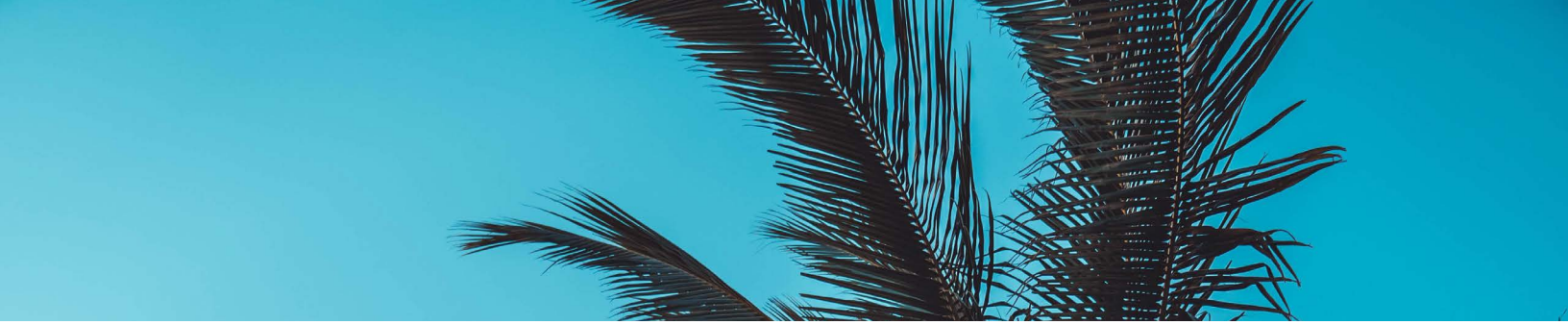


WMIC 2021
World Molecular Imaging Congress

TOGETHER MIAMI

October 6-9, 2021

As we prepare for the World Molecular Imaging Congress in Miami, FL, October 6-9, 2021, we are highlighting each Abstract Category. We have reached out to labs asking them to share their experiences in discovery, imaging, and translation as they relate to the WMIC Abstract Categories.

ABSTRACT CATEGORIES

BASIC BIOLOGY, SYNTHETIC BIOLOGY AND BIOENGINEERING

- Reporter Genes and Protein Engineering
- Cell-Based Reporters, Therapeutics and Cellular Engineering
- Tissue Engineering and Regenerative Medicine
- Systems Biology
- Basic Biology

CARDIOVASCULAR & PULMONARY

- Probes & Targets
- Preclinical Imaging
- New Biology
- Translational and Human Studies

COMPUTATIONAL & DATA SCIENCE

- Machine Learning: Applications
- Machine Learning: Basic Developments
- Modeling & Quantification
- Image Pre/Post-processing

IMMUNOLOGY: INFLAMMATION & INFECTION

- Probe & Targets
- Preclinical Imaging
- New Biology
- Translational and Human Studies

INSTRUMENTATION

- Innovation in Instrumentation
- Image Guidance
- Microscopy

ONCOLOGY

- Probes & Targets
- Preclinical Imaging
- New Biology
- Translational and Human Studies

