SPEAKER TITLES AND ABSTRACTS

Ajlan Al Zaki

Title: Predicting Therapeutic Response to External Beam Radiation Therapy Using a Multifunctional Nanoplatform.

Abstract: Current radiation-based therapies are often limited by the maximum allowable dose tolerated by adjacent healthy tissues. Challenges associated with accurate tumor margin delineation often exacerbate efforts to maximize tumor dosage while minimizing the damaging off-target effects of radiation. To help overcome this shortcoming, Gold and Superparamagentic iron oxide (SPIO)-loaded polymeric Micelles (GSMs) were designed to help demarcate tumor boundaries by magnetic resonance imaging (MRI) and enhance radiosensitization via a gold-mediated photoelectric effect. Intravenous injection of GSMs into fibrosarcoma-bearing mice led to selective accumulation of GSMs in the tumors, enabling non-invasive MRI imaging and tumor delineation. Tumors were subsequently exposed to 150 kVp X-ray therapy. The ninety day survival of GSM-injected, irradiated mice was 71% versus 29% with x-rays alone, and 0% with GSM alone. Interestingly, the rate of volume decrease of irradiated tumors correlated linearly with the amount of GSM-generated contrast visualized on MRI scans. The contrast-enhancing capabilities of this dual-metal nanoparticle micelle system could thus enable a more personalized approach to a patient's cancer therapy, by assisting with treatment planning and in helping to predict tumor response.

Theresa Busch

Title: Under Construction: Light-Activated Drug Delivery in Combination with Photodynamic Therapy

Abstract: Photodynamic therapy (PDT) is a drug- and light-based approach to the treatment of diseased tissue that involves the activation of a photosensitizer with specific wavelengths of visible radiation. This leads to the production of cell- and tissue-damaging reactive oxygen species. Thus, the effectiveness of PDT can be related to the accumulation of photosensitizer in malignant tissue and the delivery of a sufficient fluence of photosensitizer-exciting light to this area. Selectivity is afforded by differentials in photosensitizer accumulation between diseased and normal tissue, as well as by the boundaries of the illumination field. Given the local and relatively selective effects of PDT, there is much interest in developing it as one component of a combined modality regimen for cancer therapy. Toward this goal, we have initiated studies of polymersomes assembled from diblock copolymers as nanoparticle carriers of both photosensitizing and chemotherapy drugs. We expect that these particles will lead to PDT-mediated cytotoxicity upon exposure to light, accompanied by the localized delivery of chemotherapeutic. Light-activated release of chemotherapeutic should provide for a local therapeutic effect while reducing systemic exposure relative to that found with delivery of free drug. Other advantages include the potential to target polymersomes to tumor cells and the ability to deliver multiple drugs of differing hydrophobicities via a single particle.

Dennis Discher

Title: A Passport to Nanomedicine Success

Abstract: The body's immune system exists to identify and destroy foreign objects, whether they are bacteria, viruses, or even nanoparticles designed to deliver drugs or dyes. Unlike the learned response of the adaptive immune system, which includes the targeted antibodies that are formed after a

vaccination, the innate immune system tries to destroy everything it doesn't recognize as being part of the body. Macrophages find, engulf and destroy invaders, and proteins in blood work in tandem, adhering to objects in the blood stream and drawing macrophages' attention. Earlier attempts to circumvent it involved coating the particles with polymer brushes, but these brushes only slow down the macrophage-signaling proteins so we tried a different approach: Convincing the macrophages that the nanoparticles were part of the body and shouldn't be cleared. There may be other molecules that help quell the macrophage response, but human CD47 found on all cells is clearly one that says, 'Don't eat me'. After chemically synthesizing a minimal CD47 peptide, we attached it to conventional nanoparticles that could be used in a variety of experiments. Anyone can make the peptide and put it on whatever they want, but our initial studies have improved circulation and show better imaging of model mouse tumors as well as improved efficacy of an anti-cancer drug-delivery nanoparticle.

Jay Dorsey

Title: Selective Targeting of Brain Tumors with Gold Nanoparticle-Induced Radiosensitization.

Abstract: Successful treatment of brain tumors such as glioblastoma multiforme (GBM) is limited in large part by the cumulative dose of Radiation Therapy (RT) that can be safely given and the blood-brain barrier (BBB), which limits the delivery of systemic anticancer agents into tumor tissue. Consequently, the overall prognosis remains grim. Herein, we report our pilot studies in cell culture experiments and in an animal model of GBM in which RT is complemented by PEGylated-gold nanoparticles (GNPs). GNPs significantly increased cellular DNA damage inflicted by ionizing radiation in human GBM-derived cell lines and resulted in reduced clonogenic survival (with dose-enhancement ratio of ~1.3). Intriguingly, combined GNP and RT also resulted in markedly increased DNA damage to brain blood vessels. Follow-up *in vitro*experiments confirmed that the combination of GNP and RT resulted in considerably increased DNA damage in brain-derived endothelial cells. Finally, the combination of GNP and RT increased survival of mice with orthotopic GBM tumors. Prior treatment of mice with brain tumors resulted in increased extravasation and in-tumor deposition of GNP, suggesting that RT-induced BBB disruption can be leveraged to improve the tumor-tissue targeting of GNP and thus further optimize the radiosensitization of brain tumors by GNP. These exciting results together suggest that GNP may be usefully integrated into the RT treatment of brain tumors, with potential benefits resulting from increased tumor cell radiosensitization to preferential targeting of tumor-associated vasculature.

