Seeing the Multiple Dimensions of Cancer:

How Targeted Imaging Technologies Are Bringing New Clarity to Cancer Care

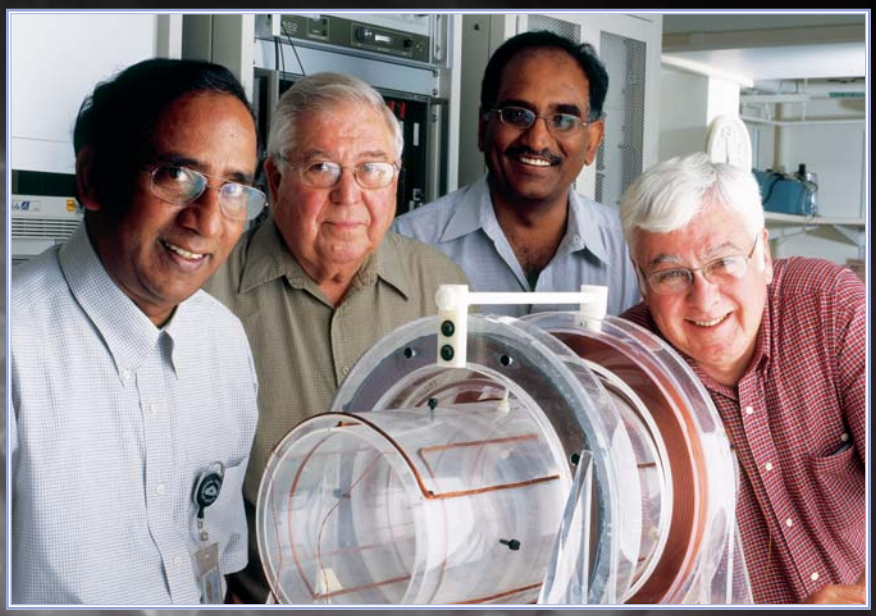
A surgeon, a radiologist, and an oncologist sit in a dimly lit room, banks of monitors in front of them. Their attention is focused on a collection of pictures: black-and-white, color, human outlines, brightly lit spots in some places, dark in others. At the press of a button, the radiologist sends a command to a group of computers. Data are exchanged, and the images merge together effortlessly into a single picture of a human form, superimposing physiology on anatomy. The bright spots fuse, revealing the location, viability, and vulnerabilities of a tumor.

This seamless scenario does not yet represent standard clinical practice. But it represents the ideal treatment planning or drug assessment scenario, one in which clinicians from different fields of oncology are able to share and integrate the data generated by a host of molecularly targeted imaging technologies—such as targeted optical fluorescent tagging, magnetic resonance imaging (MRI), and an emerging technology, electron paramagnetic resonance imaging (EPRI)—into single, holistic images that provide researchers and clinicians with a complete representation of the patient's tumor, including its location, its size, and its physiology.

Together, these technologies are fueling a new understanding of how tumor physiology and structure affect drug action while also bringing new precision to clinical treatment planning. The physician-scientists of CCR's Molecular Imaging Program (MIP) and Radiation Biology Branch (RBB) are leading the charge to refine these technologies and translate them into clinical practice, making the above scenario a reality.

The New Way: Seeing Is Believing

The traditional way of drug development, while effective and straightforward, is time-consuming and cumbersome. Researchers give the trial cohort a drug



(Photo: R. Baer)

Left to right: **Sankaran Subramanian, Ph.D., Staff Scientist; Mr. Frank Harrington, NIH Machinist; Murali Krishna, Ph.D.; and Jim Mitchell, Ph.D., show their original self-built magnet and field gradient assembly, which they used for electron paramagnetic resonance imaging (EPRI). This magnet was used to first demonstrate the feasibility of *in vivo* oxygen imaging.**

or treatment of interest, follow them for months or years by MRI or computed tomography (CT) scanning, and look for changes in tumor size.

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Traditional methods of treatment planning, particularly for radiation therapy or surgery, have similar limitations. Radiologists image the tumor using the same or similar techniques, with the goal of creating detailed three-dimensional representations of tumor size and location.

At the same time, functional imaging—technologies like positron emission technology (PET)—have rapidly advanced the ability of doctors and scientists to see the activity within a tumor, as represented in the case of PET by the relatively insatiable appetite of cancer cells for glucose.