NEW CHEMISTRY, MATERIALS AND PROBES

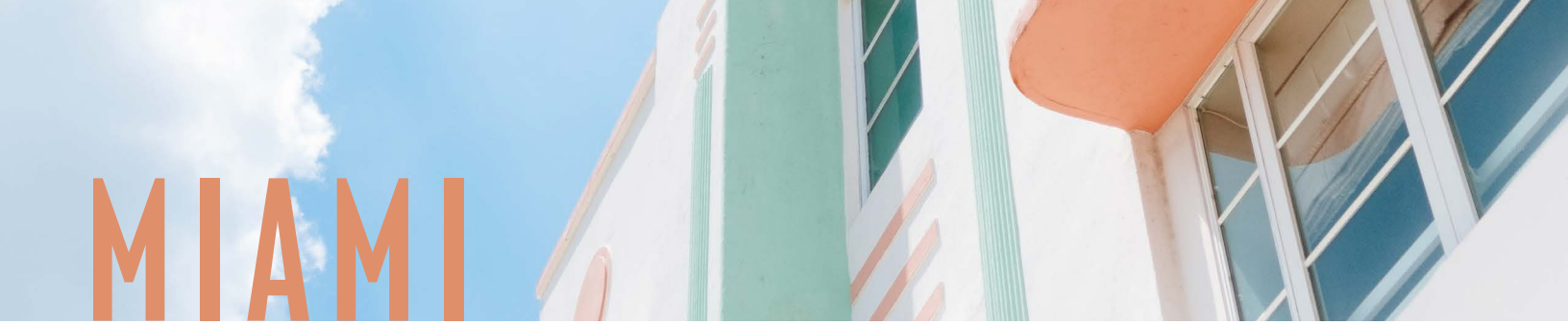
- New Chemistry for Optical Imaging
- New Chemistry for Magnetic Resonance Imaging and Magnetic Particle Imaging
- New Chemistry for Nuclear Imaging
- New Chemistry for Ultrasound Imaging
- New Chemistry for Multimodal Imaging
- New Approaches to Biochemical Analysis

NEUROSCIENCE

- Probes & Targets
- Preclinical Imaging
- New Biology
- Translational and Human Studies

SYSTEMIC DISEASES (KIDNEY, LIVER AND PANCREAS)

- Probes & Targets
- Preclinical Imaging
- New Biology
- Translational and Human Studies



Developing an MR Probe

Peter Caravan, Ph.D.

with Peter Caravan, Ph.D.
Professor of Radiology, Harvard Medical School
Co-Director, Institute for Innovation in Imaging,
Massachusetts General Hospital

Moderator: Lisa Baird
CEO, World Molecular Imaging Society (WMIS)

Lisa Baird (LB): Can you tell us about your work translating an MR Probe?

Peter Caravan (PC): In my lab, we work on both MR probes and PET probes. I've had experience in inventing new MR probes and PET probes and taking them into early-stage clinical trials, i.e., translation.

LB: How is molecular MR similar and different to PET?

PC: Both MR and PET use targeting to localize the probe to a specific pathology, but there are a few differences. MR probes are often called contrast agents. MRI gives an image that's typically based on water and fat and the probe that we use gives contrast changes in that image. We don't see the probe directly, but we see its effect on water, and that's why we call it a contrast agent. This is different from nuclear and optical imaging techniques, where you can visualize the probe directly. Since there is already a signal in MRI, there's a threshold of how much probe is needed in order to see a change in that signal. That threshold concentration typically falls in the micromolar range of the imaging reporter. That's much higher than is needed in nuclear techniques like PET. As a result, there's a limit to the types of targets we can see with MR. We can't see the very sparse targets that might be present at picomolar concentrations that one can sometimes visualize with PET. But there are many ways to get around the sensitivity issues, and we have several tools available with MRI that we don't have with PET.

LB: How can MRI overcome these sensitivity issues?

PC: The MR signal can be affected in various ways. Since we see the effect of the probe indirectly, there are several different mechanisms that we can use. We can design probes that change the relaxation times of the water molecules or have probes that undergo chemical exchange with the hydrogen atoms of water. We can also design probes that change their property in response to certain stimuli. The effect can increase or decrease in the presence of the stimulus and that stimulus could be:

- pH change
- Local enzymatic activity
- Temperature change
- Change in oxygen partial pressure
- Binding of the probe to a molecular target

All of these are ways in which we can manipulate the signal, and that manipulation can make it more sensitive to smaller changes. This is one way to get around the issue of needing a higher concentration of probe.

The other way to get around this issue is to bring more reporter molecules along. That leads to nanotechnology approaches where you bring a big payload of signal-generating moieties to the target.

LB: What approach do you use in your lab?

