have partnered to provide training opportunities for high school and undergraduate students from underserved populations in nanotechnology. CRCHD provides a range of training opportunities for underrepresented and underserved groups (http://crchd.cancer.gov). Through mentoring, training, and different funding mechanisms; CRCHD provides opportunities necessary for trainees to launch careers as independent researchers. The overarching goals of the Emerging Technologies Continuing Umbrella of Research Experiences (ET CURE) initiative are to:

- Create a pipeline of underserved students and investigators in the fields of emerging and advanced technologies
- Increase the number of scientists from underserved populations with training in emerging technologies and cancer health disparities
- Enhance the application of emerging technologies to cancer research through increased training and educational opportunities; and
- Foster academic, scientific and multi-disciplinary research excellence, culminating in the emergence of a mature investigator capable of securing competitive advanced research project funding.

The ET CURE program at the University of California San Diego (UCSD) is developing a pipeline for training underserved investigators. Currently, there are seven undergraduates training in the ET CURE program; all of whom are either juniors or seniors. The curriculum for these students includes several distinct features which are designed to prepare the students for a successful career in cancer research. The UCSD research training program has been led by Drs. Andrew Kummel and Tim Johnston. Dr. Saadik Esener is one of the Alliance investigators involved in the ET CURE pilot at the Moores cancer center and states, "most projects are multi disciplinary and there is a continuous needs for helpers in the labs coming from diverse fields including chemistry, physics and engineering. In some cases the undergraduate student may be very smart with a background that matches exactly the project in hand and he can make important intellectual contributions. We were fortunate to have such a graduate student who came up with a practical and important idea that he implemented that we are using now in testing our motherships." All students were active participants in nanotechnology research, weekly group lab meetings and journal clubs. Dr. Kummel added; "the ET CURE students in the summer became key participants in several nanotechnology

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projects, one on developing an assay for antibody drugs in blood, one on developing DNA nanoparticles for detecting cancer center and/or delivering therapeutics, and one on developing soft nanoparticle with very high drug loading." An integral program component is to provide the students with a research experience that enables them to become independent competitive cancer researchers. Melissa Aillaud is one of the ET CURE students currently in the program and credits the program as having a massive impact on her future plans to pursue graduate studies in cancer research (See page 8). It is paramount that the application of emerging technologies to cancer research through increased training and educational opportunities be utilized to increase the number of underserved in the pool of future investigators.

In addition to the ET CURE program, UCSD has also utilized Research Supplements to Promote Diversity in Health-Related Research. One graduate student that is currently being supported by a supplement is Sergio Sandoval. Sergio currently works in the lab of Dr. Kummel with a primary focus of creating nanoparticles for drug delivery and using them to selectively target cancer cells (**Figure 1**). His parents immigrated to the US in order to make a better life for him and his siblings and views that support from such programs has provided an opportunity for his dream to be a cancer researcher into fruition. In addition to receiving support, he has also been instrumental in serving as both a scientific and personal mentor to the ET CURE students. Sergio's work in the lab will one day help to alleviate the burden of those suffering from cancer and his work as a mentor will develop further the future generation of cancer researchers.

Overall the ET CURE program has helped provided a pathway in to research for several undergraduate students at UCSD and provides a model for increasing the diversity within the cadre of cancer research scientists in emerging technologies and by extension cancer health disparities.

For more information about opportunities through CRCHD contact Dr. LeeAnn Bailey, baileyl@mail.nih.gov.

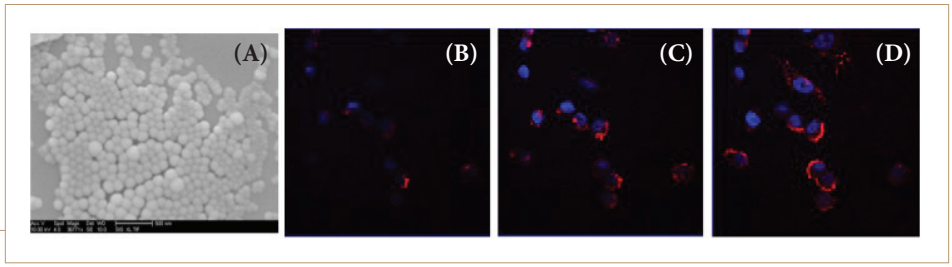

FIGURE 1. *Images of PLGA-lipid hybrid NPs. (A) SEM Image of 100 nm PLGA-lipid hybrid NPs. (B-D) Three consecutive confocal cross sectional cuts (B to D), within MCF-7 cells, showing endocytosed NPs in the cell cytoplasm.*

Red: Nile Red Loaded NPs. Blue: Hoechst Nuclear Stain

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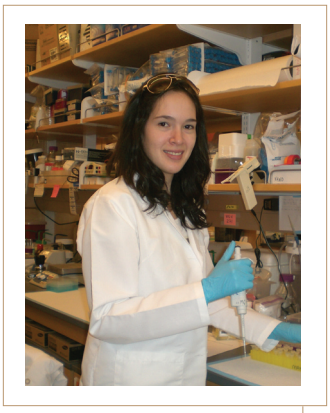

Melissa Aillaud

Melissa Aillaud is a senior majoring in Bioengineering at UCSD. As a recipient of both the National Action Council for Minorities in Engineering and a Math, Engineering, Science Achievement NSF scholarship, Melissa represents one of the aspiring cancer research trainees currently part of the ET CURE program.

What is your involvement with ET CURE?

The ET CURE program at UCSD has funded my participation in this research project during the summer and academic year. Also, my peers and I presented our research projects during a NCI site visit. Lastly, other ET CURE undergraduates and I hold a weekly journal club meeting, where we discuss scientific papers regarding topics mostly in the nanotechnology field.

How has the ET CURE program influenced your undergraduate experience?

ET CURE has enriched my undergraduate experience so much! It has allowed me to be involved in a research lab during the academic year — a great thing to do if you are enrolled in a research institution like UCSD. Most importantly, I have gained mentors who are guiding and supporting my academic decisions.

Has the program motivated you to apply for graduate school?

Yes. ET CURE has allowed my continuous involvement in a research lab which helped me confirm my interest in developing a research project.

Investigator Highlight

Dmitri Simberg

ISOLATION OF CIRCULATING TUMOR CELLS FROM BLOOD USING MICROBUBBLES

By: Dmitri Simberg

University of California, San Diego (NANO-TUMOR) San Diego, CA

Investigator's background

Dmitri Simberg received his Ph.D. in Biochemistry at the Hebrew University of Jerusalem, Israel, where he researched the mechanisms of transfection using cationic liposomes and membranes. During his graduate studies he developed transfection reagents that are now sold commercially. Dmitri received postdoctoral training in the laboratory of Dr. Erkki Ruoslahti in the Burnham Institute on targeting tumors with nanoparticles for imaging and treatment of cancers, and an additional training in the laboratory of Dr. Mattrey, where he developed targeted ultrasound contrast reagents and conceived the idea of microbubble-assisted cell separation. He currently holds a position of Project Scientist at the NanoTumor Center in UCSD.

