

NCI 2007 PRE-OP THERAPY IN BREAST CANCER  
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DR. KATHY PRITCHARD: Thank you very much, David. Next, I'd like to welcome Matthew Ellis, who I think is a Professor in the Department of Medicine at Wash U, the Vice Chair of the breast committee from CALGB, and the Chair of Medical Oncology for ACOSOG. He's versatile.

DR. MATTHEW ELLIS: Thank you very much. So, I'll try and keep on time, too. So, I have an interesting topic to discuss -- this issue of window-of-opportunity studies and what we may learn from them. Well, the definition of a "window-of-opportunity studies" would be one where there's a relatively brief exposure to an intervention with no real therapeutic intent, as opposed to a preoperative treatment strategy where the idea is to shrink the tumor.

Of course, there are studies which are sort of hybrids between the two. You've seen some elegant examples of that from Professor Dowsett this morning, where he combines a window study using a two-week biopsy and then the patient stays on that therapy for therapeutic intent.

Now, in general, why would we want to conduct window studies? I thought of four areas to discuss. There's probably others:

One interesting idea [is] that you could study potential chemoprevention agents in this setting of the perioperative environment, where you could get a pre- and post-treatment sample to try and demonstrate that a given chemoprevention agent might have relevant biological effects.

Secondly, you might be able to use a window study to identify tumor resistance or sensitivity profiles. And I'll show you some examples of that.

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A third might be a demonstration of proof of principle with respect to a biological agent showing it as an expected mechanism of action.

And then, finally, you might even use it as a drug development strategy. For example, having defined a target, you could define a dose that actually effectively inhibited that target. This concept's sometimes referred to as, "biologically effective dose".

But there are a lot of problems -- practical constraints -- for any no-therapeutic-intent study, and perhaps particularly for these window studies. First of all, there are ethical and practical difficulties of conducting studies where there's no expected patient benefit. Of course, one is also restricted to non-toxic agents -- or relatively non-toxic agents -- in this setting of curative breast cancer surgery; so that you need a very well-established toxicity profile.

There are complex issues regarding logistics of sample collection and consent, which I'll discuss. And then, of course, you're relying on the tissue to give you your answer; so, you need robust surrogate endpoints which have some meaning with respect to clinical outcomes. And that's one of the larger barriers that we face in these studies -- what are you actually measuring and what does it mean?

And then, finally, the surgical setting may present some special difficulties with certain agents. For example, perhaps anti-angiogenesis agents.

So, examples of studies [agents] assessed in window studies. And I do apologize: there were quite a few of these in the literature, and I just picked out just one or two to make some points. But there's been some fairly extensive work with endocrine agents. Because, of course, they have relatively low short-term toxicity. There's been some study of dietary components. There have been studies that are in planning or being

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considered with drugs that have sort of incidental anti-cancer activity that might have prevention value, like COX-2 inhibitors and perhaps statins.

And then finally, there's beginning to be some work on signal transduction inhibitors, particularly those that have a very well-established toxicity profile. This kind of study using window of opportunity would not be appropriate for something that's very early on in its clinical profile.

So, first of all, [we've] discussed this issue of using the setting to study potential chemoprevention agents. And this just illustrates for me some of the barriers.

Vered Stearns here is in the audience; and if Ann Gallagher was here, I'd also point her out to you as someone who actually screened a very large number of patients -- 267 -- for a perillyl alcohol study. Perillyl alcohol was hypothesized to be a potential chemoprevention agent. It actually can be found in lavender oils, and it has a relative in the skins of citrus fruits called limonene. And these have been shown in various rat models to prevent induction of breast cancer. And so the idea was, could you demonstrate biological effects of perillyl alcohol in a window-of-opportunity study?

So, in order to find a relatively small number of patients who actually received this drug - - maybe 20 patients or so -- we had to screen 267 patients. The reasons for non-participation are given on the right-hand side and included ineligibility, no response to the study coordinator, difficulties with scheduling, patients who declined -- that was actually a relatively small number -- and miscellaneous logistical problems.

