DR. CLIFFORD HUDIS: In the interest of staying close to on schedule, we're going to go ahead and get started. Our next speaker is David Mankoff. He's a Professor in the Radiology Department at the University of Washington School of Medicine. He's going to talk about PET, MRI, and other imaging. David, thank you for being the sacrificial lamb and starting as the room drifts in… We understand.

DR. DAVID MANKOFF: So, all of you that are back in the room -- you're going to get it, because the first slide or two is critical, so you'll just have to fill everybody else as they come back in. (Laughter)

I wanted to thank Julie, Eric, and Jo Anne for including me in this process and actually for being enlightened enough to have in vivo imaging in addition to the in vitro assay as part of the lecture. And so I wanted to thank you all very much. I've really enjoyed this conference. And I hope that you'll see, by the end of this talk, especially following those very nice set of discussions of tissue assay, how in vivo imaging is really complementary to the type of information that you get from those assays that we've just heard about.

So, the goals that I've been charged with today are to, number one, talk about research goals -- to really set the stage for why we would want to do imaging in a research study. And, as we said in the organizing committee, I'll give you an "Imaging 101" and just tell you what some of the modalities are, especially functioning on those… emphasizing those functional imaging modalities.

And then I'll go through, very briefly, some examples where research is beginning to have… bear fruit, to show some examples of how imaging can be helpful in these goals. And then briefly talk about future directions. And I promised the organizers I would do that all within my 20 minutes of allotted time.

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Now, being in Washington, I have to start with the appropriate caution: these are almost all investigational imaging methods. Not all of them. I'm going to show you some very exciting results, but I'm not endorsing these as clinically effective tests, at least not at this point.

So, I think this is actually probably my most important slide. I want to spend just a little bit of time on it; and I think you'll see that this slide echoes Dr. Wolff's talk from yesterday -- talking about what the imaging research goals are in the preoperative setting.

So, I have my timeline at the top, starting with diagnosis, my orange line of therapy here, and then looking, as we've heard throughout this conference, at pathologic response, especially CR, and then, importantly, looking at outcomes like disease-free survival and overall survival.

My goal number one -- and I'm just doing these along the timeline here -- is really, can we use imaging to do the kind of things we've been talking about doing from path? And that is, to identify our targets -- especially as we move towards targeted therapy -- and/or predict response.

And a closely related goal number two is to be able to measure response very early, perhaps as early as a day, or at least a few days or a week or two after the start of treatment. Now, actually, this may be more appropriately
-- especially when we're using these functional measures -- thought of not as early response but perhaps as the pharmacodynamics of the treatment that we're doing. Now, one of the things that's come up in some of the past lectures, is that, when you look that early, that may not be entirely predictive of response.

And, very importantly, I think what we're trying to do here is not necessarily find the responders, but find the non-responders. If we can use these two front goals to help us better select our patient population -- identify early who's not going to respond to the treatment we may have chosen -- that's what we're after here.

Now, as we move further down into the therapy and get towards the end of the therapy, we get to my goal number three, which is, predict outcome. Much of the work that you've seen in imaging in the neoadjuvant setting is predicting the outcome of pathologic response. And, as we've heard over and over again, a pCR is a very predictive outcome. But the hope here is that this in vivo functional imaging can not only get us as good as a path response, but when we start to look, more importantly, at more robust and downstream outcomes, that the imaging should add to what we already get from pathCR. And I'll show you at least one example of that in my talk.

And then, finally -- perhaps the most scientific goal -- is, we have these elegant in vivo tools -- can we use them to learn something about what happens in vivo to tumors as they get treated, in this very unusual situation of preoperative chemotherapy, where we have the tumor in place while the treatment is going on?

So, those are the goals, and those of you that made it back in the room for this slide -- you'll now get the rest of the talk.

Now, there's a number of different imaging modalities. There's CT; MR -- both magnetic resonance imaging and spectroscopy; my specialty, radionuclide imaging; ultrasound; and optical imaging. I'm really going to emphasize human imaging and emphasize functional imaging. So, with all due respect to the CT'ographers and the ultrasonographers in the crowd, we're going to focus on the ones that are in yellow [on the slide].