Piotr Grodzinski

Title: Cancer Nanotechnology – An Opportunity for New Diagnostics and Therapeutics – View from the NCI Alliance for Nanotechnology in Cancer

Abstract: National Cancer Institute is engaged in efforts to harness the power of nanotechnology to radically change the way we diagnose and treat cancer. Novel and multi-functional nanodevices will be capable of detecting cancer at its earliest stages, pinpointing its location within the body, delivering anticancer drugs specifically to malignant cells, and determining if these drugs are effective. Functionalized nanoparticles would deliver multiple therapeutic agents to tumor sites in order to simultaneously attack multiple points in the pathways involved in cancer. Such nano-therapeutics are expected to increase the efficacy of drugs while dramatically reducing potential side effects. *In vivo* biosensors would have the capability of detecting tumors and metastatic lesions that are far smaller than those detectable using current, conventional technologies. Furthermore, they will provide rapid information on whether a given therapy is working as expected.

In order to further these research goals, NCI formed a program called Alliance for Nanotechnology in Cancer which was initiated in 2004. The Alliance invests approximately $150 million for the funding period of 5 years to pursue applied nanotechnologies for cancer detection, therapy, and prevention with an aim to achieve clinical translational stage of these technologies towards culmination of the program. The Alliance funds Centers of Cancer Nanotechnology Excellence, the development of nanotechnology platforms, and two training programs: Cancer Nanotechnology Training Centers and Path to Independence Awards. An intramural arm of the Alliance - Nanotechnology Characterization Laboratory provides a characterization support to evaluate clinically promising nanomaterials and establish their physical, pharmacological and toxicological characteristics.

The nine Centers of Cancer Nanotechnology Excellence are: *Carolina Center of Cancer Nanotechnology Excellence* at the University of North Carolina, *Center for Cancer Nanotechnology Excellence and Translation* at Stanford University, *Center for Cancer Nanotechnology Excellence at Johns Hopkins University, Center for Translational Cancer Nanomedicine* at Northeastern University, *Dartmouth Center for Cancer Nanotechnology Excellence* at Dartmouth College, *MIT-Harvard Center of Cancer Nanotechnology Excellence, Nanomaterials for Cancer Diagnostics and Therapeutics* at Northwestern University, *Nanosystems Biology Cancer Center* at California Institute of Technology, *Texas Center for Cancer Nanomedicine* at the University of Texas Health Science Center.

This presentation will describe the details behind the organization and science and technology of the Alliance.

Daniel Hammer

Title: Designer Vesicles and Nano-materials from a Recombinant Surfactant Protein.

Abstract: Vesicles are biomimetic capsules with large payloads for drug delivery and imaging. Vesicles are self-assembled from surfactants of appropriate physical properties, such as phospholipids. We have developed vesicles from a recombinant protein surfactant, oleosin, in which the materials properties, structure, and targeting can be widely tuned. We show that some variants of this protein can assemble into vesicles, but others can make unique materials, such as micelles, worm-like micelles, fibers and sheets. We address the opportunities for building functionality into the protein, such as a pH switch and protease cleavable domains.

Tayyaba Hasan

Title: Photoactivatable Multi-inhibitor Nanoconstructs in Cancer Therapy

Abstract: Photodynamic Therapy (PDT) is a photochemistry based therapeutic modality for cancer and non-cancer pathologies. It involves the light activation of specific molecules to generate activated molecular species that are toxic to neighboring biological targets. There have been significant advances in the understanding of PDT-induced cell death and cell injury mechanism. These studies indicate that in the process of dying or injury, the target cells mount a robust response secreting cytokines and regulating cellular molecules that can be deleterious to the cell death process. This leads to an incomplete tumor destruction response. To combat this barrier to optimal tumoricidal activitiy, we have developed multi-compartmental nanoconstructs encapsulating multiple inhibitors. Results from initial studies using these constructs *in vitro* in 3D models and *in vivo* murine models will be presented.

Costas Koumenis

Title: Nanoparticle Delivery of Curcumin and Cisplatin for Head and Neck Cancer.

Abstract: Head and neck cancer (HNC) is the sixth most common form of cancer worldwide, with an estimated 500,000 new cases diagnosed annually. While advances in the study of altered fractionated radiotherapy (and the use of concomitant cisplatin and radiotherapy (RT) have significantly improved survival, the 5 year survival rate remains below 50%. Therefore, novel targeted approaches are needed to improve local control and overall survival in HNC.

Our group has been investigating the potential radiosensitizing effects of natural polyphenolics particularly curcumin. Curcumin, a polyphenol extracted from rhizomes of the plant Curcuma longa L is a widely studied chemopreventive agent which was shown to have a low toxicity profile in multiple human clinical trials. Recently, we demonstrated the effectiveness of curcumin as a radiosensitizer in flank and orthotopic models of HNC by targeting Thioredoxin reductase 1 (TxnRd1), an antioxidant enzyme which is overexpressed in HNC and which plays a critical role in HNC progression and as a chemo/radiosensitizing target. Interestingly, we also found that curcumin afforded significant protection to normal fibroblasts irradiated under identical conditions. However, the use of curcumin clinically has been limited by is its low bioavailability outside the GI tract.
To improve the bioavailability of curcumin formulations, we have been working to generate a nanoparticle-based formulation of curcumin that will allow for a rapid translation to the clinic. Our approach is to load curcumin into the membranes of nano-sized polymer vesicles (polymersomes) assembled from diblock copolymers of polycaprolactone (PCL) and polyethylene oxide (PEO). Polymersomes retain many of the advantages of nanoparticles for drug delivery, but they additionally have significant advantages and can extend nanoparticle functionality through their ability to encapsulate high payloads of both hydrophilic and hydrophobic drugs in tandem. PCL and PEO are both FDA approved homopolymers for use in biomedical materials. Hydrophilic drugs are readily encapsulated in the lumen of the vesicle by solubilization in aqueous solution. Thus, polymersomes are advantageous over both liposomes and solid nanoparticles, have long circulation times, have superior payloads, and can be made in sizes appropriate for cancer drug delivery.