But the current imaging modalities have limitations. PET can tell radiologists how much glucose a tumor is using but cannot shed light on other aspects of tumor physiology or anatomy. MRI and CT can help provide unsurpassed anatomical detail but have difficulty defining metabolic dimensions.

CCR's MIP is stepping in to bridge the functional and the structural. "The MIP was established four years ago to try to find new points of view and new solutions to challenges in cancer imaging," said MIP Head and Senior Clinician Peter Choyke, M.D. "Each technology has its strengths and weaknesses, and if we think broadly about how to leverage those strengths to answer specific problems, we can diagnose, track, and by extension treat cancers with greater specificity than is currently possible."

Imaging has always been a component of the translational research conducted at CCR, but resources dedicated to non-clinical work were often limited. The MIP is changing that, but it is doing so in a way that complements the long-standing efforts of the RBB. "We now have a strong, integrated, cancer-focused, *in vivo* imaging program made up of people with a broad but critical range of expertise," said Choyke. "With this disciplinary breadth, we can investigate the whole spectrum of imaging technologies and probes to create new families of clinically relevant image-based biomarkers."

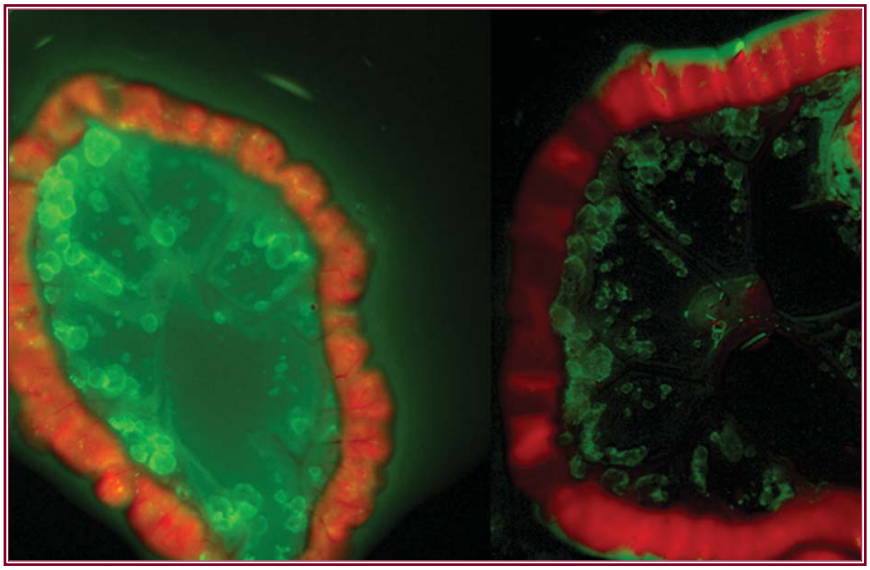
The availability of unique resources like the MIP's new dedicated clinical drug development imaging facility allows the program to serve as a focal point for research that is both high-risk and high-reward, like exploratory studies of new therapeutic agents and technology development (see "To Systematically Look Within").

Bringing Micrometastases into the Light

While radiology-based treatment planning methods provide anatomic information of unprecedented detail, once in the operating room, the most effective surgeries are those in which the surgeon can remove as much tumor as possible, including any metastatic colonies that may be present near the original malignancy. Currently, surgeons remove a margin, a buffer zone of apparently healthy tissue around the tumor, in the hopes of removing any micrometastases that may have spread, unseen, from the original cancer.

Hisataka Kobayashi, M.D., Ph.D., a Staff Scientist in the MIP, understands the challenges and importance of eliminating micrometastases early and efficiently, particularly for ovarian cancer patients. "Ovarian cancer is not a very aggressive cancer, but it is dangerous because it spreads silently. For this reason, gynecologic surgeons try to pick up as many metastatic nodules as they can." Also, because surgery, even when done endoscopically, is invasive, surgeons want to do as much as they can during a single operation.

But how can a surgeon know where the micrometastases are? Visual inspections by endoscope cannot reliably detect tiny tumors without some kind of guide or aid that makes the tumor stand out from the surrounding tissues. To address these visual limitations, Kobayashi and his colleagues—including MIP Visiting Postdoctoral Fellows Mikako Ogawa, Ph.D., and Nobuyuki Kosaka, M.D., Ph.D., as well as former MIP Clinical Fellow Yukihiro Hama, M.D., Ph.D., now an Assistant Professor at Japan's National



(*Image: H. Kobayashi, CCR*)

Figure 1: By using a cancer-specific ligand, like an antibody, conjugated to a fluorescent probe that glows only at low pH *(green)*, researchers can see metastatic ovarian cancer cells *(right, in a mouse model)* and determine whether the cells respond to therapy.