Project summary

During cancer disease, malignant cells are shed into blood (*1-3*). These extremely rare cells (few cells/ml blood) could be isolated and analyzed to provide invaluable information for diagnosis and prognosis of cancer patients. However, because of low concentration in blood, the isolation of circulating tumor cells (**CTCs**) is a laborious and expensive process.

Immunomagnetic separation is the currently used method for CTC isolation from blood samples of cancer patients. This method is very sensitive (1 CTC/ml blood or lower), but produces significant contamination of non-specific cells in the isolated sample, it is laborious and is practically limited to small volumes of sample.

In order to address the existing problems of CTC isolation from blood, Dmitri Simberg and his team propose to develop a cell isolation technique based on capture of rare cells in blood by gas-filled microbubbles (***μ*bubbles**). The *μ*bubbles coated with tumor-targeting ligands will selectively bind tumor cells in a blood sample. Due to the buoyancy of the *μ*bubbles, they will drag bound CTCs upwards, while the rest of the cells will sediment to the bottom (**Scheme**). The microbubbles will be concentrated, and tumor cells will be detected and counted.

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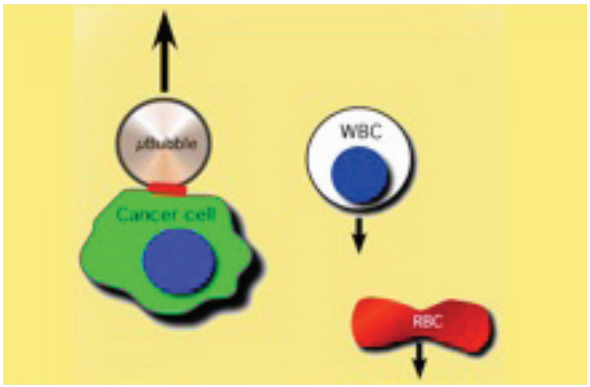
To prove that μbubbles could selectively bind and separate the cells in whole blood, Dmitri first attached a specific antibody to μbubbles in order to detect chemically modified red blood cells. Indeed, the specific antibody-coated μbubbles bound and separated the modified erythrocytes from blood. Next, he used an antibody that can recognize tumor cells. Microbubbles coated with this antibody efficiently bound tumor cells that were blended with normal blood.

The ultimate goal of this project is to develop a technology for quick and efficient isolation of rare cells from large volumes of biological samples. This technology could open new possibilities for diagnostics and treatment of cancers. If successful, the technique can be used for efficient large-scale isolation of tumor cells and biomarkers, as well as depletion of cells from blood and other tissues.



Scheme. *Microbubble-attached tumor cells are separated from blood due to acquired buoyancy.*

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Investigator Highlights

MULTIFUNCTIONAL NANOPARTICLES FOR TUMOR IMAGING AND PHOTOTHERAPY (PDT)

By: Ravindra K. Pandey, Ph.D.

Photodynamic Therapy Center, Roswell Park Cancer Institute (RPCI-CNPP) Buffalo, NY

Dr. Ravindra K. Pandey, Distinguished Professor at the Photodynamic Therapy (PDT) Center, and Director of Pharmaceutical Chemistry, Roswell Park Cancer Institute, Buffalo, was trained as a Medicinal Chemist at University of Rajasthan, Jaipur India, University of California, Davis and University College, Cardiff, Wales. He has extensive experience in drug design and development. He has been working in the area of porphyrin chemistry for the last 29 years and looking at their application in tumor-imaging and therapy for the last 23 years. Some of the compounds developed in the laboratory of Dr. Pandey are at advanced preclinical and human clinical trials. A few of them are recently licensed to various pharmaceutical companies. One of Dr. Pandey's current research interests is to develop ORMOSIL and PAA-based multifunctional nanoplatforms (in collaboration with Dr. Prasad, SUNY, Buffalo and Dr. Kopelman, University of Michigan, Ann Arbor) and investigate their utility in tumor-imaging and phototherapy. This project is funded by the NCI as one of the nantotechnology platform grants in which Dr. Pandey is the principal investigator.

Porphyrin-based compounds as PDT Agents:

Among the current cancer treatment modalities, PDT has attracted increasing attention for the curative and palliative treatment of cancer and some non-cancerous conditions. It exploits the biological consequences of localized damage inflicted by the photodynamic process. Three critical elements required for the initial photodynamic processes to occur (i) a tumor-avid compound (photosensitizer), (ii) appropriate light source and (iii) tissue oxygen. The photosensitizer is activated by an appropriate wavelength of light. Exposing the tumors with light converts the tissue-oxygen (triplet state) to highly reactive singlet oxygen (type II reaction), which is believed to be responsible for destroying the tumor (Pandey, et al. Lasers in Surgery and Medicine, 2006, 38, 445-467).

The major challenge of cancer therapy is preferential destruction of malignant cells while sparing the normal tissue. Critical for successful eradication of malignant disease are early detection and selective ablation of the malignancy. PDT is a clinically effective and a still evolving locally selective therapy for cancers. It is FDA approved for early and late stage lung cancer, obstructive esophageal cancer, high-grade dysplasia associated with Barrett's esophagus, age-related macular degeneration and actinic keratoses. Photosensitizers have been designed which localize relatively specifically in certain subcellular structures such as the mitochondria, which are exquisitely sensitive targets. On the tumor tissue level, direct

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photodynamic effect can lead to tumor cell kill, destruction of the tumor supporting vasculature and possibly activation of the innate and adaptive anti-tumor immune system interaction to destroy the malignant tissue. The preferential killing of the targeted cells (e.g. tumor), rather than adjacent normal tissues, is essential for PDT, and the preferential target damage achieved in clinical applications is a major driving force behind the use of the modality. The success of PDT relies on development of tumor-avid molecules that are preferentially retained in malignant cells but cleared from normal tissues.

