Finding the Positives in Triple-Negative Breast Cancer:

A Three-Part Live CME Webcast Series

Seminar II
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Faculty
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SESSION 2: DNA repair pathways and translation research in triple-negative breast cancer (TNBC)

DR LOVE: Welcome to Finding the Positives in Triple-Negative Breast Cancer. I'm Neil Love from Research To Practice in Miami, Florida, and it has been a long time for patients with triple-negative breast cancer, but things seem to be changing, particularly in the last year.

Let's talk a little bit about what we're going to try to do tonight. We have two distinguished faculty members joining us. From Dallas, we have Dr Joyce O'Shaughnessy and, from Houston, Dr Jenny Chang: Two important contributors in this field.

Last week we had Cliff Hudis and Lisa Carey, and next Tuesday we'll be back for the third of our series, an hour-long program with Hope Rugo and Kathy Miller.

Here are my disclosures.

We're really looking forward to this tonight. We know it's probably been a long day for everybody and we hope you can hang on and listen to some of the science involved. We're going to start out in a few minutes with Jenny, who's going to talk about, particularly, some of the pathways and the translational science in this field that's just been exploding. We'll talk about a case from her practice, and we'll take some questions from the audience out there on the Web.

Then Joyce will talk about the information she first presented at last year's ASCO meeting in the plenary session on a PARP inhibitor in triple-negative breast cancer, BSI-201. Again, we'll talk about one of her cases and some questions from the field.

If you have a question or case, please just type it into the lower left-hand corner of your screen. If we don't get to it tonight, we'll try to come back maybe next week and get to it then.

All of the slides — and between the six speakers in these three webcasts there are almost 150 slides — will be posted on the web for you to go back and review. So just try to sit back and relax. Don't worry about every single detail that's up there on the slides. We're going to really just try to provide a conceptual overview.

And Joyce, I don't know if you remember this, this is a polling question we asked at our satellite meeting in San Antonio in December that you were present at, and we were covering five topics. And we asked the audience, "Which is the one you want to hear the most about?" And you might remember that it was overwhelmingly triple-negative breast cancer. I don't think that would have been the same answer about a year ago.

Introduction of poly(ADP-ribose) polymerase (PARP) targeted therapy in the treatment of TNBC

And I know Joyce, you're such an advocate for patients and I think you really — over the years, I've heard you anguish about the lack of excitement in research of triple-negative breast cancer. Just before we start to get into the science, I'm just curious what it felt like when you first saw the data? What was it like when you were backstage there at the plenary session getting ready to go up there and really offer some hope, I think, the first time to these women and to their physicians?

DR O'SHAUGHNESSY: That's exactly how I felt, Neil. I felt like, "Wow! I'm so fortunate to be able to go out and deliver this really important good news." I felt so privileged because I knew that everybody was going to be very, very excited to hear that we'd made a start. And I know in my own practice, we've got a long way to go, but we've made a start. And it was thrilling to have the opportunity to go out and tell people some good news about triple-negative breast cancer.

DR LOVE: So, Jenny, in memory I have of that plenary session — if you remember, it was kind of different in that the discussion by Merill Egorin was actually before Joyce's talk, and he stood up — I don't think anybody even knew what to expect from Joyce's talk — and started to go into PARP biology. I mean I don't know if the other people in the audience felt like I did, which was like, "Wow! What is this man talking about?" There's a lot of science in triple-negative breast cancer, and we're going to try to go through some of it tonight, but it seems like all of a sudden, in the last couple of years, maybe since — I guess around 2005, there's just been this huge influx of information and translational work in triple-negative breast cancer.

DR CHANG: Yes. And we're beginning to understand that triple-negative breast cancer isn't just one type of disease, but it's a heterogeneous disease. And maybe you can use different treatments to tackle different subtypes of breast cancer, especially triple-negative breast cancer. But it's really, really exciting, I think.