Defense Medical College—have developed a system that literally makes ovarian micrometastases light up.

The system makes use of a natural response to antibody or receptor-ligand binding, namely that once an antibody is bound to a cell, it will be taken up by the cell and then sent to the lysosome, a cellular compartment or organelle that uses low pH to digest internalized proteins. In Kobayashi's system, exposure to the acidity of the lysosome triggers the fluorescent tag attached to the antibody, making the cell glow (Figure 1). "Because we use only a cancer-specific antibody, we only highlight cancer cells, not normal cells," Kobayashi said. The system also takes advantage of a second aspect of cellular physiology. Only viable, healthy cells are able to maintain a low lysosomal pH; if a cell is damaged, its lysosomes become alkaline. Thus, if a cancer cell that has internalized Kobayashi's tagged antibodies is damaged—by chemotherapeutics, for instance—the lysosomal pH rises, and the tag's signal fades.

"If we give this tagged antibody to an ovarian cancer patient before surgery," according to Kobayashi, "the surgeon can look for glowing areas and know that they represent micrometastases. At that point, the surgeon can remove them or paint them with a chemotherapeutic agent and observe, in real time, whether the drug has any effect."

Though the system is only in the preclinical stage, it already shows promise. In the December 2008 issue of *Nature Medicine*, Kobayashi and his team reported on the system's specificity at highlighting lung metastases as peritoneal metastases of ovarian cancer in mouse models.

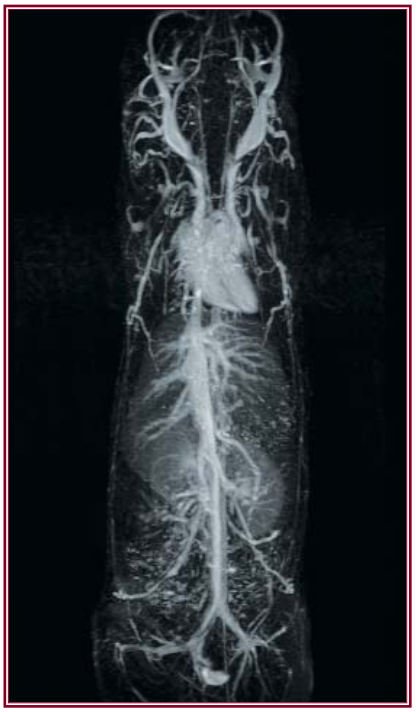
Kobayashi believes the fluorescent system could have widespread applications. "Endoscopic surgery lends itself well to image guidance, which is effectively what we are developing with this technology. It can be applied to any cancer for which there is an appropriate antibody or ligand. We could adapt this method as a way for surgeons to better determine the edges of a tumor while conducting resections. It could be used as a way of guiding robotic surgery, an area NCI is interested in pursuing. We could even use multiple fluorescent tags responsive to different aspects of physiology to increase the scope of visual information we can gain in real time."

Revealing Vascularity

Before the surgery can even take place, though, a surgeon needs to gather as much information as possible about the tumor's shape, location, and activity. Similarly, while deciding whether to employ chemotherapy, targeted therapy, or other treatment strategies, a medical oncologist should have as much information on tumor structure and physiology as possible.

To add physiological sensitivity to the anatomic detail provided by MRI, Choyke and his colleagues have turned to an imaging technique called dynamic contrast enhanced-MRI (DCE-MRI). "DCE-MRI falls somewhere between molecular imaging and anatomic imaging," said Choyke. The true difference between the two is reflected in time. Standard MRI takes a snapshot of a tumor's anatomy and location. By comparison, DCE-MRI is, as the name implies, dynamic, producing a representation of a tumor's blood flow over time.

At the heart of DCE-MRI is a running series of MRI snapshots taken at very short intervals using a contrast agent called gadolinium. This rare earth element interacts with the protons in water molecules, making them stand out more clearly on an MRI scan than they normally would. "Gadolinium actually changes the properties of the water in the body," Choyke explained. "When we run a DCE-MRI scan, the movie we produce actually captures the effects of gadolinium on the surrounding water, giving us a dynamic view of the agent's uptake into and clearance from the tumor."