Clinical PDT initially was developed at the PDT Center, Roswell Park Cancer Institute (RPCI), Buffalo and has set-up one of the world's largest basic and clinical research program. Initially the RPCI group developed Photofrin®, the first generation FDA approved hematoporphyrin-based compound. Subsequently, improved agents were developed on the basis of SAR and QSAR studies with high selectivity and desirable pharmacokinetics. Although the mechanism of porphyrin retention by tumors in not well understood, the balance between lipophilicity and hydrophilicity is recognized as an important factor. Dr. Pandey's laboratory has developed a series of effective PDT agents with the required photophysical characteristics and used chlorophyll-a and bacteriochlorophyll-a as the substrates. An extensive QSAR studies on a series of the alkyl ether derivatives of pyropheophorbide-a (660 nm) led to the selection of a highly effective candidate, HPPH (hexyl ether derivative), which is currently in Phase II human clinical trials. The other PDT agents currently under development at RPCI are selected from a series of purpurinimides (700 nm) and bacteriopurpurinimdes (780-800 nm) with high tumor-specificity, high singlet oxygen ($^1O_2$) producing capability and significant tumor-avidity. The long wavelength absorption exhibited by these molecules is important for treating large deep-seated tumors, because it increases the light penetration due to less scattering, which should minimizes the number of optical fibers needed for light delivery within a bulky tumor. (Pandey et al. The Porphyrin Handbook, Academic Press, Vol. 6, 158-184).

Tumor Imaging and PDT

Medical imaging has undergone tremendous changes since the discovery of X-rays. Depending on the organ of interest, lesion localization, size, availability of imaging systems, a large number of imaging techniques are now available to guide diagnostic and therapeutic procedures. With the discovery of flexible fiber optics, endoscopy is now used for imaging hollow organs. Cross-sectional imaging modalities such as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) are regularly used for imaging the organs of interest. In recent years several fusion-imaging approaches, e.g. MR/CT, MR/optical imaging, MR/PET imaging and PET/optical imaging have created enormous interest. A significant progress has also been made to use these imaging modalities

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for monitoring the tumor response or to use a single compound as a dual-function agent for tumor-imaging and therapy, including photodynamic therapy (PDT) or phototherapy. Dr Pandey's recent work has shown that tumor-avid photosensitizers can be used as vehicles to deliver the desired imaging agents (MRI, Fluorescence and PET) to tumors. (Dr. Pandey and co workers, Bioconjugate Chem., 2005, 16, 32-42, Bioconjugate Chem. 2005, 16, 1264-1274), These dual-function agents show a great potential for tumor imaging and PDT. **Fig. 1** shows the structure of a photosensitizer (HPPH) and cyanine dye (fluorophore) conjugate and its ability to image the tumor and monitor the tumor response by using the same compound for PDT.

Advantages and limitations of Bifunctional photosensitizer-flurophore conjugates.

In a separate study Dr. Pandey's group has developed certain bifunctional conjugates that use tumor-avid photosensitizers to target the NIR fluorophores to the tumor. The function of the fluorophore is to visualize the tumor location and treatment site. The presence of the photosensitizer allows subsequent tumor ablation. The optical imaging allows the clinician performing photodynamic therapy to continuously acquire and display patient data in real-time. The idea of a "See and Treat" approach is to determine where to treat superficial carcinomas and how to reach deep-seated tumors in sites such as the breast, lung and brain with optical

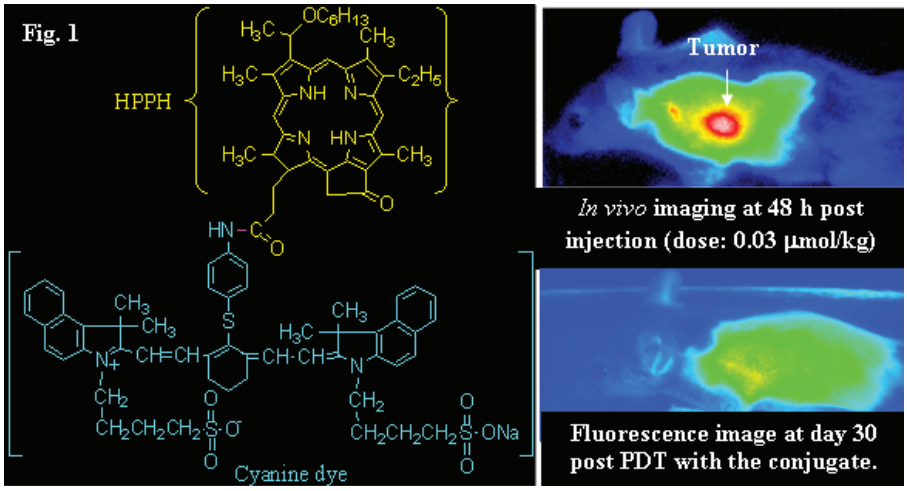

FIGURE 1.

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fibers delivering the photo-activating light. The limitations with these compounds are (i) they involve several synthetic and purification steps and (ii) a significant difference in the imaging (very low dose) and therapeutic dose, because a part of the singlet oxygen (key cytotoxic agent for PDT) produced on exposing the photosensitizer in the conjugate is quenched by the cyanine dye (fluorophore). However, Dr. Pandey's group in collaboration with Dr. Kopelman's research team (University of Michigan) was able to solve both of these problems.

Advantages of PAA NPs for developing fluorescence — imaging/PDT agents:

For investigating the utility of PAA NPs three different approaches were used. First, the photosensitizer HPPH and the cyanine dye (fluorophore) were post-loaded in variable ratios (HPPH to CD: 1:1; 2:1; 3:1 and 4:1 molar concentrations). Among these formulations, the 2:1 formulations produced the best tumor imaging and long-term tumor cure in BALB/c mice bearing Colon26 tumors. This formulation, in a single dose contained the therapeutic dose of HPPH (0.47 μmol/kg) and the imaging dose of Cyanine dye (0.27 μmol/kg), which were similar to the components if used alone for PDT tumor-imaging respectively, but with much more tumor selectivity (skin to tumor ratio of HPPH was 4:1 instead of 2:1 without NPs). See **Fig. 2.**

Photosensitizer for PET Imaging and PDT:

Dr. Pandey's group has shown that most of the photosensitizers derived from chlorophyll-a remain in circulation for a long time and show maximum uptake in tumor at 24 to 48h post injection. To investigate the utility of these compounds in PET imaging, a series of radionuclides with variable half-lives were introduced. Among the radio-labeled compounds investigated, the I-124 analog shows improved tumor-imaging potential than F-18 FDG for imaging certain indications: e.g., brain, pancreatic and lung tumors (**Fig. 3**, images of BALB/c mice bearing Colon 26 tumors. This compound also showed its remarkable ability in imaging tumor metastasis (i.e metastasis of breast cancer to lung in BALB/c mice bearing 4T1 tumors in the belly). As a non-labeled analog it can be used for photodynamic therapy, providing a unique ability for a "See and Treat" approach (Pandey et al. J. Med. Chem. 2005, 48, 6286-6294, Pandey et. al. 2009, 52, 445-455, Bioconjugate Chem. 2009, 20, 274-282). The only disadvantage of ^{124}I-photosensitizer (PS) is its long half-life, and the high uptake

FIGURE 2.

