Quick the Probe, Sharp the Molecular Image

To the Lab and Back to the Clinic, Imaging Technologies Make Ready Improved Biomarkers

Summer E. Allen, Ph.D.

Researchers are developing a host of new uses for molecular imaging techniques—including magnetic resonance imaging (MRI), positron emission tomography (PET), and single photon emission computed tomography (SPECT)—that are already commonly used in patient care. Once these techniques have picked up new capabilities in the laboratory, they will return to the clinic, where they will likely transform the diagnosis and treatment of cancer and other diseases.

"The approval rate for new therapeutic entities in oncology is the lowest of all disease areas. Ninety percent of new chemical entities that go into clinical testing for cancer fail—despite the fact that they are all backed with tons of animal data suggesting that they should work," says Andrew Kung, M.D., Ph.D., director of pediatric hematology, oncology, and stem cell transplantation, New York-Presbyterian Morgan Stanley Children's Hospital/Columbia University Medical Center.

Dr. Kung has been at the forefront of a movement to improve preclinical animal studies—primarily by using more representative animal models of cancer and by measuring treatment response using molecular imaging and other techniques that might also be used to measure desired responses in patients.

Molecular imaging was an important component of a "co-clinical" trial of a potential therapeutic for lung cancer that was performed by Dr. Kung's group. A co-clinical trial involves running an animal study in parallel with a human clinical trial. The animal study generates new data that can then be used to improve subsequent clinical trials.

Using this model to examine the effectiveness of the MEK inhibitor selumetinib on lung cancer caused by KRAS (Kirsten rat sarcoma viral oncogene homolog) gene mutations,

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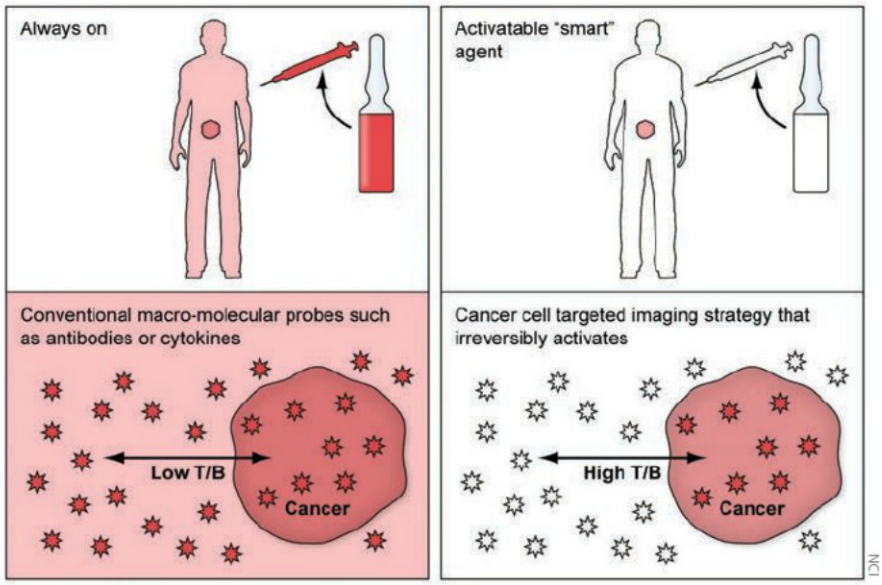
Dr. Kung and colleagues identified genetic determinants that likely modify how different groups of patients respond to the drug. They also found that PET imaging could be used as an early indicator of drug response.

"We were able to demonstrate that a change in PET signal was actually something we could see very early, before there was a change in tumor volume," states Dr. Kung. "I think the overarching goal in molecular imaging is to move us past the point where we use imaging just to measure the size of tumors. [Molecular imaging should] tell us something about the characteristics of the tumor, the behavior of the tumor, and the pharmacodynamic efficacy of drugs."



Scientists at the NCI believe that an "activatable" optical imaging probe may soon be able to help surgeons discriminate between cancerous and normal tissue when they remove tumors from cancer patients.

"Sprayable" Probes

When surgeons remove tumors from cancer patients, they often have difficulty discriminating between cancerous and normal tissue. Soon, "activatable" optical imaging probes may help solve this problem. Hisataka Kobayashi, M.D., Ph.D., chief scientist of the molecular imaging program at the National Cancer Institute, and his group are developing an imaging probe that can be sprayed directly onto a surgical site.