PC: We use both direct and indirect targeting. In my lab we've worked on a number of different approaches in molecular MR. We've worked with direct targeting, where a peptide recognizes a specific protein—in one instance, fibrin, and in another instance, type 1 collagen. We then get a non-covalent binding interaction between the probe and the protein of interest, which localizes the probe at the region of the pathology, and we see a signal change there. We've also used probes that alter their properties in the presence of either a change in pH or in the presence of enzymatic activity.

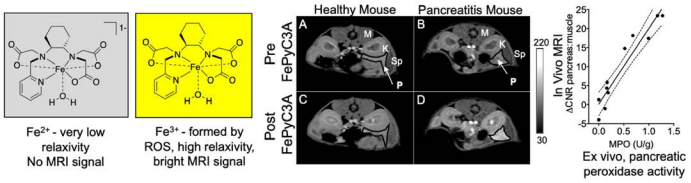


Figure 1. The iron-based MR probe FePyC3A is MRI silent in its +2 oxidation state but provides strong MRI signal in its +3 oxidation state. When Fe2+-PyC3A is administered to normal mice there is no appreciable change in MR contrast, but in a model of pancreatitis the pancreas (P) is strongly enhanced due to reactive oxygen species (ROS) converting the probe to its high signal form. The magnitude of the MR signal change is directly proportional to the amount of myeloperoxidase enzymatic activity in the pancreas. Courtesy Eric Gale, Ph.D., Massachusetts General Hospital and adapted from J. Am. Chem. Soc. 2019, 141, 5916.

A probe that we've been working on recently in collaboration with Eric Gale, Ph.D., at MGH, is an iron-based molecule that changes its oxidation state from +2 to +3 in the presence of reactive oxygen species (ROS). We start with a probe that has no effect on the MR image in the absence of ROS, but which gets brightly turned on in their presence, Figure 1. This is a mechanism that goes from a completely OFF state to an ON state—that is much harder to do in other modalities. The utility of such a probe is shown in the figure where in normal, healthy mice there is no change is MR signal when the probe is administered but in a model of pancreatitis where the pancreas is inflamed and has high levels of ROS, the probe is "turned-on" and we see the pancreas, labeled P in the figure, strongly enhance. The MR signal measured in vivo correlates strongly with enzymatic activity in the pancreas measured ex vivo. This type of turn-on MR probe is analogous to fluorescent probes where you have a fluorescence quencher that gets cleaved by an enzymatic reaction to turn on a fluorescent signal. We can do the same thing with MR.

LB: Can you tell us more about your work with Enzymatic Activity?

PC: We have also been working in collaboration with Alex Bogdanov, Ph.D., at UMass Medical Center, with a probe that becomes oxidized and forms a free radical in the presence of a reactive oxygen species. This species binds to tissue pathology and allows localization and an increase in signal because its relaxivity increases. These are the sort of areas we've been working in.

LB: Can you give us tips you use when validating your probes?

PC: One thing to always remember in any kind of molecular imaging is that the signal we see has different components. You're going to see signal just from distribution, where the probe distributes—and there's always going to be some component of your signal that's non-specific. Then you're going to have some component of the signal that's due to specific targeting / binding. If you have a probe that changes its properties when it's localized to the target, then that creates even more complexity, and you'll have a change in signal due to that. ***One of the first things we need to be concerned about is whether the signal change we observe is actually due to the specific mechanism of action we're probing.*** In pathological tissue, there will often be differences in the rate of delivery of the probe. We'll see alterations in perfusion of that tissue, and compared to normal tissue, there'll be differences in the tissue's permeability and in its extracellular volume. The rate of washout of signal from the pathological tissue will also show differences compared to normal tissue. A good example of this is how we use extracellular gadolinium-based contrast agents in radiology. These are simple gadolinium chelates that have no targeting but are used every day in the clinic. Non-specific gadolinium-based contrast agents work because many tumors have a very leaky vasculature, giving them a much larger extracellular volume compared to surrounding normal tissue. This makes those tumors appear bright when you give a gadolinium-based contrast agent and that has nothing to do with any kind of targeting effect—it's just due to distribution.