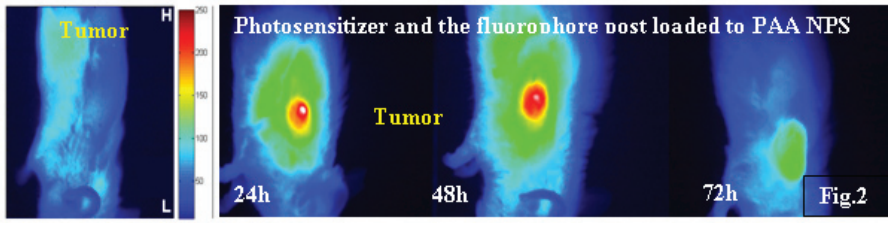
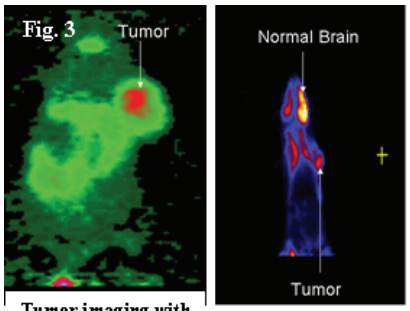


FIGURE 3.



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of the agent in other organs (especially in spleen and liver) could be toxic. However, by using PAA NPs, Dr. Pandey's group was able to enhance the uptake of the agent in tumor and reduce it significantly in spleen, liver and other organs.

PAA NPs enhanced the tumor uptake and contrast.

On injecting the PAA NPs first in BALB/c mice bearing Colon 26 tumors and then the ^{124}I-photosensitizer after certain time interval showed enhanced tumor-contrast. Interestingly, the size of the nanoparticles used (30 to 150 nm) produced a remarkable difference in biodistribution of the labeled PS. Larger size of PAA NPs showed less uptake of the imaging agent in spleen and liver. (**Fig. 4**). The ^{124}I-chlorophyll-a analog shows great potential in imaging lung, brain and pancreatic tumors also.

HPPH conjugated PAA NPs containing F3-Cys peptide at the outer surface show targeted specificity:

In collaboration with Dr. Kopelman (University of Michigan, Ann Arbor), the specificity of the targeted NPs was tested

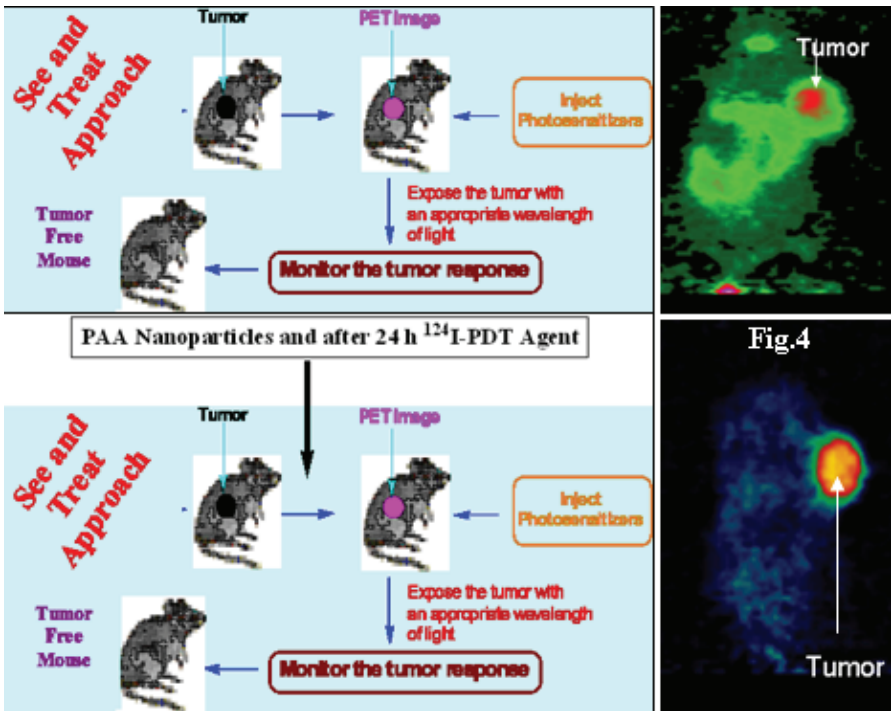

FIGURE 4.

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by fluorescent imaging (**Fig. 5**). F3 targeted HPPH-conjugated PAA NP specifically bound to MDA-MB-435 cells (expressing nucleolin) while non-targeted NPS did not, indicating that F3-mediated specificity is retained in the presence of conjugated HPPH. F3 targeted NPs did not accumulate in the nucleus. On activation of cells with light at 660 nm only F3-targeted NP caused cell kill (Fig 11). Cell internalization of F3-targeted NPs was confirmed by fluorescence confocal microscopy at different heights from the surface of the cell.

F3-Cys shows target-specificity in 9L glioma cells:

Similar to F3-Cys, a pegylated form of F3-Cys PEG on Rhodamine-PAA NPs also showed remarkable target-specificity in the 9L rat glioma cell line which also expresses nucleolin,

Summary: The use of porphyrin-based compounds as photosensitizer in PDT has been known for quite sometime. During the last decade the Roswell Park group has developed a series of highly efficient photosensitizers with long wavelength absorption in the range of 660-800 nm. Dr. Pandey's group was the first to demonstrate the utility of certain tumor-avid molecules for developing multifunctional agents (MRI/PDT, fluorescence imaging/PDT, PET imaging/PDT). These conjugates show a great potential for tumor imaging at a very low dose and show improved contrast than Magnavist® (MR agent) and ^{18}F-FDG (a clinical standard for PET). The funding obtained through the NCI funded platform provided an opportunity to Dr. Pandey and his collaborators (Dr. Prasad, SUNY, Buffalo and Dr. Kopelman (University of Michigan, Ann Arbor) to look at the utility of various NPs in developing multifunctional agents. Among the NPs investigated so far, the PAA NPs show a great potential in developing multifunctional nanoplatforms with improved tumor-specificity, enhanced tumor-imaging and photosensitizing efficacy. The preliminary in vitro results suggests that target-specificity can be increased by introducing certain target-specific moieties at the surface of the nanoparticles. These studies (*in vivo*) are currently in progress.