In preliminary studies we generated curcumin-encapsulated polymersomes which released curcumin into the aqueous media in a time-dependent manner as evidenced by the characteristic peak absorption spectrum of curcumin at a peak wavelength of 425nm. Moreover, polymersome-encapsulated curcumin is able to inhibit TxnRd1 activity and elicit cytotoxicity in FaDu cells in a dose-dependent manner. Following an 8h pretreatment, the curcumin-loaded polymersomes induced significant radiosensitization of FaDu cells, albeit with lower dose-enhancement ratio compared to free curcumin, due to slower release kinetics. Moreover, we have also demonstrated that these polymersomes encapsulate both curcumin in the hydrophobic membrane and cisplatin in the aqueous core and can deliver them to tumor cells. Dual-loaded with curcumin and cisplatin polymersomes decrease cell survival more than singly loaded vesicles. Future studies will determine whether these bifunctional polymersomes demonstrate enhanced cytotoxicity to animal models of solid tumors.

Vladimir Muzykantov

Title: Targeting drugs and probes to vascular endothelium

Abstract: Endothelial cells represent an important therapeutic target in vascular, pulmonary and other disease conditions including inflammation, thrombosis, ischemia and tumor growth. Alas, most drugs and imaging probes have no endothelial affinity, which hinders therapeutic and imaging modalities. To optimize endothelial delivery we devise nanocarriers targeted to the endothelial surface molecules. In particular, cell adhesion molecules (CAMs) represent attractive targets for endothelial drug delivery in the context of inflammation and oxidative stress. Nanocarriers targeted to endothelial CAMs afford superior therapeutic interventions and PET-imaging of inflamed endothelium in animal models. Tuning of nanocarrier design (avidity, geometry, and binding to specific epitopes) helps to control

parameters of drug delivery including binding to selected endothelial cell phenotypes, intracellular addressing and therapeutic effects.

Shuming Nie

Title: Nanotechnology for Multiplexed Cancer Detection and Image-Guided Surgery.

Abstract: The development of biocompatible nanoparticles for in-vivo molecular imaging and targeted therapy is an area of considerable current interest across a number of science, engineering, and biomedical disciplines. The basic rationale is that nanometer-sized particles have functional and structural properties that are not available from either discrete molecules or bulk materials. When conjugated with biomolecular targeting ligands such as monoclonal antibodies, peptides or small molecules, these nanoparticles can be used to target malignant tumors with high specificity and affinity. In the "mesoscopic" size range of 10-100 nm diameter, nanoparticles also have large surface areas for conjugating to multiple diagnostic (e.g., optical, radioisotopic, or magnetic) and therapeutic (e.g., anticancer) agents. Recent advances have led to the development of biodegradable nanostructures for drug delivery, iron oxide nanocrystals for magnetic resonance imaging (MRI), quantum dots (QDs) for multiplexed molecular diagnosis and in-vivo imaging, and nanoscale carriers for short-interfering RNA (siRNA) delivery. We have developed biocompatible and nontoxic nanoparticles for in-vivo tumor targeting and detection based on self-assembled nanostructures and pegylated colloidal gold. In particular, colloidal gold has been safely used to treat rheumatoid arthritis for 50 years, and has recently been found to amplify the efficiency of Raman scattering by 14-15 orders of magnitude. Here we show that large optical enhancements can be achieved under in-vivo conditions for tumor detection in live animals. A major finding is that small-molecule Raman reporters such as organic dyes are not displaced but are stabilized by thiol-modified polyethylene glycols. These pegylated SERS nanoparticles are considerably brighter than semiconductor quantum dots with light emission in the near-infrared window. When conjugated to tumor targeting ligands such as single chain variable fragment (ScFv) antibodies, the conjugated nanoparticles are able to target tumor biomarkers such as epidermal growth factor receptors (EGFR) on human cancer cells and in xenograft tumor models. ***Acknowledge:*** This work was supported in part by grants from the National Cancer Institute (U54 CA119338, RC2 CA148265, and R01CA163256). S.N. is a Distinguished Scholar of the Georgia Cancer Coalition (GCC).

Sergei Vinogradov

Title: Dendritic upconverting nanoparticles form multiphoton imaging and sensing

Abstract: Lanthanide-based upconverting nanoparticles (UCNPs) form a class of imaging agents with unique non-linear optical properties. However, utilization of UCNPs in biomedical arena has been hampered by the lack of robust methods of their solubilization and surface functionalization. We show that non-covalent modification of UCNPs with polyanionic porphyrin-dendrimers converts them into stable, water-soluble, non-toxic imaging probes. UCNP-to-porphyrin excitation energy transfer enables analyte-sensitive detection by upconverted luminescence. As an example we demonstrate that UCNP/porphyrin-dendrimers make up ratiometric pH nanosensors for physiological pH range. Exceptionally high apparent multiphoton absorption cross-sections of dendritic UCNPs combined with their excellent bio-compatibility make them directly suitable for physiological imaging. Using a low power continuous wave (CW) laser for excitation we performed mapping of mouse cortical vasculature with micron-scale resolution down to 400 mm under the brain surface, setting the first precedent of true in vivo two-photon microscopy with CW sources.

Rong Zhou

Title: MRI-based Appraisal of Nanoparticles: Promise and Pitfalls.