LB: How do you know if it is a real targeting effect or a distribution effect?

PC: When you move on to molecular imaging, you need to be sure you're actually having real targeting—not just a distribution effect. There are a couple of ways to ensure you're having a real targeting effect. The classical way to look at specificity is what one does in nuclear imaging experiments like PET, where you give high doses of a non-imaging-active, version of the probe. By doing that, you saturate the target and then get very little uptake. You perform an imaging study showing high signal in the area of pathology, and then you perform the study again—but this time, co-administering or pre-treating with a large, 100-1000-fold, amount of the non-imaging active compound (e.g., in the case of PET the nonradioactive form of the probe). Sometimes, these blocking studies just can't be done with MRI, if you're imaging a target that's present at a very high concentration. For example, collagen in the context of tissue fibrosis or fibrin in the context of blood clotting—these targets are present in high micromolar concentrations and a complete block would require very high doses of the probe. In a mouse study we typically dose on the order of 0.1 mg probe per gram mouse so increasing this dose to 100 mg/g may not be feasible for solubility or toxicity reasons. Sometimes it's possible, but sometimes it's just not possible to achieve such a high dose level.

Validating Your Probe: Specificity & The Importance of a Control Probe

PC: Another way is to make a probe that is a matched control and has the same pharmacokinetics as the targeted probe. We first have to establish that both probes do indeed have the same pharmacokinetics. We then use that probe in the same animal models (ideally in the same animal) and show the signal changes we observe are not as great. You can, of course, expect to see some changes in signal. You might expect to see significantly different changes in diseased animals compared to normal animals, even with your control probe—again, because there may be differences in the physiology. But ideally the targeted probe will provide a much larger signal change due to its specificity. There are many different ways to think about making a matched control and it should be as physically similar as possible to the targeted probe. In my work, we often use peptides as a targeting group, and in some cases, we have used cyclic peptides. We've made the linear version of the peptide which does not bind as a control. You can also change the order of the amino acids, so you don't have binding. Here again, it's an isomer and it might have similar pharmacokinetic properties. This is one way to get at that specificity.

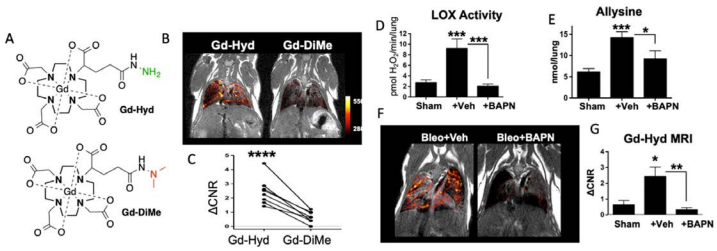


Figure 2. Allysine-targeted probe Gd-Hyd for imaging tissue fibrogenesis. A) The permethylated analog Gd-DiMe has the same relaxivity and pharmacokinetics as Gd-Hyd but cannot engage the allysine target. B) Gd-Hyd enhanced MRI detects fibrogenesis in bleomycin injured mouse lung. C) Gd-Hyd shows higher lung signal than Gd-DiMe in pairwise study. D) BAPN treatment inhibits LOX lung activity in bleomycin injured mice and prevents formation of E) the allysine target. F, G) Gd-Hyd enhanced MRI shows high signal in bleomycin injured animals treated with vehicle (veh) but BAPN treatment markedly attenuates this signal. Courtesy of Peter Caravan, Massachusetts General Hospital and adapted from Chen et al. JCI-Insight, 2017, 2, pii 91506.