Case discussion: A 48-year-old woman, a known BRCA mutation carrier, develops contralateral right inflammatory TNBC six years after diagnosis of disease

DR LOVE: So before we tackle the science that evolved, we also are going to try to deal with some just day-to-day clinical management issues about triple-negative breast cancer. And I asked both of you to submit a case and thought we could, before — Jenny, before you start your talk and kind of go through — I mean, Jenny, I asked you to come here tonight because you're great at explaining pathways. To me, a lot of this just sails over my head. But you — we asked you for a couple of cases and the case that you brought to us — which actually we got a very similar case from the audience last week — was a woman who's actually had two triple-negative breast cancers. Maybe you can just briefly go through what happened with her.

If you can put up the slide.

DR CHANG: This is a young, premenopausal woman with breast cancer, and she presented with breast cancer back in 2002, and she had a left lumpectomy for a node-negative breast cancer. She had adjuvant radiation therapy, but no adjuvant chemotherapy.

About six, seven years after that, she presented to see me with a contralateral right inflammatory breast cancer, and it was big, it was red, it was hot. It was about 12 by 12 centimeters at the time of presentation.

So I enrolled her in a clinical study and she received neoadjuvant docetaxel chemotherapy. And basically, her cancer just grew straight through docetaxel and so, after two cycles, we switched her to AC chemotherapy. And even after the first cycle the cancer began to shrink, and after four cycles of AC chemotherapy with doxorubicin and cyclophosphamide, she then went to surgery.

Now, we know that both doxorubicin and cyclophosphamide are DNA-damaging agents, and so, at the time when she went for surgery, very, very surprisingly, she had a pathologic complete response with no residual tumor in the breast or in the axilla. And this patient is a known BRCA1 mutation carrier. Her mother died of breast cancer when she was very young. And other than that, we don't know very much more about her family history.

But this is a very — I think a very stark example of somebody who really is completely resistant to one kind of chemotherapy, like taxanes, and yet very, very highly sensitive to another, like anthracyclines. And are there differences in the biology of the tumor that can help us select who will respond to one treatment but not to another? And hopefully we'll be able to cover some of that during the talk later.

Selection of chemotherapeutic agents for patients with BRCA mutation-positive TNBC

DR LOVE: Joyce, what do we know about chemotherapy in triple-negative breast cancer, chemotherapy in BRCA breast cancer? I mean, I looked at this case and I was thinking, "Wow! This does not happen very often, I wouldn't think," but what do we know about this, Joyce?

DR O'SHAUGHNESSY: This is really a wonderful case, and it really recapitulates the preclinical data because BRCA1 is a spindle checkpoint, and when you have lost your BRCA1, when your spindle gets damaged by the taxane and you don't have BRCA1, the cell loses the ability to realize it's damaged and so it just goes on its merry way. And so that's — actually the preclinical cell-line data suggest that BRCA1-mutated breast cancers don't really respond well to taxanes, but they respond very well to DNA-damaging agents because they don't

have the BRCA1 or 2 there to repair the single-strand and double-strand breaks that our chemotherapy agents make.

So this — Jenny's case is really right on. Neil, we don't know a whole lot. That's the thing. This is a whole new area. There is a preclinical — sorry, a clinical study of preoperative single-agent cisplatin, 75 mg/m^2 every three weeks times four in 25 BRCA1 germline mutation carriers. I believe they were from Poland. And with just four cycles of single-agent cisplatin preoperatively for BRCA1 mutation carriers, there was an 87 percent pathologic complete response rate — and I beg your pardon, I think it was 72 percent — 72 percent pathologic complete response rate. That's been published now and also updated at ASCO last year.

So that's very, very impressive data with single-agent cisplatin. So we are really, I think, in the infancy of sorting out what chemotherapeutic agents really are best utilized for triple-negative disease and that subset of triple-negative, which is the BRCA1 germline mutation carrier.