So, if you're going to do window-of-opportunity studies, you have to have a whole sort of setup to get at patients and discuss the study with them early in the course of their work-up.

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And during the course of this early window study, we came across a number of issues. One was the potential for patient harm in the early-disease setting. So, we had to do a sort of phase 1 dose-escalation approach to make sure that patients would actually tolerate the medication in the perioperative setting. There was issues regarding the discussion of research with patients who were experiencing a high level of distress due to a recent diagnosis of breast cancer. It's not the best moment to discuss clinical research with patients.

And then (unint.) was an interesting issue that also came up. If the patients entered the perillyl alcohol study, there was a question of whether they were subsequently eligible for Cooperative Group adjuvant studies. So that needs to be addressed, too.

One of the logistics problems we were confronted with -- the whole idea of this study, we would use existing pathological material. So, there was no dedicated tissue accrual. All our assays were based on formalin-fixed materials.

And we included patients who needed re-excision, not all of whom had material when they ultimately went to surgery for a second time. And so as a result, we found that our ability to get paired tissue -- before and after the intervention -- was somewhat limited. And that really restricted our ability to study our surrogates.

So, one of the things I did when I went to Duke was to look at the idea of whether you could get frozen tissue at the time of diagnostic biopsy. So, in other words, everyone would be approached at the time that the radiologist or the surgeon was taking tissue for diagnosis and say, well, take some extra cores that would be then frozen and could be potentially used for research. This approach required a system of so-called "abeyance pathology", where these samples are put on one side. And if the pathologist feels that a

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diagnosis can't be made with the routine cores, those abeyance samples then become part of the pathology record, and they're analyzed, too.

So, for example, this happened when the standard cores didn't show any cancer. And, interestingly, every time we did this, the abeyance cores also did not show any cancer. But, what you can show, if you have a good system for doing this, is you can actually get frozen tissue through abeyance pathology in a very large number of cases.

And you get high-quality cores as well. You can see here for T1 tumors, N=85. We're able to get up to four average cores per patient for research. And most of these had at least one core with 60 percent cancer. And I think, as the tumor size went up, we got to the point where almost all patients had a sample.

And so this is also, obviously, of great value in the setting of standard neoadjuvant therapy. Because if you have a system in place like this, you don't have to ask a patient for a research core. You already have one.

The other issue was getting the post-treatment sample at lumpectomy. And John Olson developed this device, where the lumpectomy specimen -- when it comes out of a patient, the routine thing to do is a specimen mammogram -- and John developed this device that holds the lumpectomy specimen and allows you to take core biopsies.

And we actually challenged the pathologists to see if they could see which ones we'd actually cored for research and which ones hadn't been subject to this. And they actually could never tell which ones we'd actually taken little bits of tissue out for research. So, this increased the number of patients for which you've got paired, before-and-after frozen material for window studies.

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And this just shows what you can do with this biopsy device in this relatively small experience when the biopsy device was used on the lumpectomy specimen versus not. It really increased the number of patients who had a cancer-rich core quite substantially. Because using the image you get, you can place the core needle right in the middle of the lumpectomy specimen where the tumor is.

So, how about using window-of-opportunity, or let's just say tissue surrogates, for identifying tumor resistance and sensitivity profiles? And here, the best data comes from the endocrine therapy field. Like Mitch [Dowsett], we've been working on Ki67 as both a surrogate endpoint biomarker but also PD marker -- or pharmacodynamic -- marker. And in this study that compared neoadjuvant letrozole and tamoxifen, as I think everyone appreciates now, the aromatase inhibitor produced superior clinical response rates.

But in the course of this study, we also developed a concept of cell-cycle complete response. So, the idea here is that ER-positive tumors, as we've learned, relatively uncommonly undergo pathCR, even with chemotherapy and certainly very rarely with endocrine therapy. So, was there something in the post-treatment sample that we could use that would have similar properties to pathological complete response?