So, here is "Imaging 101". We've heard a lot about magnetic resonance imaging at this talk, and, as you know, this creates a three-dimensional image related to the proton chemical environment. We can alter that environment using magnetic nuclides such as gadolinium and iron. And we can actually be very clever about our pulse sequence, even in the absence of contrast, and measure things like diffusion, which may be an indirect indication of cellularity.

The capability is influenced by the field strength -- this is a little bit like computer processing speeds: they get larger all the time. The latest clinical field strength is 3T.

And, in particular, I'm going to emphasize, in this discussion, something that you've heard about already -- Dynamic Contrast Enhanced MR, or DCE MRI. These are the same curves you saw before, from Dr. Partridge at our institution, who is one of Dr. Hylton's, our earlier speaker's, post-doctoral fellows. And, again, the idea is we get functional information here to complement the type of anatomic information we've heard about earlier.

Now, the advantages of this technique is that it has very high spatial resolution. It does not have a radiation dose, which is very nice. The disadvantage is, is that, of all the imaging modalities, this is probably the most confined. If you've ever been in a magnet, it's pretty tight space; it clangs a lot; people can get used to it, but it takes a little bit of doing. And then the contrast possibilities are somewhat limited by the choice of nuclide that we have for contrast.

Now, if you take that same piece of machinery and then go back to your organic chemistry -- and I realize, for the non-chemists in the crowd, that may create a few nightmares -- but, if you go back to your organic chemistry lab, you remember the idea of

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using the magnet as a spectroscopy device; and that has to be able to look at the signals to identify the chemical species and their concentration in the tissue. Now, if you thought that was a challenge with your little organic chemistry samples, it's actually even more of a challenge in the body. And so this is an area where the higher field strength makes a huge amount of difference.

Now, this is a very elegant technique that does not require contrast, that allows us to look at a whole bunch of different molecules at the same time and measure their concentration with some regional capabilities. The disadvantage is, is that even with our very high magnets, it still has fairly limited spatial resolution. And if you thought it was a challenge to, you know, look at those spectra when you were in organic chemistry, imaging having a whole bunch of them and having to look at them quickly over many, many tissue planes and samples. So, it is a challenging data analysis paradigm even in our current state of the art of computers.

Now, when we talk about radionuclide imaging, we're thinking about single photon emission computed tomography, or SPECT, using such highly biologic nuclei [nuclides?] as technetium and I-123, which limits, to a certain extent, what you can make.

Now, positron emission tomography, or PET, uses positron emitters that come in flavors like carbon and fluorine, and, because of that, we can actually have a very wide range of probes, which is really, I think, perhaps the key strength of PET as a technique. We get a 3-D image of radionuclide concentration, and, perhaps even more so than MRI, you can get quantitative measures over time to look at kinetics through dynamic imaging.

Now, this is a very sensitive technique. It needs very few molecules to generate the image. And so you really are operating under tracer conditions where you do not perturb the physiology that you're looking at, unlike most of the other imaging modalities. We

can be quantitative and, again, especially with PET, we can have a very wide range of molecular probes. The problem here is, we don't have a whole lot of spatial resolution; this is inherently limited in its spatial resolution and anatomy. The combination of PET and CT in the same device -- in a PET/CT device -- helps a little bit, but it doesn't completely overcome that.

And there is some radiation dose. I did get a question about what the radiation dose is there. Actually, from a typical PET procedure, the radiation dose is less than diagnostic CT; but it's still a radiation dose, unlike some of the other modalities.

And then, finally, I'm going to very briefly touch on optical imaging. Simply said, this uses the light that your eye can see, but in much more sophisticated ways than we've ever been able to do before. And we can use contrast agents, we can look at bioluminescence, we can do near-infrared spectroscopy -- we can get very sophisticated as to how we look at the light emanating from tissue.

The advantages here: it's very portable; it's inexpensive
-- and this can actually be a bedside technique; it's minimally invasive; and it is possible to generate optical molecular contrast agents. So why is not… why is this not the modality that everybody's been using for the past 10 years? It has pretty limited tissue penetration, and some of the technology that's evolved has overcome that, to a certain extent; but it still remains one of its chief disadvantages.

Now, one of the things I was asked to say is, what is the patient burden for a patient who's going to participate in an imaging research study, as part of what she does in a neoadjuvant therapy trial? So, you can see the imaging times vary, with perhaps PET and SPECT being the longest imaging times; but you can spend up to an hour in the magnet,

more typically 15 to 30 minutes. And even some of the other… shorter tests will take some time.