Abstract: As an imaging modality, magnetic resonance imaging is unique for the appraisal of nanoparticles. MRI has superb soft tissue contrast and true tomographic capability that is not limited by tissue penetration. Importantly, the versatility in pulse sequence design allows extraction of physiological relevant parameters. This talk will provide an overview on two aspects of MRI-based appraisal of nanoplatforms. 1) Apparent diffusion coefficient (ADC) of water as a biomarker for detection of early responses of tumors to nanomedicine will be discussed along with technical pitfalls. 2) Kinetics of blood clearance and tissue (tumor or liver) uptake of nanoparticles modulated by host physiology or properties of nanoparticles will be examined

POSTER TITLES AND ABSTRACTS

1. Lauren Brady, DO Oldridge, VG Cheung

Title: RNA Processing in Response to Ionizing Radiation

Abstract: Radiation exposure is common in diagnostic and therapeutic settings. However, the mechanisms underlying individual response to radiation remain poorly understood. In this project, we are studying the mechanistic basis of cellular and gene expression responses to radiation with the ultimate goal of improving ways to predict and influence therapeutic outcomes in radiotherapies. We carried out RNA-sequencing of cultured B-cells from normal individuals before and after exposure to ionizing radiation. We detected 166 genes with various changes in RNA processing following radiation exposure, leading to isoforms with differences in expressed exons, transcription start sites and UTR lengths. Among these genes, the shorter isoforms were preferentially expressed in irradiated cells ($P_c < 0.01$). These shorter gene isoforms differ in transcript stability and encode proteins with altered functional domains, thus likely to have different molecular functions. One example is the histone methyltransferase, SUV420H1, whose shorter isoform maintains the catalytic domain but lacks a region that mediates heterochromatin binding. Even though the short and long isoforms of this gene share some common targets, by RNA interference, we found several hundred genes whose expression levels are influenced by the short isoform alone. Many genes play roles in cell signaling and alternative splicing, such as an interferon alpha-inducible protein, *IFI6*, a mediator complex subunit, *MED17*, and a serine/threonine kinase involved in chromatin assembly, *TLK1* ($P < 0.01$). Thus, the differential expression of the short and long isoforms of *SUV420H1* affects genes that mediate chromatin dynamics, cell cycle progression and transcriptional regulation. In this presentation, I will show data on the roles of RNA processing and isoform-specific regulation in radiation response.

2. David Busch

Title: Statistical Tumor Localization and Monitoring in Diffuse Optics

Abstract: Diffuse optics permits measurement of multiple physiologically important parameters without invasive or ionizing procedure. We have applied statistical techniques to both extract the location of tumors from multi-parameter 3D tomograms and to track the evolution of tumors over the course of neoadjuvant chemotherapy treatment. Our results in small populations are encouraging and point the way towards future studies.

3. Peter Chhour, Pratap C. Naha, Muredach Reilly, Victor A. Ferrari, David P. Cormode

Title: Labeling monocytes with gold nanoparticles for tracking their recruitment to atherosclerotic plaque with computed tomography

Abstract: Atherosclerosis is the primary cause of heart attacks. It is characterized by thickening of artery walls due to plaque development. Monocytes migrate into the arterial intima and eventual differentiate into foam cells, which lead to rupture prone plaques. A plaque rupture in the coronary arteries can block the artery and lead to myocardial infarction and death. Computed tomography (CT) is the most commonly employed technique to image the coronary arteries. The use of gold nanoparticles (AuNPs) as CT contrast agents is appealing due to gold's biocompatibility and strong X-ray absorption. CT tracking of monocytes with gold nanoparticles may lead to improved knowledge of monocyte behavior and early detection of rupture prone plaques allowing for clinical intervention.

Gold nanoparticles were synthesized via the Turkevich method and subsequently capped with thiol ligands, providing stability in biologically relevant media, i.e. phosphate buffered saline (PBS). Ligands found to stabilize AuNPs in PBS were 11-mercaptoundecanoic acid (11-MUDA), 16-mercaptiohexadecanoic acid (16-MHDA), 4-mercapto-1-butanol (4-MB), poly(ethyleneimine) (PEI), and 11-mercaptoundecyl-tetra(ethylene glycol) (MTEG). TEM images revealed an average core diameter of 15 nm for ligand stabilized AuNPs. These formulations were incubated with a monocyte cell line (RAW 264.7). Cell viability was analyzed through the LIVE/DEAD assay, which showed that the AuNPs were well tolerated by monocytes up to 0.5 mg/ml for each formulation. Cells were incubated for 24 hours with each AuNPs formulation and the resulting cell pellets were imagined with a clinical CT scanner. The results demonstrated strong contrast generation for 11-MUDA and 4-MB as compared to other particles.

We have demonstrated the preliminary principles of labeling monocytes with AuNPs to study their recruitment to atherosclerotic plaques with CT. This work shows that AuNPs can be extensively loaded into monocytes with low toxicity and create strong CT contrast

4. Hoon Choi, Hui Qiao, AnnMarie Chacko, Ting Liu, Rong Zhou, I-Wei Chen

Title: Biomimetic, synthetic and self-assembled nanoplatform for targeted delivery of high-payload paclitaxel to Her2/neu breast cancer.