The probe, γ-glutamyl hydroxymethylrhodamine green(gGlu-HMRG), fluoresces when glutamate is cleaved by γ-glutamyltranspeptidase—an enzyme expressed only by proliferating cells. "This agent is activated within three to five minutes…We can clearly see the fluorescence of the cancer," says Dr. Kobayashi. "It varies. Mostly we find that 50–60% of the cancer is activated, but the normal tissue is not activated."

The gGlu-HMRG probe is smaller, less toxic, and less expensive than more specific antibody-based imaging probes. Additionally, because it is sprayed directly onto the surgical site, it can be reapplied in the middle of the procedure to ensure that all cancerous tissue is removed. Dr. Kobayashi's group has performed toxicity studies on small animals and is now testing the spray probe on tumor samples from patients.

Dr. Kobayashi is also developing antibody-based activatable photoimmunotherapy probes that can be used to kill cancer cells. These probes contain a monoclonal antibody that binds to a protein that is overexpressed in cancer cells—such as HER2 in breast cancer or PSMA in prostate cancer. The antibody is coupled to a photosensitizer molecule that disrupts cellular membranes and causes cell necrosis when exposed to near-infrared light.

These photoimmunotherapy probes are set to enter human clinical trials in early 2015. "We are starting with head and neck cancer because it is easy to shine the light [on these organs]—the oral cavity and the upper throat," explains Dr. Kobayashi. "We are already planning the second trial for pancreatic and colon cancer deep in the body (combined with surgery)."

Patient Selection

Another promising application for molecular imaging is patient selection. "What we are trying to do is predict which patients are likely to

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respond to a certain therapeutic," says Paul Acton, Ph.D., scientific director and global head of molecular imaging at Johnson & Johnson. "We have been developing techniques to essentially take the drug itself and label the drug so it becomes its own companion imaging diagnostic."

The goal is to give a patient a very small amount of a potential drug that has been labeled with a radioactive marker. A doctor can then use a standard imaging technique, such as PET, to see whether the drug is binding to its molecular target in that patient. If it is, a full dose of the drug will be administered to the patient; if it is not, the treatment is unlikely to be effective.

One challenge in developing these companion diagnostics is separating signal from background, especially for biologic drugs. "They stay in circulation for such a long time—you get this huge background signal from just circulating biologic therapeutic; it takes a long time to see a decent signal," explains Dr. Acton. "That whole time, the background is washing out, giving your patient a significant radiation dose from the tracer."

According to Dr. Acton, various companies—including Johnson & Johnson—are working to develop tracers that wash out faster. Such tracers could rely on shorter half-life isotopes that would give a lower radiation dose to the patient.

Companion diagnostic probes may provide a better sense of the true efficacy of therapeutics during clinical trials and improve treatment selection for patients. "The true benefit, of course, is to the patient," remarks Dr. Acton. "You're giving them a drug that you are much more confident is going to work for them, and you're not giving them unnecessary side-effects with a drug that is unlikely to work. This could be a major step forward in the development of personalized medicine."

Tracking Amyloid

Visceral amyloidosis is a fatal disease characterized by the accumulation of fibrillar protein aggregates (amyloid) in the heart, kidneys, pancreas, and other visceral organs. As of now, clinicians in the United States have no way to obtain whole-body images of amyloid deposits in these patients. "We need to develop a way to provide noninvasive imaging to these patients because it could help doctors provide accurate prognoses and monitor therapeutic responses, and it could give the patient a better view of the extent of their disease," says Jonathan Wall, Ph.D., director of the Preclinical and Diagnostic Molecular Imaging Laboratory at the University of Tennessee Graduate School of Medicine.