Perspective on BRCA testing for a 42-year-old patient with contralateral TNBC

DR LOVE: So, Jenny, last week we talked a little bit about adjuvant therapy of triple-negative breast cancer and what the prognosis is and how do you approach treatment. And we actually got this case that we didn't get a chance to talk about, from Dr Elkhatib, who — a young woman, actually very similar to your case in that she had two primary triple-negative breast cancers. The first one she got dose-dense AC/paclitaxel. Now the woman has a second triple-negative breast cancer, and one of the questions we got, it wasn't put in this, but was, is there any role of doing BRCA testing in triple-negative breast cancers? I mean I guess you could justify it based on this woman's age to start with. And what would you think about adjuvant therapy in a woman who's developed a second primary tumor after dose-dense AC/paclitaxel, Jenny?

DR CHANG: I mean, in this lady, I think if you did a BRCA proanalysis on her, I think she's going to make the mark for testing for BRCA1 and 2. She's got bilateral breast cancer and it's triple-negative and she's premenopausal. So even in the absence of a family history, I think she would require BRCA1 and 2 sequencing.

I think we are in the area where we don't have very much data. Personally, she has high-risk disease. I'd probably reconsider adjuvant chemotherapy, because she has high-risk disease and she's very, very young. But that is in the absence of data, and I would choose something with DNA damaging in it.

DR LOVE: And we're going to get to your talk in just one second, but final comment from Joyce about this case and about this issue. Another question that we got last week was the question of BRCA testing in a patient who might normally not think about — an older patient without a family history — just who has triple-negative breast cancer. In this era of the excitement about PARP inhibitors, is there any reason to think about BRCA testing in a patient like that?

DR O'SHAUGHNESSY: I don't think so, Neil. Judy Garber brought data to San Antonio about two years ago, I think, where they looked at triple-negative breast cancer patients under the age of 50, over the age of 50, with a family history, without a family history. And it was quite striking, because under the age of 50, even without a family history, if you had triple-negative breast cancer, you had about a 13 percent chance of having a BRCA1 germline mutation, and that was much higher than had been expected, based on traditional modeling. But interestingly, in women over 50 with triple-negative disease, it was only if they had a family history of breast cancer that they had an elevated enough risk for having a BRCA1 that it became really kind of compelling to recommend testing for them.

So, over 50 without a family history wasn't particularly compelling, but that was a practice-changer for me because my cutoff had been 40 — under 40, without a family history: Test. Now, for the triple-negative, it's under 50, without a family history: Test.

Clinical approach to adjuvant therapy for patients with small, triple-negative tumors

DR LOVE: And Joyce, how do you approach the issue of adjuvant therapy in patients with triple-negative tumors, particularly the smaller node-negative tumor? How do you kind of guesstimate the actual prognosis and the impact of therapy? Another question we got a lot is, "Do you use more aggressive chemotherapy?" A lot of node-negative patients get something like TC. In triple-negative should it be shifted to more an anthracycline/taxane?

How do you approach this issue in your practice, Joyce?

DR O'SHAUGHNESSY: It's a really important question, because one of the things I've learned, for example, in the subcentimeter HER2-positive patients, is that you can kind of throw size out the window a little bit, because the subcentimeter HER2-positives have a pretty poor prognosis, up to a 14 percent distant risk of relapse in

five years and 23 percent risk of relapse in general. And so we can't be too lulled into feeling comfortable with subcentimeter triple-negative disease.

Now in my own practice, in T1C/N0 triple-negative, I'd give them ACT. I give them three drugs. Under a centimeter, I will say that I give strong consideration to four cycles of TC — docetaxel/cyclophosphamide. I think that we really don't know a lot about doxorubicin in triple-negative breast cancer. There are some data sets that suggest it's really not critically important in the context of also receiving cyclophosphamide.

So the smaller the cancer subcentimeter, the more likely I am to use four TC. Dr Stephen Jones's data showed that TC was superior to AC in ER/PR-negative — remembering we didn't have HER2 data in that study, that older study.