So, we alighted on the idea of a tumor in which the Ki67 staining had essentially gone away -- 1 percent or less. And you can see here, in this slide, in fact, when you're comparing letrozole and tamoxifen -- relatively small number of cases. But, statistically speaking, more underwent cell-cycle complete response with letrozole than tamoxifen. And, in fact, this measure was more sensitive than the other measurements in terms of discerning the difference between these two drugs.

So, how good is cell-cycle complete response in terms of predicting long-term outcome? So, this is an interesting slide. It's preliminary data. So I want you to treat it that way.

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But it essentially shows something very similar to what Dr. Dowsett showed earlier. These are patients who, in their surgical sample, the Ki67 is 1 percent or less. So, they've undergone a cell-cycle complete response. And these are patients who have a Ki67 value above that level. And what you can see here, in this overall survival plot, that patients who have very low post-treatment Ki67's have a superior survival.

Now, I'd like to point out something interesting here. This is from the letrozole arm of the P024 study. All these patients received adjuvant tamoxifen, not letrozole. So this... and, in fact -- and counter to what you might have thought -- this predictability of the 1 percent cut-off worked much better on the letrozole arm than on the tamoxifen arm. So, it would seem that this estrogen deprivation test is quite predictive for long-term outcome, even when the patients receive tamoxifen.

Now, we have been taking advantage of this concept of the Ki67 data to try and find new markers for endocrine therapy resistance. And the reason for this is that we've been looking at letrozole -- effect of letrozole -- on proliferation, by HER2 status. And, what we've found is that tumors that are ER-positive and HER2-gene-amplified very rarely undergo what we call this "cell-cycle complete response". Almost all of them are showing letrozole-resistant proliferation; whereas, the majority of tumors that don't have gene amplification, in fact, do undergo a good response, and most of them showing very little Ki67 by the time they go to surgery.

And this finding that ER-positive tumors that have HER2 gene amplification tend to have high post-treatment proliferation levels exactly mirrors what you see in the adjuvant setting. That is to say, that these ER-positive, HER2-positive tumors do have a higher event rate compared to those that are ER-positive, HER2-negative. And this is true whether the patient's received tamoxifen or letrozole.

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But you can see that HER2 gene amplification is a minor player in the problem of endocrine therapy resistance. There's many more tumors that are HER2-negative that nonetheless are having problems with post-treatment proliferation. So, what are those other events that drive proliferation -- this estrogen deprivation resistance?

And we started a comprehensive approach to this problem, essentially using the tumor analysis as a screen for genetic events that drive aromatase-inhibitor-resistant proliferation. And in this screen, obviously, we measure the Ki67 before and after treatment. It probably doesn't matter too much whether it's a two-week [timepoint] or the three or four months. In fact, Mitch [Dowsett] has just told me that his four-month Ki67 analysis is just as predictive of relapse-free survival as the two-week.

So, the point is that these tumors can grow independent of estrogen, which is not what you'd expect of a fully responsive ER-positive tumor.

The genetics screen is based on whole-genome transcriptional analysis and array CGH. Because our primary hypothesis is there's other amplicons that can drive estrogen-independent proliferation. And we've also started some targeted gene resequencing. And we do IHC with phosphoprotein-specific antibodies to interrogate pathways.

Now, the array CGH is beginning to show some rather interesting things. Firstly, it's remarkable how complex the breast cancer genome is. And currently, we're tracking about 35 separate amplicons in ER-positive breast cancer for impacts on Ki67.

I show you these slides to illustrate to you that here's the HER2 gene -- here's the HER2 amplicon. You can see it's relatively rare compared to other amplicons. For example, the chromosome 8 rearrangement. This is a particularly interesting one at 8-P11.1 and 8-P11.2. I haven't got time to go into it -- but this has recently been shown to be



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potentially associated with the lobular carcinomas. So, you would expect it to be enriched in this population of locally advanced, ER-positive tumors.

The problem, of course, with any kind of correlation, as has been reiterated many times this morning, is sample size. We need to get to large sample sizes in order to overcome the problem of multiple testing, to show that these amplicons are associated with poor Ki67 response and, by corollary, poor long-term outcome.