As I mentioned, there is a radiation dose associated with PET. And there is somewhat of a confined and highly magnetic field in the MRI, which makes life a little bit inconvenient at times, although we're getting better and better at that over the course of time.

So, let me now go through some examples where we're able to accomplish some of these goals in very early trials, pointing toward what I think what the future will be.

And we'll start with goal number one -- to identify the target and predict response. Well, perhaps the oldest target in breast cancer is the estrogen receptor. And, fortunately, with a PET isotope, fluorine-18, we can make fluroestradiol, FES, which has properties very similar to estradiol. And this has been around for a while, and was actually developed in a collaboration between Katzenellenbogen and Welch at the University of Illinois and at Wash U.

And I'm going to start in a little bit of reverse order. This is a paper from our center, from my colleague Hannah Linden, that was published in *JCO* last spring, in the metastatic setting, that took two patients with ER-positive primary tumors, one of which had evidence of FES uptake in a sternal recurrence. So, black is hot [showing slide]; we're looking in coronal planes -- just so you can get oriented here. And, looking at the course of glucose metabolism as a measure of response -- it has a very nice, and actually rather early, response to hormonal therapy.

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Here's a patient, on the bottom, that has bone metastases from an ER-positive tumor; did not have an FES signal indicating an absence of the ER, and, without the target, this therapy doesn't work, and she didn't respond to multiple hormonal therapies.

Well, a number of years back, a study done by Mortimer, Dehdashti, and colleagues at Wash U had shown very similar results in a patient population getting primary tamoxifen therapy, including patients who were getting neoadjuvant tamoxifen therapy.

And, echoing the themes that I've told you about at the front end, what they found is, in everybody who is
ER-positive -- they're all candidates for hormonal therapy -- there was a subset of patients with low or absent uptake of the estradiol compound; and none of those responded to treatment.

Not everybody with uptake responded; but, importantly, we were able to identify a subset who did not respond, and who really would not have -- in a prospective study -- might have been candidates for other types of treatment other than endocrine treatment.

Now, this is an example of a study from Aberdeen -- we've heard a lot about their work. This is from an article by Semple. And it's a little bit of a different approach. Here, he is looking at the dynamic enhanced… contrast enhancement, looking at a couple of parameters, where most of these parameters relate to combinations of blood flow and permeability. And he was comparing that contrast enhancement map to an FDG-PET taken pre-therapy and post-therapy.

Now, in patient number one -- he had a relatively under-perfused tumor that had a lot of metabolism, and, with the hypothesis that perhaps the chemo just wasn't simply getting to that location, this patient did not respond very well.

Patient number two has a relatively well-perfused tumor, not as metabolically active; but, as you can see in the change of color over the course of time, this is a patient that responded.

So, for slightly different reasons, this pre-therapy study was predictive -- by looking at the ratio between fluorine metabolism, we found similar results in our center using a combination of PET agents.

I'm going to move on to goal number two, of measuring early response. And this is actually where there probably have been the most data and perhaps the most maturer studies.

I think the first of this came from FDG-PET -- fluoro-deoxy-glucose PET, the PET that you're used to seeing. And two articles published back in 2000 in *The Journal of Clinical Oncology*, both examining neoadjuvant therapy in locally advanced breast cancer, and both using measures of sort of minimal versus gross residual disease, macroscopic complete response versus non-macroscopic complete response; and showed that, after a single cycle of chemotherapy, they were able to see a significant difference between responders and non-responders that predicted what happened several weeks and months down… later, at the time of pathologic assessment. And, in one case, the early time point was actually the best separation between the two curves, giving an indication that this may be a very early measure of who's not going to respond, and with some prediction of who's going to respond.

You've probably seen, more recently, a larger study confirming these results, published by Rousseau recently in *The Journal of Clinical Oncology*. We're beginning to see some of these same things coming in more targeted agents, where FDG, again, has been a very

good predictor of early response -- perhaps, more appropriately, early pharmacodynamics.

You heard a lot about MRI in Dr. Hylton's talk. I won't dwell on this; but a recent study from Padhani and colleagues in Middlesex, UK, who, again, looking at this Transfer Constant Map, a measure that combines blood flow and permeability, showing that you see significant responses after one cycle that are predictive of outcome, at least in terms of pathologic complete response.