So TC was superior to AC in ER/PR-negative patients. But T1C and greater, I use three drugs.

Function of BRCA1/BRCA2 in specialized DNA repair

DR LOVE: Okay, Jenny, so we're going to let you take a crack at talking about some of the translational science, a lot of which you're involved with. And I've been asking people about this now ever since Joyce's presentation. In our interview programs, we've had programs on triple-negative disease. But each time I talk about it, I think my knowledge — translational knowledge — level goes up like about one percent. I don't know whether people who graduated med school in the last two or three years just say, "Oh, this is real easy."

But why don't you take a shot a little bit about trying to explain the biologic basis of what's going on right now in this disease?

DR CHANG: I'll try, Neil. This is a difficult task, but I'll try.

So let's talk a little bit about some of the things that we do know about BRCA tumors. So we know that germline BRCA1 mutations probably account for about two — 20 percent of inherited breast cancer, and yet, as a whole, this is only two percent of all sporadic cancers. So what we can learn from germline BRCA1? Maybe it can tell us a little bit about sporadic cancers.

Now, in order for a BRCA1 mutation carrier to develop cancer, there has to be a somatic inactivation of the second wild-type allele. What does BRCA1 and 2 do? The main function for BRCA1 is double-strand DNA break repair. It senses DNA damage. It's important in checkpoint control, et cetera. So BRCA1 and BRCA2 maintain DNA repair.

In healthy cells, with a normal BRCA gene, specialized DNA repair is maintained. Now in cancer cells, the BRCA1 or BRCA2 gene is lost and specialized DNA repair is also lost. So let's talk about the types of DNA repair and damage that can occur.

For the first type is single-strand breaks. And for single-strand breaks, for it to repair, the mechanism is base excision repair and this requires the PARP enzyme.

Another type, which is double-strand breaks, requires recombinational repair, and this is homologous recombination and it is this that is deficient or damaged or deficient in BRCA mutation carriers.

So BRCA mutation carriers have defective DNA repair, primarily through homologous recombination. So that is defective.

Induction of synthetic lethality with PARP inhibitors

So let's try to understand, and I hope I can explain a little bit about what PARP does and a little bit about how this pathway plays in, in BRCA mutation carriers.

So PARP — poly(ADP-ribose) polymerase enzyme — it is involved in DNA base excision repair, single-strand repair. It binds directly to the DNA at the point of damage and produces large branched chains of poly(ADP-ribose).

So this is a cartoon, and I hope that this maybe can explain the concept of synthetic lethality. If you have a normal DNA repair, you have four legs to a table. Your homologous recombination is intact, your base excision is intact. So if you attack these cancer cells with PARP inhibitor, the leg, the one leg is lost, and that is the leg that's involved in base excision repair. But your table is still standing because you have three legs — because of your intact homologous recombination.

What happens, looking at the bottom row now, in a BRCA mutation carrier who has defective homologous recombination? She's born with a defective repair in this double-strand break. So there's one leg missing already. So with a PARP inhibitor, which now hits the other leg, that inhibits base excision repair, the table falls and the tumor, essentially, dies. And this is the concept of synthetic lethality — exploiting the intrinsic weakness of the tumor to therapeutic advantage.

And so, based on all this work, because we believed that BRCA mutation carriers were more sensitive to PARP inhibitors, cell-line data was done and this is the BRCA2 deficient model, which shows high sensitivity to PARP inhibition. And this is using the AZ compound, the KuDOS compound. Likewise, BRCA1 cells are also extremely sensitive to PARP inhibition.

Emerging data on PARP inhibitors in TNBC and BRCA-deficient mBC

And now we have seen this paper, which has now been published in *The New England Journal*, and this is looking at olaparib, the PARP inhibitor, when given to BRCA1 and 2 mutation carriers with advanced breast cancer refractory to the many, many lines of chemotherapy, and this shows a — shows a very robust, objective response rate of 42 percent.