In Figure 2 we show a case study in probe validation. The probe Gd-Hyd recognizes the aldehyde allysine formed in the extracellular matrix as a result of lysyl oxidase (LOX) activity that occurs during tissue fibrogenesis. As a control we made the probe Gd-DiMe which is structurally very similar but cannot bind to aldehydes. We measured the pharmacokinetics of both probes in mice and found that they had the same rate of blood clearance, the same distribution in tissue, and that they both were eliminated exclusively via the kidneys. Next, we used these probes in a mouse model of pulmonary fibrosis. We took mice that had bleomycin lung injury resulting in fibrosis and randomized them to imaging with either Gd-Hyd or Gd-DiMe. The next day we imaged each mouse again, but this time with the other probe. In this pairwise study, all the mice showed higher lung signal with Gd-Hyd than with Gd-DiMe.

LB: When validating your probe how do you modulate the amount of target present?

PC: Another lesson around target validation is, if you can modulate the amount of target that's present, that's very important. Sometimes in the natural history of disease, you'll get a target that comes up in expression, and then over time it goes down. By imaging as a function of time in disease progression, you might expect to see different readouts. If you can observe that change over time, it's a good validation. In the bleomycin lung injury model from Figure 2, fibrogenesis peaks about 2 weeks post bleomycin injury and then declines with time as the injured lung tissue is replaced by stable scar. We performed imaging as a function of time post injury and were able to show first this increase in disease activity and then a decline. Another way to validate the target is to give a pharmacological stimulus. This could be a drug you're treating the animal with, that is specific to the pathway you're interested in. The importance of this study is that you still have pathology, but now you're modulating how much signal is present, which is a very useful validation. In Figure 2 we show an example of this in the mouse bleomycin lung injury model where we treated injured mice with the lysyl oxidase inhibitor β-aminopropionitrile (BAPN). We showed ex vivo that BAPN treatment inhibited lung LOX activity and prevented formation of allysine which could be noninvasively detected by Gd-Hyd enahanced MRI.

LB: What other types of experiments do you use to validate your target?

PC: When validating, it is best to combine your studies with ex vivo measures that quantitatively measure how much target is present. Ideally, try to go beyond some sort of immunostaining, which is a very subjective method—where all you can tell is that there's more of the target than in the control animals, but not how much more. It's better to take a quantitative approach, for instance, a quantitative western blot, a quantitative proteomics approach, or a biochemical analysis. In the case study in Figure 2, we developed an assay to measure allysine levels quantitatively ex vivo and further corroborated our results with quantitative and semi-quantitative histology, biochemical and gene expression measures of disease. Once you have that quantitative analysis, as you further go on to validate, you can apply those same analyses to human tissue. This is critical because you don't want to fall into the trap of designing a probe, optimizing it, and having it be the best probe for imaging a mouse model of disease, but having no ability to go beyond that. Being able to test for the presence of the target in human disease requires that you design some ex vivo measures to show that your probe can indeed bind to diseased human tissue but not to normal human tissue.

Validating Your Probe: Randomized Approach and True Blinding

PC: One thing that I think is really important, especially for animal work, is to follow the ARRIVE guidelines (source **here**). These are guidelines for reporting animal studies, full of useful information in terms of how to describe your study. Many journals now require that the ARRIVE guidelines have been followed. The ARRIVE guidelines offer great advice on randomization during the process of validation. For example, let's say you're doing a study, and you have one group of animals that have been treated with a drug, and another group of animals that have been treated with vehicle. How do you choose those animals for treatment? How do you choose them for imaging? You should have a true randomized approach for that, like using software to generate a list of random numbers and picking the animals based on that software output in order to remove all bias from your experiment.

The second key in these validation studies is to have true blinding. When doing this kind of study, if possible, ensure that the person who is doing the imaging study does not know the source of the animal, whether the animal has disease or not, and whether the animal is being treated with an active drug or not. In this way they are blinded as to the treatment. In the next step, image analysis, anonymize those images so the analyst can analyze them without knowing whether the animal was treated and whether it was a control animal or not. You then have a true blinded study. Further down the path, in the ex vivo analysis, again perform those analyses in a blinded fashion. When you finally have all the data together, then lift the blind and see how your study performed. In this way, you remove any kind of bias that the data analyst might have.