Abstract: Lipoproteins are natural nanoparticles for transporting water-insoluble lipids such as cholesterol and triglycerides encapsulated inside a monolayer shell of phospholipids; together they form a micelle that is stabilized by Apoproteins. In the case of high density lipoprotein (HDL), the Apo-A1 protein contains a large number of amphipathic α-helical peptides that cover a substantive portion of the micelle surface and may serve as a surfactant by interaction with the phospholipids. Mimicking HDL but avoiding any protein component, we test whether an amphipathic α-helical peptide (DWFKAFYDKVAEKLKEAF**)** known as 3F can stabilize a phospholipid micelle encapsulating a core of superparamagnetic iron oxide (IO) covered with cholesterol; the IO-cholesterol core is oily and replaceable by a hydrophobic core consisting of paclitaxel molecules. The nanoplatform is further decorated with anti-Her2/neu peptides (AHNP) resulting in AHNP-3F-IO (for magnetic resonance imaging) and AHNP-3F-Paclitaxel (for therapy). We show that less than 5 % of 3F can sufficiently stabilize sub-50 nm micelles to high paclitaxel concentrations. The IC50 of the 25 nm AHNP-3F-Paclitaxel nanoparticles for BT-474 human breast cancer cells (Her2/neu positive) is 23 nM but is not reached at 5000 nM for Her2/neu negative MCF-7. In mice bearing BT-474 xenografts, pharmacokinetics and biodistribution suggest that AHNP-3F-Paclitaxel nanoparticles have favorable tumor/ muscle ratio (> 8) at 24 hrs post injection. To evaluate their therapeutic effect, 25 nm AHNP-3F-Paclitaxel nanoparticles were injected i.v. at 20 mg/kg paclitaxel dose each day for 3 consecutive

days; control groups received either free-paclitaxel (clinical formulation) or empty-nanoparticles (no drug). The apparent diffusion constant (ADC) of water in the tumor was measured by MRI before and 2 days after the last injection; ADC_{50} (the 50^{th} percentile ADC) post injection is significantly higher in AHNP-3F-Paclitaxel group compared to free-paclitaxel (P=0.01776) or empty-nanoparticles (P=0.0000545) group. In summary, apoprotein mimetic peptide (3F) stabilizes phospholipid micelles, leading to a nanoplatform that has a high-payload of paclitaxel.

5. SH Chung, KA Cengel, CB Simone, JS Friedberg, SM Albelda, ME Winters, J Menko, JC Finlay, TC Zhu, E Glatstein, AG Yodh, TM Busch

Title: Cytokine-reported inflammation is correlated with hypoxia during intraoperative Photodynamic therapy: a study by diffuse optical spectroscopy in mesothelioma patients.

Abstract: The presence of pre-existing or treatment-initiated hypoxia can limit therapeutic response to photodynamic therapy (PDT). In a clinical trial of intra-operative HPPH-mediated PDT for malignant pleural mesothelioma, we have used Diffuse Correlation Spectroscopy (DCS) and Diffuse Optical Spectroscopy (DOS) to measure the effect of PDT on blood flow and hemoglobin concentration, respectively. Patients initially underwent debulking surgery for mesothelioma, including a radical pleurectomy. PDT immediately followed by delivering 661 nm illumination through a dilute intralipid solution filling the thoracic cavity. DCS and DOS measurements were made using a probe sutured to tissue within the illumination field. Resulting data showed the extent of treatment-created hypoxia, measured as reductions in the concentration of oxy-hemoglobin, to vary substantially among the first 8 patients that we have analyzed. Furthermore, among these patients, those with less PDT-created hypoxia trended toward a better prognosis than those with more severe treatment-associated hypoxia. In an effort to assess potential causes for inter-patient variability in PDT-created hypoxia, blood samples were collected at multiple timepoints and analyzed for cytokine induction. Unsurprisingly, these revealed that surgery could lead to increases in the pro-inflammatory cytokine IL-6. In fact, a correlation was found between the relative increase in IL-6 as a result of surgery and the subsequent development of PDT-created hypoxia (R=0.72, p-value: 0.044). This suggests to us that IL-6 induction by surgery may have led to vasoconstriction during PDT, and DOS measurement of oxy-hemoglobin during PDT may be able to communicate the effect of surgery and related prognosis of the patients.

6. James Z. Hui, Andrew Tsourkas, Penn Dep. Of Bioengineering

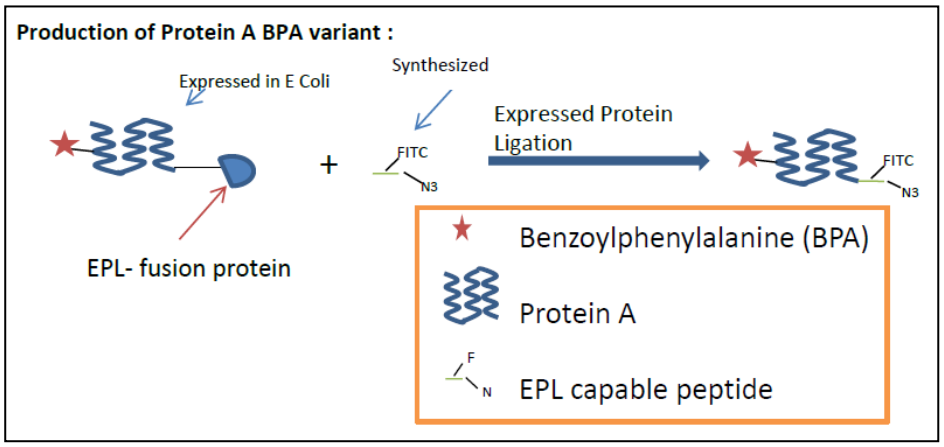
Title: Site-Specific Covalent Conjugation of IgG using a Recombinantly Expressed Protein A Domain Incorporating a Non-Natural Amino Acid