And from the key observations, this is a very well tolerated drug, with very, very few toxicities and a high response rate, according to standard response criteria, and stabilization of disease.

I know that some of the other data show that its response is not only in breast but also in prostate cancer.

And this is a waterfall plot, basically showing the responses in some tumors using olaparib at 400 milligrams BID in BRCA1 and 2 mutation carriers, with increasing tumor shrinkage. There were a few patients that had increase in tumor volume, but by and large, most patients actually had a decrease.

And this is an example of a 46-year-old patient with BRCA1 mutation, highly refractory to multiple lines of chemotherapy. She had chest wall recurrence, extensive cutaneous metastases. And even the cutaneous metastases were refractory to chemotherapy.

As you can see here, she received nine weeks of olaparib and you see a very nice, beautiful response in a cutaneous metastases to this small molecule.

And I'm not going to talk very much about Joyce's seminal paper. I really think it's one of the most heartening data that was presented, giving a lot of hope to women with triple-negative breast cancer. But here you can see, and she's going to go through in great detail, how she basically exploits the biology that may be present in triple-negative breast cancer, targeting PARP inhibitors. And actually — she will go into more details — the overall survival that BSI-201 has in triple-negative breast cancer.

So basically this is just laundry list of some of the PARP inhibitors that are out there. The AZ/KuDOS compound, olaparib, which we basically talked a little bit about, the BiPar/Sanofi BSI-201 compound, and there are a few other small molecules out there in development as well.

BRCA phenotype as a predictor of response to chemotherapeutic agents

And very briefly — and I know Neil and I will talk a little bit more about this in a while — but not all triple-negative breast cancers are alike. Probably some — a subset of triple-negative breast cancers — will have this homologous recombination defect, similar to BRCA1 mutation carriers. And if that is the case, can we actually identify those patients with inherent DNA repair defects that therefore are more sensitive to DNA-damaging agents, like anthracyclines and PARP inhibitors?

And very briefly, I'm going to go through some of this, trying to exploit this so-called known BRCA1 phenotype.

So what we did was trying to find whether or not there was a signature that could predict who would respond to DNA-damaging agents, and what we did was, we took the gene-expression data from BRCA1 mutation carriers, and basically to look at the subset of triple-negative breast cancers that had the same BRCA gene profile. And we came up with the 25-gene array and we applied it to patients who actually received neoadjuvant anthracyclines. And what we found was that patients who had this BRCA mutation picture, or gene-expression profile, were more likely to respond to anthracyclines and more likely to be resistant to taxanes.

So in summary, I think PARP inhibitors exploit defective DNA repair mechanism, which is present in BRCA mutation carriers. A subset of sporadic triple-negative cancers may shift similar defective DNA repair mechanisms, with identified and validated set of genes by microarray and by RT-PCR that can identify sporadic triple-negative breast cancer patients with this so-called BRCA-ness. And we may, in the future, be able to use this or other tests that could predict who will respond to anthracyclines/PARP inhibitors or other DNA-damaging agents.

I'd like to thank everybody in the slides. Thank you very much.

DR LOVE: Thanks, Jenny. That was awesome. I love the pictures. I love the table. We actually are spending a lot of time trying to come up with a slide set. We're working with an artist from — trained at Johns Hopkins Art

Department to really come up with some slides that explain this whole thing. And I'm going to come back to you about this concept of BRCA-ness.

Heterogeneity of TNBC

But Joyce, I've got to let you take a shot at some of the Chuck Perou tie-ins here. Last week, Lisa Carey from North Carolina went through the Perou Classification System and tried to address this issue of basal — the basal subtype versus BRCA versus triple-negative. I have to say, we got into claudin-low, too. I don't know if we're going to do that tonight. I know you and I talked about that before. But Joyce, just in a minute or two, can you kind of summarize the way you — what your take is on the Perou Classification and how it ties in to this data set that we're about to talk about?