When translating your probe, if possible, test it in different animal models—and ideally in different species—again, to convince yourself that what you're seeing isn't just unique to the mouse or the zebrafish or the particular subject you're imaging.

LB: Can you talk about how you plan for manufacturing?

PC: Another important facet of translation is effective planning. You will need to develop the process for manufacturing your probe, and this process needs to be a repeatable one, and it needs to have good controls.

The FDA uses the term chemistry, manufacturing and controls (CMC). Controls are basically analytical methods to show:

- The purity of your probe
- Its identity, i.e. that it conforms to the structure you propose
- That the process is reproducible
- That the probe is stable under conditions of storage

You will need to think about stability and how to scale up the material. Here are some questions you might ask yourself: If I'm going to store my probe before I use it, how should I store it? Should I keep it in the freezer? Should I keep it in the refrigerator? Is it okay at room temperature? You will have to perform tests to measure this. You will have to test how long it's stable under different conditions. If your probe is not a discrete molecule but a nanoparticle, then you have to consider how to characterize this material and how to show that the material produced is the same from batch to batch. These are the kinds of things to start thinking about.

LB: Can you discuss the difference in safety and tox studies between the imaging modalities?

PC: Certain safety and toxicology studies need to be done for imaging probes. The FDA has guidance documents for this (source **here**), and I assume regulators in other countries have similar guidance documents.

One of the real challenges when you work outside of PET imaging or SPECT imaging is that the mass doses used in other imaging modalities are relatively high. And as a result of that, we have to perform a number of safety studies, usually in two different animal species—one rodent and one non-rodent—in order to convince regulators that this is safe and to identify what sort of safety or toxicological effects we see at very high doses. The purpose is to understand the size of the window between the toxic dose and the dose that we're giving for imaging. We want that window to be as large as possible in order to know what the target organ is, and if we're likely to see any effect. Is this going to be something that we see in the cardiovascular system? or in the CNS? Or is it going to damage the liver? What is the effect that we might see? These questions will give us something to look for in clinical trials, so a number of studies need to be done.

For PET, usually only a very abbreviated safety package is required to start human clinical trials. This is why so many human research studies are done with PET probes, because the barrier to first-in-human is much lower than for optical or MR or CT probes. Because the studies require less material, and you don't need to do all these toxicological safety studies, the cost to getting first-in-human studies is also much lower. Keep in mind you'll need to manufacture enough material for these studies prior to performing them.

LB: Do you have any advice you can give the readers on how to work with regulatory?

PC: One of the things I've certainly learned is that it is incredibly valuable to have a pre-IND (investigational new drug) meeting with FDA. The pre-IND meeting can have a number of different structures.

Once you request a pre-IND meeting with FDA, you will then send them what's called a briefing book, which is really a summary of all of the work you've done to date around the manufacturing and the nonclinical safety and efficacy study, or maybe you have a plan for your clinical trial—whatever information you want to include. Another very important part of the pre-IND meeting is that you want to be very specific with the questions you ask the regulator. Avoid open-ended questions. For example, if you start a question with "What does the FDA think of . . .", you're not going to get a very satisfactory answer. If you ask a question projecting into the future with no current research—for example, if you haven't started your human trials and you ask questions about phase two or phase three trials—then you're not going to get a very specific answer. The FDA will only be able to comment on the information you provide to them. The more specific you are, the more specific they will be.

A better way to phrase a question to the FDA might be: "These are the studies we've done and the studies that we plan to do to address nonclinical safety and toxicology, does the FDA agree with this plan?" If they believe there are other studies that should be performed, then they would tell you under those conditions. If you already have a very detailed plan for manufacturing, that's going to allow them to give you a better answer than if you just give them vague, general information.