(*Image: P. Choyke, CCR*)

Figure 2: Dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI) allows researchers to visualize entire circulatory systems, as with the mouse above, or the vasculature of tumors, making this technology an excellent tool for assessing anti-angiogenic therapies.

Because water is the main component of blood, the contrast agent makes anything containing significant amounts of blood, like blood vessels, shine brightly on the scans (Figure 2). "The angiogenic vessels in a tumor tend to be leaky, so they accumulate contrast agents rapidly and wash them out rapidly," said Choyke. "Measuring this ebb and flow of agent,

Standard MRI takes a snapshot of a tumor's anatomy and location. By comparison, DCE-MRI is... dynamic, producing a representation of a tumor's blood flow over time.

DCE-MRI becomes a way of identifying and monitoring highly angiogenic tumors." This capability to directly image angiogenesis positions DCE-MRI well as a tool for assessing anti-angiogenic therapies. "To tell if non-antibody-based tyrosine kinase inhibitors or antibody-based anti-angiogenics like bevacizumab are working," Choyke noted, "you need to be able to see the tumor and the drug's effect on the tumor over time. You need to know the tumor's angiogenic state before, during, and after treatment, and the closer you can get to gathering that information in real time, the better. With DCE-MRI, you can rapidly make those assessments."

The technique also provides greater flexibility for tumor diagnosis, staging, and screening. Choyke sees particular utility for the method in prostate cancer. "Prostate tumors are often hypervascular in comparison to the rest of the gland," said Choyke. "As an organ, the prostate is very challenging to image. It is located deep in the pelvis; it is an anatomically heterogeneous gland, and it is prone to hyperplastic changes that become more pronounced with age, the same age group that is at risk for prostate cancer. So, we are effectively trying to take a picture of an abnormality in a heterogeneous background in a small, remote organ."

There are additional reasons to have the tools available to image the prostate in detail. Prostate cancer tends to be localized, yet the majority of therapies are applied to the whole gland. "Ideally, we'd like to reduce the number of men undergoing whole gland therapies or radical prostatectomies, or we'd like to eliminate such therapies altogether and replace them with minimally invasive ablation techniques that could take care of the cancer without the side effects associated with more radical techniques."

Hypoxia in View

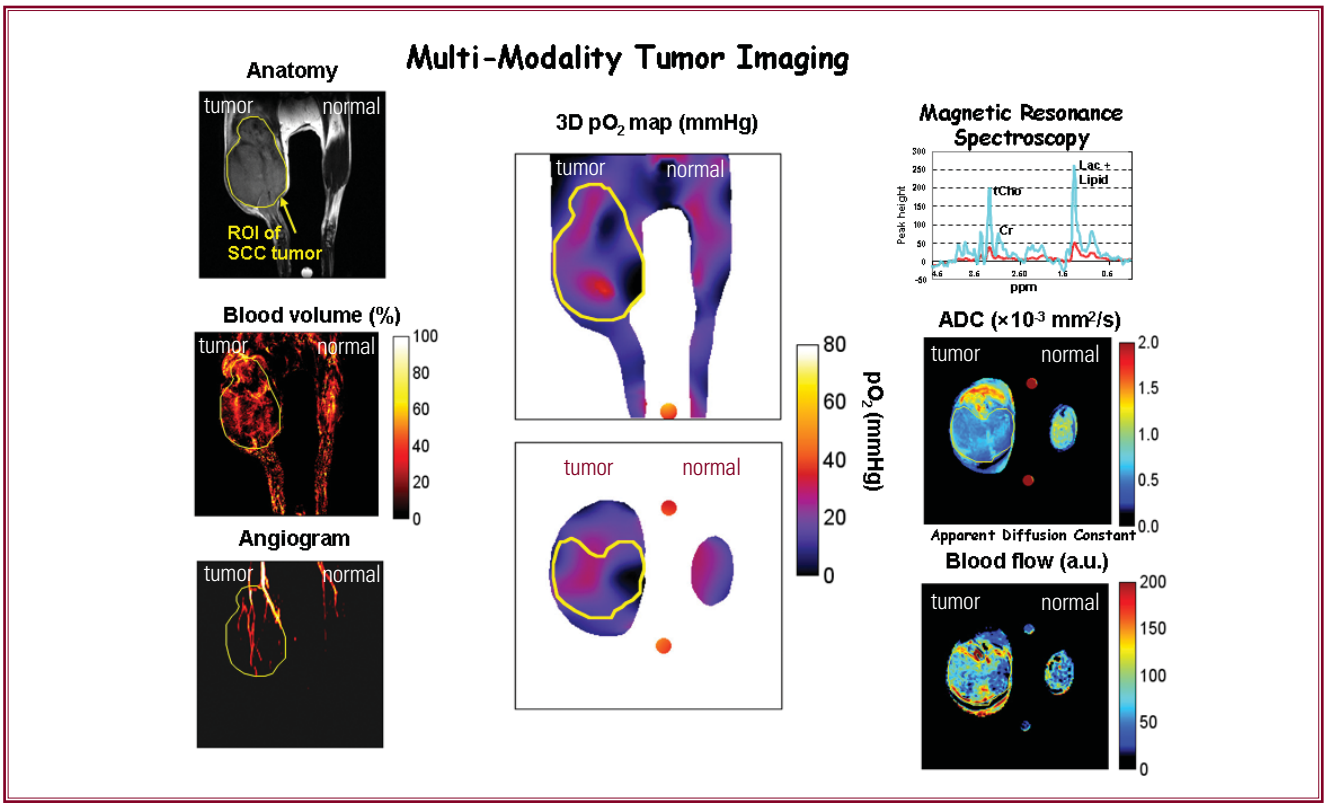
The translation of angiogenesis to oxygen concentration is not a one-to-one conversion. But knowledge of a tumor's oxygen level, or pO_2, can be crucial when planning treatment or assessing the effectiveness of an investigational therapy. "Tumors with significant hypoxia, or low pO_2, are very resistant to radiation therapy and maybe to chemotherapeutic agents as well," according to RBB Chief James Mitchell, Ph.D. "And for twenty years we have known that tumor hypoxia is directly tied to poor clinical outcomes, even in patients who undergo surgery."