Introduction: Antibodies, most commonly IgGs, have been widely used as targeting and therapeutic ligands. When conjugating antibodies onto imaging or therapeutic agents, it is necessary to ensure that the conjugated molecules or surfaces are not near the antigen binding domains. However, current conjugations methods are either non-site-specific or complex. We developed a reliable method to ensure site-specific antibody conjugation by incorporating a UV active non-natural amino acid into the Fc-binding domain of recombinantly expressed Protein A. Upon exposure to long wavelength UV light, a covalent link is formed between protein A and IgG. Combining this technology with expressed protein ligation (EPL), we can achieve site-specific labeling of IgGs onto nearly any surfaces, diagnostic and therapeutic agents**.**

Results: We used E Coli. and EPL to recombinantly expressed Protein A variants possessing an UV active non-natural amino acid benzoylphenylalaine (BPA), a fluorescent label, and a Click-capable

azide. These allow our Protein A to be visualized and to be easily attached to various molecules using the highly efficient copper-free "Click" chemistry. We have demonstrated that our Protein A variants have high expression yields. We have also shown that Protein A variants can specifically bind to and be UV cross-linked (350nm) onto a broad range of IgGs. We further demonstrated that these IgG-Protein A conjugates can be efficiently "clicked" onto dibenzocyclooctyne-derivatized nanoparticles and microplates and achieving higher detection sensitivities as a result of the site-specific conjugation. Lastly, we demonstrated that the crosslinking is not inhibited in complex biological solutions (i.e. ascites, tissue culture supernatants) hence greatly simplifying the conjugation procedure with monoclonal antibodies.

Conclusions: We have successfully demonstrated that recombinantly expressed, photoactive Protein A domains can be used to site-specifically and covalently conjugate IgGs. Our conjugation method is ideal because: 1. It is broadly compatible with most native full-length antibodies. 2. It conjugates IgGs at their Fc domains, hence avoiding steric hinderance or destructions of the antigen binding domain. 3. The non-harsh yet versatile nature of our technique suggests various potential applications both in therapy and diagnostics fields.



7. B Judy, O Okusanya, ET Segal, J Quatromoni, B Madajewski, D Holt, S Singhal

Title: A Novel Small Portable Imager of Fluorescence (SPIF) For Intraoperative Imaging of Lung Tumors

Abstract: Introduction: Up to 20% of patients that undergo "curative" surgical resection leave the operating room with cancer cells at the margins, satellite lesions or lymph nodes. Optically visible fluorescent dyes exist that can visually enhance tumor deposits, however, require complex imaging systems which have limited utility in the operating room. Our group hypothesized a light-weight, hand-held near-infrared (NIR) camera system would be adaptable in the operating room for real-time intraoperative imaging.

Methods: Over several months, we developed several CCD cameras with a broad range of filters, narrow band light sources and tracers to examine murine and canine tumor tissue. Once constructed, we tested our imaging system on three canine patients with spontaneous lung tumors scheduled for surgery. At the time of operation, each had their lung cancers imaged in vivo and on the back table. Histopathology was used to confirm the diagnosis.

Results: Ultimately, we used a CCD camera mounted on a metal plate with a reticulating 760nm bandpass filter and a 720nm LED spotlight (The Small Portable Imager of Fluorescence — SPIF). The images captured by the camera were integrated with commercially available software. The dogs were injected with indocyanine green prior to their planned pulmonary resections. All 3 patients underwent uneventful pulmonary resection with intraoperative imaging with no adverse events. All tumors were fluorescent and could be easily visualized during imaging. All findings were consistent on final pathology.

Conclusion: The results suggest that NIR imaging using our cost effective portable system can accurately and reliably detect canine lung cancer. This imaging technology has the potential to dramatically improve the care for patients with cancer who undergo for surgery and we show that it can readily and efficiently be implemented.

8. Roshan Karunamuni, Ajlan Al-Zaki, Anatoliy Popov, David Cormode, Jim Delikatny, Andrew Tsourkas, Andrew D.A. Maidment

Title: Silica-encapsulated silver nanoparticles as contrast agents in dual-energy breast x-ray imaging.

Abstract: Contrast-enhanced dual-energy (CEDE) breast radiography increases the conspicuity of radiographic imaging agents by suppressing the anatomical signal variation in the body. Traditionally, CEDE breast imaging has employed iodinated contrast agents. Through analytic modeling, we have demonstrated that silver is a superior DE imaging agent to iodine in the mammographic energy range.
We have developed non-toxic, biologically-stable, silica-encapsulated silver nanoparticles as contrast agents in breast x-ray imaging. The silver core is synthesized by reduction of silver nitrate using polyvinylpyrriodine at elevated temperature. The PVP-stabilized silver cores are then encapsulated within a silica shell by mixing with tetraethoxysilane for 12 hours. Finally, the silica-silver nanoparticles are made biologically-compatible by the addition of a polyethylene glycol (PEG) surface layer. This is a two-step process, in which the nanoparticles are first made lipophilic by covalently linking octadecanol to the silica surface. Next, an amphiphilic ligand that contains a lipophilic polycaprolactone chain and a hydrophilic PEG chain is bound to the surface of the nanoparticle. Transmission electron micrographs of the particles showed a solid silver core with an average diameter of 39 ± 6 nm and a total diameter (including the silica shell) of 102 ± 9 nm.
Immunocomprimised mice were injected with 600 mg/kg of silica-silver nanoparticles. Immediately post-injection, the animals were imaged initially using a small animal, micro-CT scanner at 45 kVp. The images demonstrated significant enhancement in the heart, liver, and primary and peripheral blood vessels. The mice were re-imaged 24 hours after the injection, and the majority of the particles were located in the spleen and liver, with minimum enhancement observed in the heart and other organs. We are currently working to complete dual-energy imaging studies.