This pre-IND meeting is really an incredibly valuable process in two ways. One, it's valuable in terms of just putting together the briefing book, which is essentially going to form the basis of your IND application. And two, in asking and receiving feedback from FDA you will often learn things that you weren't necessarily expecting. It is always better to learn it in advance than at the time that you submit your IND application.

Developing A Probe: The Challenge of Finding The Right Clinical Questions

PC: Overall, it's challenging to develop a good probe. Often, what we make won't work in the beginning, then when we do get something that functions in an in vitro assay, once it is administered to an animal, it might be metabolized rapidly, or it might have unfavorable pharmacokinetics distribution. You may have to make other iterations and improve on your work. It's very important before you go down the translation path to first think: ***What question are we trying to answer? Can that question be answered with other methods? If so, are those methods limited in some way? Do we need this novel approach, or is it going to be an incremental advance? On the other hand, is it an approach that's going to have a huge clinical impact if it's successful? Who's going to use it and for what reason and how narrow is that indication?*** It could be okay to develop a probe for a very specific narrow indication, but a probe is often more valuable if it has utility in more than one area.

To give you some examples of this from our lab's work, one area that we've been very interested in is abnormal wound healing. This is referred to as fibrosis, or desmoplasia in the context of cancer. Essentially, we are referring to chronic tissue injury—or even acute tissue injury. As the organ heals, it follows a wound-healing process, but that process can get out of control because of repeated damage to the organ, resulting in scarring. It's a general phenomenon. In chronic diseases, like hepatitis caused by alcohol, diet, or viruses, you get scarring of the liver. In the lung, known causes like radiation injury in cancer treatment, or miners who inhale silica, or idiopathic pulmonary fibrosis, all these can result in scarring of the lung. In the heart, you have fibrosis occurring because of an acute myocardial infarction, or fibrosis in the atria caused by atrial fibrillation. Scarring of tissue occurs in the myocardium in patients with heart failure. Over time, because of the scarring, the organ doesn't function as well, and you get an ongoing series of injury that can lead to organ failure and death. In the intestine, conditions like Crohn's disease and colitis can scar the intestine, and it occurs in many kidney diseases, in atherosclerosis, and in muscular dystrophy. Some cancers, like pancreatic cancer, develop very fibrotic lesions.

PC (cont.): We have been interested in imaging some of these processes. Why we want to image fibrosis in these organs depends on the disease. In some cases, a method of early detection is needed. In others, we already have good methods for detecting early, but we want to stage the disease better. So often with fibrosis, the way we stage disease is by biopsy, which is invasive and only samples a small part of the tissue. In some organs, it can be quite dangerous to do a biopsy. In these cases, we can do quantitative molecular imaging to stage disease. Another area is prognosis—will the patient be stable over the next few years? This will impact how a doctor treats the patient because treatments often come with side effects. If we treat more aggressively, the quality of life may go down, so we might not want to do that if the disease is slowly progressing. Another application is in measuring response to treatment. Many different pathologies have several new therapies and getting an early readout of whether the treatment is working or not working would allow the physician to switch to another therapy.

Circling back to where I started—as we've developed tools to image fibrosis in the liver, we found that they've also been applicable in the lung, heart, and intestine, or in imaging a treatment response in pancreatic cancer. So, it can be applied in a number of different areas. Your probe is likely to have a bigger yield if you can apply it in multiple areas.

The limitation in most fibroproliferative diseases is that we didn't have very good ways to see early disease—and frankly, we still don't. The best imaging tools often only pick up advanced disease. The deeper I dive into these questions, the more I realize there are many unmet needs throughout other organ systems and other pathologies. When I present our work, we often get interest from other physicians who are working in another field, and they ask, "Well, I see you're doing this in the liver. Does it also work in the bowel?" These questions become so important and drive our research into new directions. The great thing about working in imaging is that you can point the camera anywhere—especially in MRI where we're not limited by needing to get a light on the target.