"We have not had a readily available, noninvasive, and direct way to measure pO_2," said Murali Cherukuri Krishna, Ph.D., Head of the RBB's Biophysical Spectroscopy Section. "Indirect radiological measurements only provide qualitative information. Direct measurements with oxygen-sensing electrodes are accurate but are invasive, inappropriate for many tumors, and only give a localized snapshot of tumor pO_2."

"What we needed," Mitchell concluded, "was a quantitative method to map tumor hypoxia in deep sites in real time and in a way that can be coregistered with PET, MRI, or CT."

Krishna and Mitchell's answer to this need is a new imaging modality called electron paramagnetic resonance imaging (EPRI), an offshoot of nuclear magnetic resonance (a technology widely used for chemical analysis) that allows direct, quantitative assessment of oxygenation across a whole tumor (Figure 3).

Several features of EPRI work in its favor as a viable technology. The method employs the same equipment used for MRI. "The radio frequency we use for EPRI can be generated with an MRI scanner," according to Krishna. "In one sitting, we can generate three-dimensional EPRI oxygen maps and MRI anatomic maps of the same tumor."



(*Image: M. Krishna, CCR*)

Figure 3: Integrating images generated using multiple techniques can provide very comprehensive information about a tumor. For instance, overlaying MRI and EPRI data lets researchers assess structure, blood flow, blood volume, metabolite levels, and oxygenation in mouse model of squamous cell carcinoma. *(left leg, tumor; right leg, normal)*

But its development did not come without challenges. "EPRI looks for free radicals," said Krishna. "The body, especially the immune system, makes a number of endogenous free radicals. But none of them are stable or spectrally simple enough to be used for imaging. To make this technology work, we needed an artificial free radical that could be used as a tracer, something that would interact directly with the oxygen in a tumor and produce a simple, detectable signal."