9. Dayton D. McMillan, Daniel Chen, Michele M. Kim, Xing Liang, Timothy C. Zhu

Title: Parameter Determination for Singlet Oxygen Modeling of BPD Mediated PDT

Abstract: Photodynamic therapy (PDT) offers a cancer treatment modality capable of providing minimally invasive localized tumor necrosis. To accurately predict PDT treatment outcome based on pre-treatment patient specific parameters, an explicit dosimetry model is used to calculate apparent

reacted $^1O_2$ concentration ($[^1O_2]rx$) at varied radial distances from the activating light source inserted into tumor tissue and apparent singlet oxygen threshold concentration for necrosis ($^1O_2]rx, sd$) for type-II PDT photosensitizers. Inputs into the model include a number of photosensitizer independent parameters as well as photosensitizer specific photochemical parameters ξ, σ, and β. To determine the specific photochemical parameters of benzoporphyrin derivative monoacid A (BPD), mice were treated with BPDPDT with varied light source strengths and treatment times. All photosensitizer independent inputs were assessed pre-treatment and average necrotic radius in treated tissue was determined post-treatment. Using the explicit dosimetry model, BPD specific ξ, σ, and β photochemical parameters were determined which estimated necrotic radii similar to those observed in initial BPD-PDT treated mice using an optimization algorithm that minimizes the difference between the model and that of the measurements. Photochemical parameters for BPD are compared with those of other known photosensitizers, such as Photofrin. The determination of these BPD specific photochemical parameters provides necessary data for predictive treatment outcome in clinical BPD-PDT using the explicit dosimetry model.

10. Casey McQuade

Title: A multi-functional nanoplatform for imaging, radiotherapy, and the prediction of therapeutic response

Abstract: Clinicians face many challenges when treating patients with solid tumors. These challenges include discerning tumor boundaries through imaging techniques, treating the tumor specifically while sparing healthy tissue, and predicting a patient's response to therapy in order to optimize future treatments. External beam radiation therapy is especially sensitive to tumor localization, as imaging data determines beam targeting and therefore the amount of radiation both the tumor and healthy tissues will receive. The unique properties of metal-based, micelle-encapsulated nanoparticles may allow clinicians to overcome these barriers to effective treatment while not increasing the toxic burden on patients. To this effect, we report the design of a gold- and superparamagnetic iron oxide (SPIO)-loaded polymeric micelle (GSM) capable of providing both radiosensitization and magnetic resonance imaging (MRI) contrast. GSMs prepared with a hydrodynamic diameter of approximately 100 nm had a transverse relaxivity (r2) of 196 $mM^{-1}s^{-1}$ and a detection sensitivity of 3.48 μg Fe/mL (12.5 μg Au/mL) in PBS. Furthermore, when human fibrosarcoma cells were treated with GSMs and irradiated with 6 Gy of 150 kVp, 15 mA x-rays, a 2.2-times increase in DNA double strand breaks was measured by γ-h2ax assay, compared with cells treated with radiation alone. Intravenous injection of GSMs into flank tumor-bearing nu/nu mice led to selective accumulation of GSMs in the tumors (6.64 % injected dose / gram tissue), enabling non-invasive MRI imaging and clear delineation of tumor margins after 24 hours. Subsequent irradiation with 4 Gy of 150 kVp x-radiation therapy led to a 90-day survival of 71% in GSM-treated mice, compared with 29% for x-ray only mice and 0% for GSM-only mice. Finally, the rate of tumor volume decrease following irradiation was linearly dependent upon the amount of GSM-generated contrast visualized by MRI. The combined therapeutic, diagnostic, and prognostic characteristics of this dual-metal nanoparticle micelle system could thus enable a more personalized approach to a patient's cancer therapy, based upon their tumoral uptake of GSMs.

11. Pratap C. Naha, Anna L. Brown, Harold I. Litt, David P. Cormode and Andrea M. Goforth

Title: Glucose stabilized bismuth nanocrystals as a novel computed tomography contrast agent

Abstract: The use of nanoparticles as contrast agents for medical imaging is of great current interest. Gold nanoparticles have been reported as X-ray contrast agents for computed tomography (CT). However, the high cost of gold has encouraged the exploration of nanoparticles based on other cheaper, strongly X-ray attenuating elements such as bismuth. In this study, we present the synthesis

of large, dense, glucose stabilized bismuth nanoparticles (Bi-NPs), their intracellular uptake, x-ray attenuating properties and biocompatibility. Bi-NPs were found to have cores of 72 nm. The presence of glucose at the nanoparticle surface was confirmed by proton nuclear magnetic resonance and fourier transform infrared spectroscopy. Strong X-ray attenuation of Bi-NPs was confirmed using a clinical CT scanner. 0.1, 0.25 and 0.5 mg/ml of Bi-NPs was incubated with HeLa and J774A.1 cells for 24 hours, then the cell pellets were scanned in a clinical CT scanner. Strong X-ray attenuation was observed from the cells, which indicates that Bi-NPs were internalized. Transmission electron microscopy confirms Bi-NPs internalization and are localized in early endosomes/lysosomes. The cytotoxicity of Bi-NPs was investigated with the MTS assay. No toxic response was observed in either HeLa or J774A.1 cells at concentrations from 0.0061 to 0.5 mg/ml. The strong X-ray attenuation and biocompatible nature of glucose coated Bi-NPs indicate strong potential for *in vivo* imaging applications.