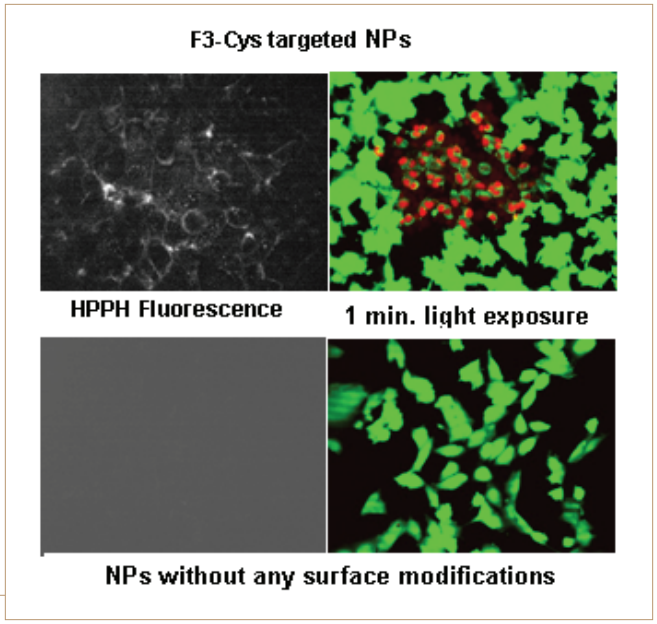

FIGURE 5. *Fluorescence (left) & Live/dead cell assay (right) of HPPH conjugated PAA NPs + or - F3-Cys peptide incubated for 15 min in MDA-MB-435 cell lines.*

MULTIFUNCTIONAL NANOPARTICLES FOR RNA INTERFERENCE THERAPY TO OVERCOME TUMOR MDR

By: Mansoor Amiji[1] and Zhen-feng Duan[2]

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Multidrug resistance (MDR) in tumor cells is a major factor which contributes to the failure of cancer chemotherapy, and numerous efforts have been attempted to overcome MDR by developing combination strategies that both inhibit MDR and deliver therapeutic drugs. MDR may be due to alterations in specific proteins (such as Pgp/mdr1 or MRP1) or alternatively due to changes in the apoptotic threshold by anti-apoptotic proteins (such as Bcl-2 or survivin). A wide range of compounds that interact with P-glycoprotein (Pgp) or MRP1 and block drug efflux have been reported to reverse the MDR phenotype. Unfortunately, the clinical toxicities associated with these compounds at concentrations required to inhibit Pgp have precluded their widespread use and to date, none of these attempts have yielded a tolerable and effective therapy to reverse MDR. Thus, identification of new strategies would be useful to improve clinical outcomes in refractory cancer patients.

Down-regulation of MDR transporters and anti-apoptotic genes by RNA interference (RNAi/siRNA) has been suggested as a more specific alternative to overcome MDR than the use of conventional small molecule pharmacological inhibitors. Although the RNAi technology is an excellent candidate for cancer therapy, several challenges need to be addressed for clinical application. First, the poor membrane permeability of siRNA limits cellular uptake. Secondly, most of the reagents for delivery of siRNA such as Lipofectamine are toxic. Third, siRNA is unstable and rapidly degraded by nucleases. Further use of siRNA as therapeutic agents will rely mostly on the development of more efficient systemic delivery systems.

Nanotechnology offers solutions to overcome the challenges of systemic siRNA delivery. Several varieties of nanoparticles are available including polymeric nanoparticles, dendrimers, inorganic/metal nanoparticles, quantum dots, liposomes, and micelles. Although some nanoparticles have been tested for systemic siRNA delivery, problems still remain such as siRNA loading efficiency. The versatility of polymeric nanocarriers offers a significant advantage over other nano-carrier platforms; polymer matrices can be selected according to utility which allows for the customization of nanoparticle properties. Additional advantages of polymeric nanocarriers include ease in surface modification, greater encapsulation efficiency of the payload, payload protection, large surface area-to-volume ratio, and the ability to modify

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the rate of polymer erosion for temporal control over the release of nucleotides. Among the natural polysaccharides, dextran based nanoparticles have been used to deliver chemotherapeutic drugs, resulting in enhancement of drug accumulation and increased apoptosis in cancer cells.

The Cancer Nanotechnology Platform Partnership (CNPP) program established between Dr. Mansoor Amiji's research group at Northeastern University and Dr. Zhen-feng Duan and his collaborators at Massachusetts General Hospital recently designed and evaluated a novel drug delivery system to overcome tumor MDR with *mdr1* silencing siRNAs. Novel biocompatible, lipid-modified dextran-based polymeric nanoparticles were used as the platform for *mdr1* silencing siRNA delivery, and the efficacy of combination therapy with this system was evaluated. MDR cell lines were treated with the *mdr1* siRNA nanocarriers and Pgp expression, drug retention, and immunofluorescence were analyzed. Combination therapy of the *mdr1* siRNA loaded nanocarriers with increasing concentrations of doxorubicin (DOX) was also analyzed. To assess the silencing efficacy of siRNA loaded nanoparticles on cell lines, we have utilized EGFP siRNA on EGFP expressing cells to test its effects. EGFP siRNA loaded nanoparticles were cytotolerant at the concentrations utilized in this study. Western blot assay was performed to estimate the effect of *mdr1* siRNA loaded nanoparticles on Pgp expression. The mdr1 gene silencing siRNA loaded nanoparticles inhibited the expression of Pgp at concentrations as low as 30 nM. *mdr1* silencing siRNA transfected with Lipofectamine suppressed Pgp expression starting from 24 hours up to 48 hours. On the other hand, siRNA loaded nanoparticles could maintain Pgp suppression for up to 96 hours. Reversal of MDR is usually manifested as an increased intracellular accumulation of chemotherapeutics, which can be achieved by disturbing Pgp mediated drug uptake and efflux. Therefore, the effect of *mdr1* siRNA loaded nanoparticles was examined for the uptake and efflux of the Pgp substrate, calcein AM. Cells treated with mdr1 silencing siRNA were shown to decrease calcein AM efflux in a dose-dependent manner as determined by image analysis, and was confirmed by microplate spectrofluorometer analysis. Using fluorescent microscopy, subcellular distribution of DOX in resistant cells was analyzed. After a 3 hour incubation period with free DOX in MDR cells, the drug was primarily concentrated in the cytoplasm with very low level of fluorescence observed in the nucleus. When DOX was administered after treatment with *mdr1* gene silencing loaded nanoparticles to

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drug resistant cell lines, an increase in fluorescence was observed in the nucleus and cytoplasm (**Figure 1**). This subcellular distribution mimicked that of the drug sensitive variant when treated with DOX. The dextran nanoparticle is non-cytotoxic by itself at the dose utilized in this study. After treatment with *mdr1* silencing siRNA loaded nanoparticles, they found that DOX showed an increased amount of anti-proliferative activity in drug resistant lines in a dose dependent manner. With the delivery of nanoparticles carrying 100 nM *mdr1* silencing siRNA, tumor inhibition with DOX was substantially more marked than with the administration of 100 fold higher amounts of free drug. Part of this work has been accepted for publication by BMC Cancer. A similar approach that targets another MDR gene (MRP1) and the anti-apoptotic gene Bcl-2 is currently being evaluated in lung cancer MDR cells.