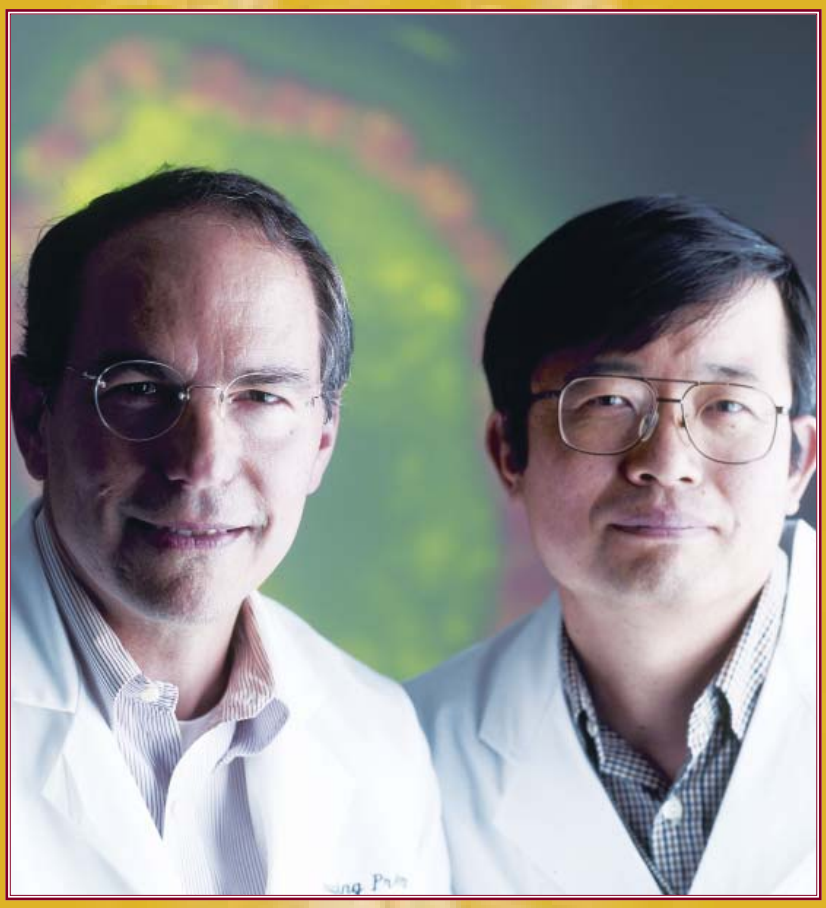
"GE has developed a family of tracers called TAM probes specifically for *in vivo* paramagnetic imaging," Mitchell noted. "Because the TAM signal on an EPRI scan increases linearly with oxygen concentration, imaging the tracer distribution within a tumor gives us a direct, quantitative, real-time image of its oxygen distribution."

Computational resources also proved to be a roadblock. "Paramagnetic signals last only one to two microseconds," Mitchell said. "The magnetic signals detected with MRI, by contrast, last about a second. The processing power needed to capture paramagnetic data simply hasn't been available until now."

Krishna and Mitchell—along with Postdoctoral Fellow Shingo Matsumoto, Ph.D., and Fuminori Hyodo, Ph.D., formerly a Postdoctoral Fellow in the Krishna laboratory and now at Kyushu University in Japan—published the results of a successful proof-of-concept mouse study in the April 2008 issue of the *Journal of Clinical Investigation*; the team is already pursuing translation to humans.

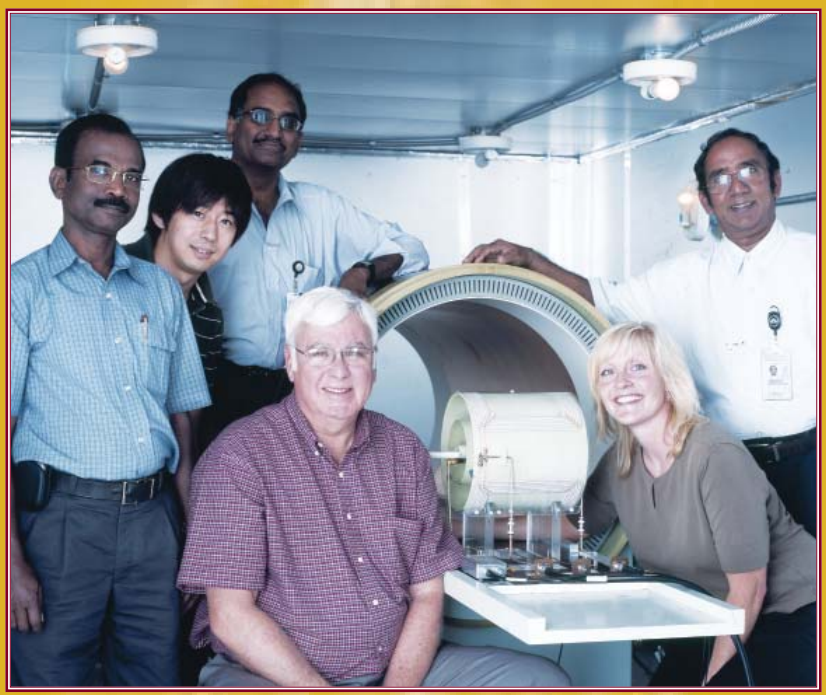
"With the ongoing development of technologies like DCE-MRI and EPRI, all here within the collaborative environment of CCR," Mitchell continued, "we now have the first real opportunity to look at tumor pO_2, metabolism, blood flow, vascularity, and anatomy and make them all correspond. We can't yet tell what the full impact will be on drug development and clinical care, but as these imaging modalities mature, we can tell that they will change the playing field."