So, to jack up our sample size, we initiated this study with ACOSOG [ACOSOG-Z1031]. It's comparing three aromatase inhibitors, which is a perfectly reasonable clinical question to say, is there one that should be favored over the others with respect to neoadjuvant treatment?

These aromatase inhibitors do differ in mechanism of aromatase inhibition as well as potency. So, we are asking whether there is a difference here. But also, of course, we're able to get the frozen material we need to do these sophisticated analyses. And this trial is actually going quite well. We're just about to reach that magical 20 percent accrual rule that CTEP uses to demonstrate that a particular study is viable.

So, additional studies have been done, for example, to demonstrate a new biological agent has an expected mechanism of action. And here I want to show you a few slides from Carlos Arteaga just because it illustrates this point. And he's able to take patients who have early-stage breast cancer and give them actually a relatively toxic drug, erlotinib -- these patients had quite a lot of skin rash. Nonetheless, we owe these patients our gratitude, or, at least, the breast cancer community does, because this was actually allowing Carlos to really study whether this drug might have the kinds of anti-breast-cancer activities we'd like to see. Now, it's a HER1 inhibitor.

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So, he wanted to demonstrate that, in breast cancer, it would actually inhibit the relevant pathway. And so he's measuring multiple components of this pathway. I'll just show you one or two of his slides. What he's able to show, for example, that -- this is HER1 -- phospho-HER1 -- and he can switch off phospho-HER1 with erlotinib in this particular patient. So, that's showing that the target is appropriately hit by the kinase inhibitor.

Interesting, erlotinib also inhibits phospho-HER2. Although, in vitro, it's a selective HER1 inhibitor. In vivo, it's actually able to inhibit HER2 phosphorylation as well, and actually has quite a strong anti-proliferative effects. So, I think the jury's still out as to whether HER1 inhibition is an important thing to consider for breast cancer treatment.

And then, finally, this concept of whether you can extend this idea further to establish biologically effective doses either of chemoprevention agents or biologics. And there's a lot of interest in this. I'd guide you to have a look at this review in *Nature Reviews Cancer* on the concept of phase 0 trials, where patients -- in this case, they were largely considering the advanced-disease setting, but you could also, with the right agent, do this in the perioperative setting -- basically, using tissue surrogates to establish dose and to establish that the appropriate target is being inhibited.

This paper is also very important. There's lots of... We've used this word, "robust", extensively during the course of this symposium, but I'd also introduce you concepts of accuracy, dynamic range, precision, reproducibility and sensitivity, and actually Mitch [Dowsett] was discussing some of these issues with respect to his Ki67 data.

And I'd actually also like to take this slide to emphasize that I don't think Ki67 -- post-treatment Ki67 -- is ready for primetime. I think that we need to develop a very robust assay before we do that... incorporate Ki67 data into patient decision-making. But I think it definitely could be done and we should consider it.

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So, the idea, then, ultimately, is that instead of the usual drug development paradigm where you try a drug in a mouse, the tumor shrinks in the mouse, and then you give it to a random population of patients in a phase 1 study, and then you sort of expand your sample size, hoping that you can find a sub-population of patients who are going to respond... Here, you do something much smarter, which is actually you understand the mechanism of action of drug, you understand the target, you show you can inhibit that target in phase 0 trials at the appropriate dose, and then you go into phase 2 in the right population of patients, whose tumors express the target, at the right dose. And then you may even, in sort of orphan drug indications, go straight to approval even without doing a phase 3 trial.

So, my conclusions are that window-of-opportunity studies are feasible but remain challenging; but there are ways around the problems. The clinical barriers are determined by the intent of the study, the nature of the agent, and the sample size.

And, most of all, window trials have woefully inadequate sample sizes. So, I was very impressed by the POETIC study design, because I think, actually, a sample size of thousands will probably be necessary. And then, finally, the scientific barriers are determined by the quality of the biomarker analysis and the mechanism of action of the agent. Thanks a lot.