The work of the CNPP is a valuable example of a rationally designed, multifunctional and combinatorial delivery system for overcoming tumor MDR and is an important step for improving the treatment of MDR tumors in the clinic. Future studies evaluating the efficacy of dextran nanoparticles as drug and siRNA vehicles *in vivo* will be needed for clinical translation of this technology.

The research was funded by the NCI's Alliance for Nanotechnology in Cancer Platform Partnership grant R01-CA119617.

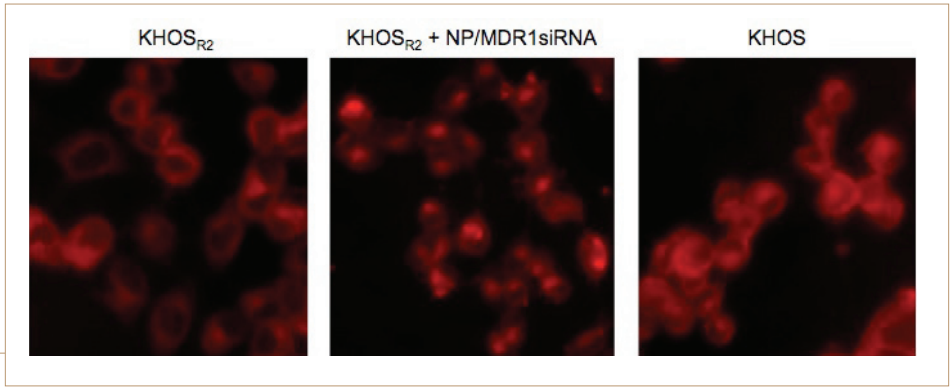

FIGURE 1. *Subcellular distribution of doxorubicin in drug sensitive (KHOS) and resistant (KHOS$_{R2}$) cell lines. A prominent increase in fluorescence was observed in the nucleus when multidrug resistant cells KHOS$_{R2}$ were pre-treated with MDR1 silencing siRNA-loaded nanoparticles followed by administration with doxorubicin.*

IMPROVING DENDRIMERS FOR CANCER THERAPEUTICS

By: Daniel Q. McNerny, Douglas G. Mullen, Mark M. Banaszak Holl, James R. Baker, Jr.

University of Michigan (UMich-CNPP) Ann Arbor, MI

Since shortly after their first development in 1979, dendrimers and dendritic polymers have been presented as an ideal delivery platform for therapeutic applications. With a highly controlled branching architecture, poly (amidoamine) (PAMAM) dendrimers have been utilized to address the shortcomings of traditional chemotherapies and non-targeted treatments. Furthermore, PAMAM dendrimers offer improvements over many other targeted polymeric and macromolecular delivery devices because they can be designed to be nonimmunogenic, biocompatible, and functionalized with molecules tailored for a specific application while maintaining a degree of homogeneity unsurpassed by other multivalent and multifunctional platforms. Despite these attractive qualities, dendrimer-based therapeutics have been slow to meet expectations. Difficulties with scale-up synthesis and reproducible biological activity have largely prevented dendrimers from reaching the clinic. To address these challenges, recent work examines the effect of stochastic ligand conjugate to dendrimers and has shown that product distributions can be identified on the well-defined dendrimer backbone. In addition, dendrons have been utilized to orthogonally couple multiple functionalities in aim of avoiding complex stacking of functional ligand distributions.

Understanding product distributions of functionalized PAMAM dendrimers using HPLC

Commonly used characterization techniques, including nuclear magnetic resonance (NMR), ultraviolet/visible (UV/Vis) spectroscopy, Fourier transformed infrared spectroscopy (FTIR), and elemental analysis are only capable of determining the mean ligand to nanoparticle ratio and do not provide information about the distribution of nanoparticle-ligand species present in the nanomaterials. Using PAMAM dendrimers and a model ligand, the distributions of dendrimer-ligand components were resolved and quantified by HPLC.[1] This study is significant because it shows that the average structure, which is commonly reported in many studies, does a poor job reflecting the diversity of dendrimer-ligand species that exist in each sample. Additionally, the average dendrimer-ligand species is often not the most abundant species. Knowledge of these distributions for functional nanomaterials can lead to improved system design and predictions of structure, function and activity of the generated material.

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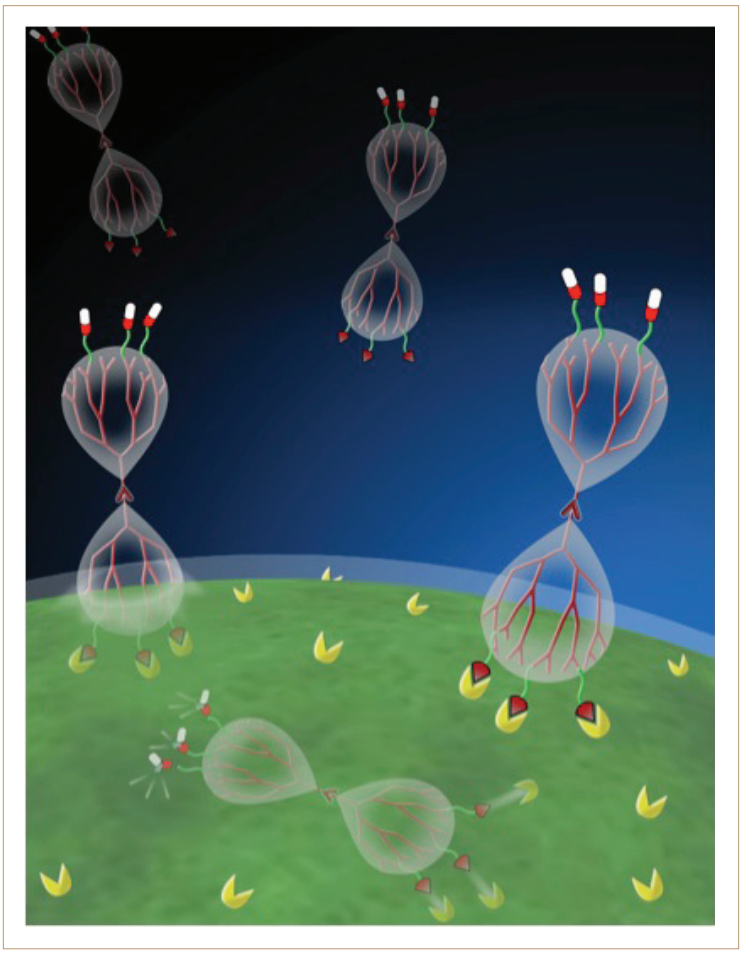
Dendrons as a platform for orthogonal coupling

An alternative from traditional functionalization of dendrimers to create multifunctional platforms is to utilize asymmetrical dendrons.[2] By designing dendrons with an orthogonal focal point, the dendron can be selectively coupled to a diagnostic and imaging agents, therapeutics, or other biologically relevant moieties and surfaces. In addition, coupling a second functionalized dendron can create a Janus face dendrimer. The ability to functionalize unique compartments of the device in parallel allows for a "mix-and-match" approach to address personalized applications.