To learn more about CCR's Molecular Imaging Program or the Radiation Biology Branch, visit their Web sites at http://mip.nci.nih.gov/ and http://ccr.cancer.gov/labs/lab.asp?labid=52.



(*Photo: R. Baer*)

Peter Choyke, M.D. *(left)*, and Hisataka Kobayashi, M.D., Ph.D. *(right)*



(*Photo: R. Baer*)

The EPR Imaging Lab
Sitting: Jim Mitchell, Ph.D., Anastasia Sowers, A.S.
Standing *(left to right)*: Nallathamby Devasahayam, M.S.; Shingo Matsumoto, Ph.D.; Murali Krishna Cherukuri, Ph.D.; Sankaran Subramanian, Ph.D.

To Systematically Look Within

(Photo: R. Baer)

Angela Stuber *(left),* Certified Nuclear Medicine Technologist, and Karen Kurdziel, M.D. *(right),* position their patient for a combined, single photon emission tomography/computed axial tomography scan (SPECT/CT scan).

Targeted imaging technologies such as dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) and electron paramagnetic resonance imaging (EPRI) are giving precise insight into how cancer works at a molecular level. While these developments have the potential to revolutionize standard clinical care, they are also fueling a paradigm shift in drug development.

To have this kind of R&D impact, though, dedicated equipment and resources need to be available. Time on scanning equipment used in clinical trials is limited, which constricts CCR's ability to conduct early-phase translational drug development studies like exploratory clinical trials (small trials in which patients receive a trace dose of a developmental drug as a way of assessing whether the drug behaves in people as it does in preclinical models).

To bolster CCR's capacity for these kinds of research studies, the Molecular Imaging Program (MIP), with NCI's Developmental Therapeutics Program (DTP), opened the Molecular Imaging Clinic this past summer. "This facility is separate from the clinical facility," said Karen Kurdziel, M.D., a Staff Clinician with CCR and the Director of this new facility. "It is dedicated to drug discovery research protocols and lets us use imaging as a marker to make early go/no-go decisions in an exploratory context."

The new facility will house a comprehensive set of scanners, including PET, PET/CT, and 3T MRI, as well as full equipment for capturing vital signs and blood chemistry, all in close proximity. Together, this equipment will let MIP researchers and their colleagues from across NCI learn relatively quickly what a drug actually does. "We can take a drug, label it with an appropriate tag, and track it as it travels through the body," Kurdziel explained. "With this kind of information, we can see how much of a drug actually reaches the tumor and also where else it goes."

These studies can provide valuable insight into a drug's mechanism of action and the biology underlying side effects. "We have already started a study of paclitaxel (Taxol®), which no one has ever studied using imaging," said Kurdziel. "Its pharmacokinetics have been studied using plasma, blood, and urine, but with PET imaging, we can visualize the real-time whole body drug distribution. We have already found that it migrates to the gut and stays there, attacking the rapidly dividing cells in the gut lining, which may help explain the gastrointestinal side effects associated with paclitaxel treatment.

"This technology," Kurdziel continued, "can be used to determine how much of a dose actually penetrates the tumor. With the emergence of molecularly targeted drug therapies, PET imaging can be used to determine the dose needed, which may be much lower than the maximum tolerable dose that we use currently."

Kurdziel notes that data like this can also be used to create standard imaging-based markers to guide all cancer drug development. "If we can get the FDA to approve certain imaging markers as biomarkers, such as FDG for metabolism and FLT for proliferation, we can establish standard imaging endpoints that would allow drug developers to look for valid responses after weeks of treatment instead of years."