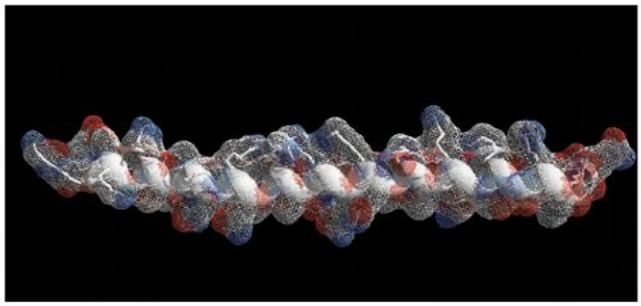
Amyloid deposits contain a special form of heparan sulfate proteoglycan that is highly charged. Dr. Wall and his group took advantage of this property to develop a small synthetic heparan-binding peptide called p5+14 that can be radiolabeled with F-18, I-124, Tc-99m, and I-123 for PET or SPECT imaging.

"The peptide is an excellent imaging agent because it is cleared very quickly when it's not bound to a target…so you achieve a high signal-to-noise ratio in the images very quickly," adds Dr. Wall. "If unbound peptide is cleared very quickly, the radiation dose to the patient is quite favorable relative to larger molecules."

An additional advantage of this peptide is that it stays bound to the amyloid deposits for a long time. "Once it's bound to amyloid in its radiolabeled form, you can image mice more than 72 hours post-injection," explains Dr. Wall. "In our mice, [the peptides] work extremely well—now we're trying to find a mechanism to get them into clinical trials in patients."

Adoption of imaging tools such as this may save both money and time in the treatment of patients with amyloidosis. "If you can image quickly and see whether [a drug]

The predicted secondary structure of peptide p5+14. The peptide is alpha helical with all the charged lysine residues on one face of the helix.



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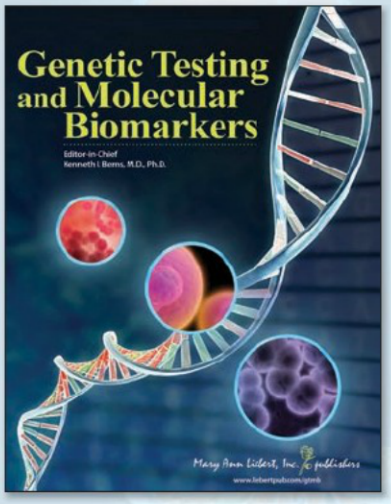

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is having an effect on the amyloid, then you can either stay the course on that drug or, if it's not working, move on to something else," states Dr. Wall. "Imaging allows you to [carry out deliberations] that aren't available for those patients right now."

Dr. Wall and colleagues are currently researching whether p5+14 can be used to monitor disease progression in other diseases associated with amyloid deposits—such as type 2 diabetes and cerebral amyloid angiopathy in Alzheimer's disease—and are exploring developing this molecule into a therapeutic since it has such high affinity for amyloid.

Theranostic Nanoprobes

"A 'theranostic' probe is by definition a probe that combines imaging with a therapy component," says Anna Moore, Ph.D., associate professor of radiology at Harvard Medical School. Dr. Moore and her group have designed multiple oligonucleotide theranostic nanoprobes to attack cancerous cells, including a small interfering RNA probe that targets the anti-apoptotic gene survivin and another probe that inhibits microRNA10b, a known regulator of cancer cell migration and invasion.

Dr. Moore and her group are working on engineering these probes so they won't be taken up by noncancerous cells. "The problem with the nanoparticles—as with anything that you inject through the bloodstream—is that they don't go 100% to your target tissue. They travel systemically through your blood and accumulate in different organs," observes Dr. Moore. "The idea is to deliver them to the target organ as much as possible and prevent the uptake by liver, kidney, spleen, and other organs that are designed to eliminate toxins and debris from your body."

Dr. Moore specifically designed her nanoprobes to be modular and adaptable: "We used iron oxide nanoparticles…They are used as contrast agents for magnetic resonance imaging. [The] dextran coating can be modified chemically to conjugate various compounds, and those compounds could be other imaging reporters such as fluorescent dyes for near-infrared imaging.

"It could be radionucleotides to combine magnetic resonance imaging and PET imaging. It could be a drug." According to Dr. Moore, nanoprobes naturally enter the leaky vasculature found in most tumors, but they can also be attached to a targeting peptide that will bind to specific tumor cells.

The microRNA10b inhibitor probe looks quite promising in mouse models—it both prevents and also destroys lymph node metastasis in mice that have been injected with primary tumors. Dr. Moore next hopes to see if combining this probe with low-dose doxorubicin could be a viable treatment for human cancers.