We have successfully synthesized and evaluated of *in vitro* biological activity for a targeted, PAMAM dendron avidity platform that maintains the binding specificity and multivalency of previous dendrimer models. After the targeting ligand was conjugated to the dendron surface, binary devices were created via a copper-catalyzed 1,3-dipolar cycloaddition, commonly referred to as "click chemistry," coupling a unique alkyne group at the dendron focal point with an azide-functionalized dye, biotin, therapeutic molecule or a second dye-conjugated dendron. By combining orthogonal coupling to a multivalent dendron platform, complex product distributions may be avoided. The platform can be utilized to eliminate biologically inactive or harmful subpopulations that may be found in stochastic dendrimer system.



Summary

Current work aims to address the challenges associated in making dendrimer therapeutics, namely reproducing biological activity and understanding the product distribution associated with the desired activity. HPLC has successful resolved dendrimer-ligand distributions with the intent of gaining an understanding of how reaction conditions can be controlled to product the intended product. Dendron platforms are also being pursued, using advantageous orthogonal coupling chemistry to provide additional product precision while maintaining the same functionality seen in previously successful dendrimer systems.

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2. RGD Dendron Bodies; Synthetic Avidity Agents with Defined and Potentially Interchangeable Effector Sites That Can Substitute for Antibodies. *Bioconjugate Chemistry* **2009**, 20 (10), 1853-1859.

Nanotechnology Highlights

MAGNETIC NANOTAGS SPOT CANCER IN MICE EARLIER THAN CURRENT METHODS

By: Joe Alper

Searching for biomarkers that can warn of diseases such as cancer while they are still in their earliest stage is likely to become far easier thanks to an innovative biosensor chip developed by Stanford University researchers. The sensor is up to 1,000 times more sensitive than any technology now in clinical use, is accurate regardless of which bodily fluid is being analyzed, and can detect biomarker proteins over a range of concentrations three times broader than any existing method, the researchers say.

The nanosensor chip also search for up to 64 different proteins simultaneously and has been shown to be effective in early detection of tumors in mice, suggesting that it may open the door to significantly earlier detection of even the most elusive cancers in humans. The sensor also can be used to detect markers of diseases other than cancer. The researchers published their description of their magnetic nanosensor in the journal *Nature Medicine*.

"In the early stage [of a cancer], the protein biomarker level in blood is very, very low, so you need ultra-sensitive technology to detect it," said Shan Wang, Ph.D., of Stanford University and a member of the Center for Cancer Nanotechnology Excellence Focused on Therapy Response (Stanford CCNE). "If you can detect it early, you can have early intervention and you have a much better chance to cure that person."

Wang said the nanosensor technology also could allow doctors to rapidly determine whether a patient is responding to a particular course of chemotherapy. "We can know on day two or day three of treatment whether it is working or not, instead of a month or two later," he said. The sensor that Wang and his colleagues created, which uses magnetic detection nanotechnology they had developed previously, can detect a given cancer-associated protein biomarker at a concentration as low as one part out of a hundred billion (or 30 molecules in a cubic millimeter of blood). Although the basics of the magnetic detection technology used in the new biosensor were described last year in a paper in the *Proceedings of the National Academy of Sciences*, the new sensor is not only more sensitive than the previous one by several orders of magnitude, it also outperforms its predecessor, as well as detection methods now in clinical use, in several other ways.

The most impressive performance gain detailed in the *Nature Medicine* paper is that the researchers have now demonstrated that the magnetic-nano sensor can successfully detect cancerous tumors in mice when levels of cancer-associated proteins are still well below concentrations detectable using the current standard methodology, known by the acronym ELISA. "That is a

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critical finding for us because it says that in a realistic biological application — that of tumor growth in mice — we can actually see tumors before anything else could have detected them," said Sanjiv Gambhir, M.D., Ph.D., principal investigator of the Stanford CCNE. "I would say that the [first paper was] proof of concept of the technology, and the *Nature Medicine* paper is proof of concept of the technology working in a real-world application," he said. "It is one thing to have the technology show that it can work in principle; it is quite another to actually utilize it with real mouse blood samples from a real mouse growing a real tumor." In the *Nature Medicine* paper, the researchers show that the new magnetic-nano sensor has a broad range of sensitivity, from part-per-billion levels to concentrations six orders of magnitude, or a million times, greater. The best existing analysis methods, or assays, in clinical use can detect proteins over a range of concentrations of at most two orders of magnitude. Most of the sensing platforms currently in use are also limited to performing a single analysis at a time. To create a multiplexed assay, Wang and his colleagues attached the magnetic-nano sensors to a microchip in an array of 64 sensors, each of which can be set up to detect a different protein. As a result, the researchers can search for dozens of different proteins simultaneously during a single analysis. The new method is also faster than standard ELISA assays, with results typically available in one to two hours.

The researchers also demonstrated that the sensor is equally effective in every likely biological fluid, or matrix, that a doctor would want to analyze for cancer-associated proteins. Those fluids include urine, saliva, blood plasma (blood with the blood cells removed), serum (blood plasma with the factors that promote clotting removed) and cell lysates (the name applied to the cellular stew produced by dissolution of cells).

The key to the versatility of the magnetic-nano sensor and the broad range of concentrations it can detect lies in its use of magnetism and the wide range of ultra-sensitive magnetic detectors developed for the computer industry. The basic mechanism of detection employed in the magnetic-nano sensors is to capture proteins and disease markers using antibodies that naturally tend to bind to these molecules, known as antigens. The antibodies, dubbed "capture antibodies," are applied to a sensor, so that when the matrix of interest is placed onto the sensor chip, the appropriate antigens bind.

While the antigens are held fast, another dollop of antibodies is applied. These antibodies are attracted to a different molecular region of the antigens held on the sensors, and when the second set of antibodies binds to antigens, they effective seal them in an antibody sandwich. The researchers then apply a wash containing magnetic nanoparticle tags that have been tailored to fit specific antibodies. The magnetic nanotags attach themselves to the outer antibody on the sandwich, where they alter the ambient magnetic field in a small but distinct and detectable way that is sensed by the detector.