DR O'SHAUGHNESSY: Neil, you're hitting the nail on the head, and I think that I'd be really interested to hear what Jenny says as well, because Chuck Perou was taking the approach that there's the basal breast cancers. And so they're triple-negative, for the most part, and they have either basal cytokeratin 5 or 6, maybe EGFR, and that's the definition of this, the basals.

And then he has this new type of breast cancer called the claudin-low. They're generally also triple-negative, but they are not epithelial — they're mesenchymal. They are spindly and invasive and metastatic, and they've lost this protein called claudin. And claudin keeps your epithelial cells together, kind of like e-cadherin. And when you lose it, the cells are no longer epithelial. They become mesenchymal.

So that's a new kind of real primitive stem cell-y type of triple-negative. And then we've got the basals, and the BRCA1 germline mutation patients are basal — they're not the claudin-low.

Now, Jenny, I was really interested in your poster at San Antonio because you took these triple-negatives and I thought that you broke them down into two different groups — the ones that had the BRCA-like signature, and then, Jenny, there was a different group of triple-negative that had some other pathways. And I think your poster had some other pathways in there and they were kind of invasive and metastatic-type pathways like PDGF-R, if I'm recalling correctly. I don't know if the TGF-beta was in there, Jenny, but it might be like you were describing kind of the basals versus the claudin-low, but you didn't call it claudin-low. Am I off base on that?

DR CHANG: Slightly. I think that the non — claudin-lows are very rare. I think they're probably more the EMT, mesenchymal metaplastic tumors. And so it probably forms a subset of the non-BRCA-like, but not all of them.

But, yes, I think the non-BRCA-like had more invasive genes, more upregulation of the EGFR and all the other — Src and a few other important pathways. But even that is heterogeneous, I think.

DR LOVE: So, Jenny, obviously a key thing in oncology now, personalized oncology, which I think we've been hearing about for 30 years anyhow, is trying to predict who's going to benefit from therapy, avoiding therapy in people that aren't going to benefit. It sounds like you have started and others have started to make a little bit of headway into so-called BRCA-ness and maybe prediction of response to PARP inhibitors.

Are we maybe going to see an actual clinical assay for this over the next couple of years, Jenny?

DR CHANG: I hope so. I mean, like Herceptin® for HER2-positive breast cancer, I'd hope that we can have a test that would predict who would respond to PARP inhibitors. And we should be able to do it because it's a biologically driven therapy, so you should be able to pick up the subtype that would respond to this targeted therapy, I hope, like Herceptin for HER2 and estrogen-targeted therapy for ER-positive breast cancers.

Concept of combining mTOR inhibitors, PARP inhibitors and chemotherapy as treatment for TNBC

DR LOVE: So, Joyce, we're going to go to your case in a second, but we had a question from somebody who knows more about the biology than I do, Dr Tedesco, and she brings up the interesting question of PTEN and mTOR inhibitors. Of course, we're hearing tons about mTOR inhibitors in renal cell cancer. Does this kind of jibe with you — the concept of maybe mTOR inhibition in these tumors?

DR O'SHAUGHNESSY: Yes. Yes, and I thank you, Karen, for the question. Dr Alan Ashworth, who is the father of the synthetic lethality concept that Jenny explained with the PARP inhibitor and the BRCA mutations, recently published a paper in EMBO, and what it showed in breast cancer cell lines is that breast cancer cell lines that had lost this really important tumor suppressor, PTEN, which sits down on the PI3-kinase pathway and keeps it quiet — that the loss of PTEN led to a 25-fold increased sensitivity to PARP inhibition. So what does loss of PTEN have to do with DNA-damage repair in inhibiting PARP?

It turns out that he had some data that when you lose PTEN, you have an inactivation of the function of RAD51. Because, see, BRCA1 and BRCA2 work — I think of it as a bouquet of proteins — they don't work alone. There's a whole bunch of other accessory proteins. And RAD51's a particularly important one.