Now, I think one of the more exciting areas of work that have come along recently -- the group at Minnesota published a couple of years ago, by Meisamy in *Radiology*, has shown, by looking at magnetic resonance spectroscopy -- so, looking at the chemical species in the tumor over the course of time -- you can see some very early changes, perhaps as early as a day.

So this "Cho" represents the choline signal -- the level of choline coming from the tumor -- knowing that choline is associated with abnormal membranes and is a marker of the tumor. And what we can see is, as early as day one, is a decrease in that choline signal -- I dare you to try to read these spectra on your own. (Laughs)

But computers and people who know what they're doing can actually look at this quantitatively over the course of time and see these changes actually quite a bit sooner than you see lesion diameter changes, which take a longer time to go on. Again, perhaps another metabolic, biochemical evidence of seeing an early response, long before we start to see size changes in those same processes.

I mentioned the diffusion-weighted MRI. Diffusion is a measure of the degree to which water molecules can flow freely through tissue. And, in fact, when you have a very

cellular tumor in the breast, that limits the diffusion, and shows up as a decreased diffusion constant, often called the "ADC".

And this is an example from Dr. Jagannathan -- and I'm probably mispronouncing his name in New Delhi -- with a little bit of help from Bob Gillies in the University of Arizona. Showing, again, early changes in diffusion, perhaps -- and likely -- related to cellular dropout, making more room for water molecules to go through. Another technique to look for early response is early decreases in cellularity, as evidence of early response.

And then, finally -- this is a slide from Bruce Tromberg at UCI, who's been working with Dr. Hylton at UCSF, and using a combination of MR and then, importantly, optical scanning
-- you can see this really is a bedside technique -- showing some early changes, in this case in the lipid content and in the total hemoglobin signal measured by optical imaging, as a reflection of early response.

So, I want to move on now to goal number three -- can we look at whether these functional in vivo measures can predict outcome? And, again, we're not only going to look at the pathologic complete response, but the disease-free and overall survival as long-term endpoints.

This is a paper from our center by my colleague Lisa Dunnwald, published a couple of years ago in *Cancer*. We're using the molecule technetium-99m-MIBI, or "MIBI". This was designed as a myocardial perfusion tracer, has been used in tumor imaging -- it's actually approved for breast cancer imaging -- and, again, probably functions, at least with early uptake, largely as a tumor-perfusing imaging agent.

Now, we had previously shown by this… you can see in this example here -- that the change in MIBI uptake -- and, in particular, the residual MIBI uptake -- correlated with the pathologic response.

In this patient above, we see these many black dots in the breast and the axilla; and that's not good. Those went away at two months, and that was a predictor of a pathologic complete response.

Here's a patient whose black dots clearly got a lot better -- we measure these quantitatively as well -- but she had some residual uptake in the breast at two months, and had residual gross tumor at the time of surgery.

Now, the proof is in the pudding in terms of long-term outcomes. So, with a later subsequent analysis -- we looked at 62 patients -- I forgot to put the "N" on the slide -- and what we found is that high residual MIBI uptake, as an indication of residual tumor perfusion -- residual tumor vascularity -- was predictive of a poor outcome; not only disease-free survival, but [also] overall survival, which is actually rather challenging to predict in these studies. And I should mention that this was actually a study using patients who had largely gotten the metronomic chemotherapy regimen that Dr. Swain talked about yesterday, of the Ellis-Livingston regimen.

So, the question is, wow, this looks pretty interesting. These curves are, you know, small numbers but look rather predictive. How does that look when we compare this to other markers? Because the idea is that this is going to be additive.

Well, in this relatively small series, we did see primary path tumor CR's being predictive, as we would expect. Ki67 was predictive, as we would expect. And some trends for some of the other predictive variables. But we actually found rather a high degree of

prediction based upon the two-month study, and even greater amount of prediction based upon the study that was done close to the end of treatment, close to the end of surgery.

These hazard ratios are small; but this brings to mind that these are continuous variables, and that's the hazard ratio per unit change in this variable. And that's the power of the kind of continuous variables that you can get out of well-done imaging studies.