12. T. Paik, J. Mikitsh, A.-M. Chacko, C.B Murray

Title: Synthesis of Anisotropic ^{90}Y-Doped GdF_3 Nanocrystals as Multi-Modal Imaging and Therapeutic Agents.

Abstract: Novel imaging agents are crucial for early and accurate disease detection, offering non-invasive diagnosis and therapeutic potential. A variety of imaging modalities such as optical, magnetic resonance (MRI), and nuclear have been utilized in different applications according to their sensitivity, spatial, and temporal resolution to perform dynamic imaging. Nuclear imaging has recently attracted attention due to its high sensitivity, enabling measurement of sub-picomolar quantities. Radiolabeled probes provide imaging potential, pharmacokinetic and biodistribution information, and therapeutic opportunity (i.e radiotherapy and/or PDT).

Here, we report the radio-luminescence of yttrium-90 (^{90}Y)-labeled rare earth lanthanide trifluoride (LnF_3) nanocrystals. Radiolabeled anisotropic LnF_3 nanocrystals are synthesized *via* high temperature thermal decomposition of $LnCl_3$ precursors using $^{90}YCl_3$. ^{90}Y, a pure β-emitting radionuclide, is incorporated within nanocrystals preventing leakage of radioisotope during *in-vivo* and *in-vitro* application. Temperature and time of reaction can be altered to modify nanocrystal structure and ultimately *in vitro/vivo* properties. Basic binding assays with endothelial HUVEC cells illustrate surface charge-dependent cellular binding. We investigated visible spectrum luminescence of ^{90}Y-doped nanocrystals with an IVIS Lumina II imaging system and saw light emission increase with dose. Coupled with the paramagnetic gadolinium host, this material can be utilized as an MR contrast agent, offering unique opportunities for nuclear, optical, and magnetic imaging modalities as well as potential therapeutic applications. Nanocrystals are readily made water-soluble and can be conjugated to alter binding and therapeutic properties. These nanocrystals present a novel multi-modal imaging and therapeutic platform with flexibility to alter structural properties easily.

13. Nimil Sood, Daniel Hammer, Theresa Busch

Title: Engineering Photoresponsive Polymersomes: Applications in PDT and Chemotherapy

Abstract: Traditional cancer therapies are often associated with toxic side effects due to the poor specificity and low therapeutic index of the drug. Engineering better delivery systems can help mitigate these side effects and improve treatment. Polymersomes are synthetic vesicles that self-assemble from amphiphilic block copolymers in solution. They have diverse functionality and serve as excellent drug delivery vehicles due to the large aqueous compartment available for the encapsulation

of hydrophilic drugs and thick and robust membrane for the encapsulation of hydrophobic drugs. We have designed a new class of polymersomes containing NIR porphyrin fluorophores that absorb light in the near infrared and are stable for long periods of time. These polymersomes become unstable under illumination by the addition of dextran in the aqueous core of the vesicle, and eventually rupture. We first present the engineering principles used to optimize the polymersomes for photodestruction, and then show their cell killing potential in vitro. We used this novel construct to initially deliver the hydrophobic PDT sensitizer benzporphyrin derivative monoacid A (BPD-MA) to Ovcar-5 cells (human ovarian carcinoma). We compared the cell killing potential of the unencapsulated drug to the polymersome-drug and found that certain polymersome formulations had a comparable PDT effect as the free drug. We believe these vesicles could improve PDT *in vivo* by protecting the drug against rapid clearance and preferentially accumulating at the tumor site due to the enhanced permeability and retention effect present in the leaky vasculature of tumors. This nanoparticle formulation also has the potential to outperform conventional liposome delivery of BPD since liposomes are known to be rapidly cleared from plasma.

14. Xue Yang, Bin Chen

Title: 5- Aminolevulinic acid (ALA) induced protoporphyrin IX (PpIX) accumulation in human breast cancer cell lines.

Abstract: A major metabolic change exhibited by many tumor cells is the abnormality in heme synthesis. Tumor cells have been found to produce more protoporphyrin IX (PpIX), a fluorescent precursor of heme, than surrounding normal cells presumably due to changes in the expression and activity of heme synthetic enzymes. Tumor accumulation of PpIX can be further increased by exogenous administration of 5-aminolevulinic acid (ALA), the first product in heme synthetic pathway. This is because exogenous ALA will bypass the feedback inhibition imposed by heme on the synthesis of ALA, leading to the overproduction of PpIX and other heme precursors. Although enhanced tumor accumulation of PpIX, either inherently or following the administration of ALA, has been explored for tumor detection and treatment, the mechanism involved in enhanced ALA-PpIX accumulation in tumor cells is still poorly understood.

By profiling ALA-mediated PpIX production in a panel of human breast cancer cells with varied genotypic and phenotypic background, we have found enhanced PpIX production in most of these cell lines. Because altered phosphatidylinositol 3-kinases (PI3K) pathway signaling has been commonly found in human breast cancers, we are interested in understanding whether/how modulating PI3K signaling affects the ALA-PpIX production. PI3K pathway signaling can be stimulated pharmacologically by growth factors or genetically by overexpressing human epidermal growth factor receptor (Her2, ErbB2). We found that insulin treatment and overexpression of ErbB2 in MCF10A human breast epithelial cells significantly enhanced ALA-PpIX production, which can be effectively inhibited by PI3K pathway inhibitors including BEZ235 and lapatinib. Western blot analysis indicates that ErbB2 overexpression as well as insulin treatment increased the expression of enzymes involved in heme synthesis and treatment with PI3K pathway inhibitors caused down regulation of these enzymes. These results strongly suggest that PI3K signaling plays an important role in regulating ALA-PpIX synthesis.