Another virtue of the technology, Wang said, is that it uses existing technology already in use in the data storage and semiconductor industries. Because of that, "It can be made relatively cheaply. It is [very similar to] the same sensor you are using in a hard disk drive to read a hard disk back," he said.

One of the next steps in the research, Wang said, is to test the magnetic-nano sensors on human blood samples taken from a long-term study in which researchers took blood from subjects prior to any of them being diagnosed with cancer. To this end, the Stanford team will be collaborating with the Fred Hutchison Cancer Research Center in Seattle and the Canary Foundation, a nonprofit organization that focuses on early diagnosis of cancer. This work, which is detailed in a paper titled, "Matrix-insensitive protein assays push the limits of biosensors in medicine," was supported by the NCI Alliance for Nanotechnology in Cancer, a comprehensive initiative designed to accelerate the application of nanotechnology to the prevention, diagnosis, and treatment of cancer. Investigators from MagArray Inc., also participated in this study. An abstract of this paper is available at the journal's Web site.

Nanotechnology Highlights

DETECTING THE UNDETECTABLE IN PROSTATE CANCER TESTING

By: Joe Alper

A team of Northwestern University researchers, using an extremely sensitive nanotechnology-based tool known as the biobarcode system, has detected previously undetectable levels of prostate-specific antigen (PSA) in patients who have undergone radical prostatectomy. This new assay, just one of many being developed by investigators at the Nanomaterials for Cancer Diagnostics and Therapeutics Center for Cancer Nanotechnology Excellence (Northwestern CCNE), is 300 times more sensitive than commercially available PSA tests.

The ability to easily and quickly detect very low levels of PSA may enable doctors to diagnose men with prostate cancer recurrence years earlier than is currently possible. Prostate cancer is the second leading cause of cancer death for men in the United States. (Only lung cancer is more deadly.) "We have defined a new zero for PSA," said Chad Mirkin, Ph.D., principal investigator of the Northwestern CCNE. "This level of sensitivity in detecting low concentrations of PSA will take the blinders off the medical community, especially when it comes to tracking residual disease." This study, which was led by Mirkin and C. Shad Thaxton, M.D., appears in the *Proceedings of the National Academy of Sciences* (PNAS).

"This new PSA assay may alter the management of patients who have been treated with surgery for prostate cancer," said William J. Catalona, M.D., director of the Clinical Prostate Cancer Program at Northwestern's Lurie Cancer Center. He was the first to demonstrate that the PSA test, a simple blood test, could be used as a screening tool for prostate cancer. "Studies have shown that postoperative radiation therapy given early to patients with adverse pathology, called adjuvant radiation, reduces the recurrence rate and improves survival," Catalona said. After the removal of the prostate gland, patients typically have PSA levels that are undetectable when measured using conventional diagnostic tools. "Because the 'nano-PSA assay' is more sensitive than the current commercially available PSA tests, it may allow physicians to target adjuvant radiation for patients destined to have a life-threatening tumor recurrence."

The study is an early indicator of how nanotechnology can be used to improve medical diagnostics and early cancer detection. In the case of prostate cancer recurrence following primary surgical treatment, patients with detectable but non-rising PSA levels could be reassured that their cancer will not recur. This reassurance potentially could be delivered much earlier than with conventional diagnostic tools. For patients with increasing levels of PSA, detected before conventional tools are able, doctors could diagnose a recurrence and intervene accordingly.

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Furthermore, the effectiveness of post-operative treatment could be assessed by monitoring a patient's PSA levels. Tracking PSA levels early, before conventional tools are able, may allow doctors to validate treatments for recurrent cancer, such as radiation, hormone therapies and chemotherapies. The most effective will be able to keep down PSA levels.

"The first route to a new therapeutic is a good diagnostic tool, and that's what we have here," said Mirkin. "This bio-barcode assay, or a variant of it, could be a commercial tool in as little as 18 months. The technology is there. Now it's a business decision."

PSA is a protein produced by the cells of the prostate gland and found in the bloodstream. This pilot study looked at serum samples from 18 post-prostatectomy patients collected over the course of a number of years.

The researchers were able to reliably and accurately quantify PSA values at less than 0.1 nanograms per milliliter, the clinical limit of detection for commercial assays. The lower limit of detection for PSA using the bio-barcode assay is approximately 300 times lower than the lower limit of detection for commercial tests. The PSA measurements were used to classify the patients as either having no evidence of disease or having a relapse of disease. The Northwestern team is now conducting a similar retrospective study of 260 patients and eventually plans to do a large prospective study.

The ultra-sensitive technology is based on gold nanoparticle probes decorated with DNA and antibodies that can recognize and bind to PSA when present at extremely low levels in a blood sample. A magnetic microparticle, outfitted with a second antibody for PSA, also is used in the assay. When in solution, the antibody-functionalized particles "recognize" and bind to PSA, sandwiching the protein between the two particles.

The key is that attached to each tiny gold nanoparticle are hundreds of identical strands of DNA. Mirkin calls this "bar-code DNA" because they have designed it as a label specific to the PSA target. After the "particle-protein-particle" sandwich is removed magnetically from solution, the DNA is removed from the sandwich and read using a Verigene® ID system, a nanotechnology platform designed to detect and quantify DNA. The amount of PSA present is calculated based on the amount of bar-code DNA. For each molecule of captured PSA, hundreds of DNA strands are released, which is one of the ways the PSA signal is amplified.

This work, which is detailed in a paper titled, "Nanoparticle-based bio-barcode assay redefines 'undetectable' PSA and biochemical recurrence after radical prostatectomy," was supported by the NCI Alliance for Nanotechnology in Cancer, a comprehensive initiative designed to accelerate the application of nanotechnology to the prevention, diagnosis, and treatment of cancer. Investigators from the Innsbruck Medical University in Austria also participated in this study. An abstract of this paper is available at the journal's Web site.

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Nanomedicine Webinar Series Session 6: Development and Application of DNA Nanoparticles. ACCME accredited Webinar Series produced by UC San Diego NanoTumor Center. Speaker: Bradley Messmer, PhD, Assistant Project Scientist, Translational Oncology Program, UC San Diego Moores Cancer Center.

The NCI Alliance Nanotechnology in Cancer Bulletin is a collaborative effort developed and facilitated by the Communications and Integration Working Group (CIWG) of the Alliance program. The group is currently led by Alliance co-chairs, Lynn Coulter (Washington U. S-CCNE) and Diane Clark Robinson (NSBCC-CCNE), with coordination from NCI co-chair, Krzysztof Ptak, Ph.D.

The CIWG's mission is to catalyze effective Alliance-wide and external communications, facilitate Alliance team science integration, create education outreach opportunities, and leverage best practices.

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