So, rather predictive in this early study, and, in a multivariate model, retained a significant p-value, suggesting that the imaging data is giving you something more than simply a prediction of pCR; but this in vivo data is complementary to in vitro path data, in terms of predicting outcome.

Now, I hesitate to use the word, "surrogate" outcome, perhaps especially after Dr. Wolff's talk yesterday; but I think you can begin to see that this kind of information can be very helpful in clinical trials, in trying to get additional information in trying to identify the best way to treat patients.

And then, finally, I just wanted to show you one example of how these types of studies might help elucidate tumor biology, especially over the course of response. And, again, this comes from our center, published by Jeff Tseng, one of our residents, who's now down working with the nuclear medicine group at Stanford. Now, we looked at glucose metabolism and blood flow using a combination of two PET tracers, fluoro-deoxy-glucose, which you're familiar with, and O15 water, which actually gives you very nice measures of blood flow. And we have plotted both pre-therapy and post-therapy flow versus metabolism for a series of approximately 30 patients.

Now, what I've drawn here, in this dashed line, is what I would see if we had plotted out the normal breast. Because, remember, most tissues are actually pretty well coupled in

their metabolism and blood flow -- that's sort of how the body works: it tends not to waste nutrients and waste blood flow. And if I were to plot the normal breast, you'd see them lining up along a nice straight line down here, and I've just extrapolated that over the course of time.

Now, unlike normal tissues, the tumors were not at all coupled, especially in the locally advanced breast cancer setting, pre-therapy. And, in fact, most of them had deviated significantly away from this line, where they were overusing glucose: they were a lot more glycolytic than one would expect per unit blood flow that they were measuring.

And, very interestingly, what we saw is a move towards this line post-chemotherapy, and actually a much closer coupling between metabolism and blood flow after the institution of chemotherapy.

The next slide's a little complex, so I'll walk you through it. But this is that same set of data, grouping patients by "no response", "pathologic complete response", and then a "partial response" showing some change in time… course over the time of the tumor.

So, there's a couple of very interesting observations. Number one is, if I look at where these patients start, the non-responding patients started furthest away from this axis, in that point where they have a lot of metabolism per unit blood flow, going back to the data from Semple that I showed you before. So, even before we start, that physiology looks predictive. And, very interestingly, the responders are moving down towards that axis and getting to the point of looking like they have both decreased blood flow and metabolism, and moving towards the line of matching for the normal breast.

Now, here's where I think it gets very interesting. The non-responders who did not have a change in size actually had some average decrease in metabolism -- they were moving

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towards more normal glucose metabolism. But they had an increase in vascularity. So, this tumor's trying to move towards a more normal metabolic environment, but it's doing it at the expense of vessel creation and increased blood flow, and that may actually be rather detrimental to the patient's long-term outcome, as I've just showed you.

So, this has been a hypothesis-generating exercise for us, because the null hypothesis that where we look at an imbalance between substrate use and delivery, and we look at aberrant metabolism, that's a stress response. Same kind of stress response that occurs in the heart that's ischemic. And that stress response for the tumor is a way of having the tumor cells avoid death, just like a heart trying to save itself. And this may be actually identifying a rather resistant tumor.

Now, in the process of chemotherapy, even in the absence of response, there is a decrease in metabolism. But, in those tumors with less favorable responses, there's actually an increase in blood flow, [which if it] gets us back towards more balanced metabolism, they may actually respond. But the question is whether we pay the price for this angiogenesis, for this increased blood flow, in terms of recurrence and metastases?

So, I'll conclude there; and I think I actually did manage to keep on my time. But hope to convince you that this is a very exciting area of research. It's quite complementary to the type of work that we've seen before.

There's a variety of modalities -- I've left some out, and I apologize to those for whom I've missed your favorite modality. But they are very good not only for measuring size, but [also] for measuring biochemical, functional, and molecular processes.

Our goals are clinical endpoints; but some are different clinical endpoints than we may have been used to using for imaging.

And so I hope I've at least begun to convince you that we can get some unique biologic insights that look at the in vivo biology of a treated tumor; and then, importantly, I think you can link this back pretty quickly to the talks you saw before the break and, so, how this in vivo clinical imaging can link us back to laboratory findings, and vice versa, and really create a very nice environment for translational research. And I thank you again.