So when you lose PTEN, apparently your RAD51 doesn't work very well, and, at least in his cell-line model — exquisite sensitivity to PARP inhibition.

So that does bring up a really interesting hypothesis for a clinical trial, which is to put together a DNA-damaging chemotherapy with a PARP inhibitor and then, in those that have PTEN loss, put in a PI3-kinase inhibitor or an mTOR inhibitor that blocks that PI3-kinase pathway downstream, because you've lost that PTEN.

I think it's an extremely important trial that cannot happen fast enough.

DR LOVE: So, I mean I don't — sometimes I think I can't get beyond upstream or downstream in terms of pathways, so I'm not going to ask where PTEN is. But, Jenny, before we get to Joyce's case, I asked her before about this question that we got about BRCA testing in triple-negative, the older patient you wouldn't think about BRCA. Is there a role now for that, Jenny?

DR CHANG: In the absence of a family history?

DR LOVE: Right.

DR CHANG: For an older patient with triple-negative, I don't think the indication is that we should test at this point.

DR LOVE: So, hopefully, we're going to have a clinical assay, maybe related to the work that you reported at San Antonio, that'll help us predict who's going to respond.

Case discussion: A 58-year-old woman with metastatic TNBC (mTNBC) one year after completing adjuvant TAC chemotherapy receives carboplatin/gemcitabine with BSI-201 as part of a Phase II clinical trial

Joyce, let's talk about your patient. Maybe this is going to be the future of where practice is in a couple of years. Why don't you talk about what happened with this woman?

DR O'SHAUGHNESSY: This is a patient who actually enrolled in the original randomized Phase II study of gemcitabine/carboplatin with or without the BSI-201. And she's a 57-year-old woman with no family history of breast cancer who underwent a left mastectomy for triple-negative breast cancer and had six positive nodes. So she was a high-risk triple-negative. And she was treated with TAC adjuvant chemotherapy and postmastectomy radiation therapy, but she did not do very well at all.

And a year later, she presented, interestingly, with nodal predominant disease — big, painful supraclavicular and large left cervical lymphadenopathy — mediastinal adenopathy as well. And really nothing — nothing else.

Node biopsy showed recurrent triple-negative breast cancer. And so —

DR LOVE: And Joyce, let me just — let me just interrupt before you continue on with the case, because one of the things we got into in that symposium in San Antonio that I was just mentioning is, what were the options before these new, exciting agents came on board? How would you be thinking through nonprotocol management of a woman like this who's progressed a year after TAC?

DR O'SHAUGHNESSY: That was — we really just didn't have any good options there, Neil, because unfortunately, the truth is that in these triple-negative patients who really have manifest themselves to be refractory to our best drugs, we have very, very little evidence, if any, that bringing in any other cytotoxic agent is really that important — that they are effective in them. We think we want to turn to a platinum, but if you ask yourself, "What's the data that a platinum's going to help anybody who's already relapsed quickly after TAC?" there are no data.

So in fact the cytotoxic therapies don't work very well. So I think most of us turn to bevacizumab — we turn to Avastin® for patients, and we'll put it together with weekly paclitaxel or, now that we have RIBBON 1/RIBBON 2, we have a variety of choices on chemotherapeutic agents. But again, you have to be — for E-2100, you had to be out at least a year from your adjuvant taxane. This woman would have just been on that cusp.

So, Neil, I think the only agent that heretofore I've been impressed with in drug-refractory, triple-negative has been Avastin. I think Avastin generally does help the patients — gets them either some tumor response or some progression-free survival — but we've very, very little data that cytotoxic therapy is non-cross resistant in that type of patient.

DR LOVE: And actually, we'll have the principle investigator of E-2100, Kathy Miller, on next week. We'll talk about bevacizumab, platinums, et cetera.

But Joyce, maybe you can just follow up briefly in this patient? Now, was she symptomatic at the time she entered the trial? And can you just bring us up to date?

DR O'SHAUGHNESSY: Yes, she was symptomatic. She really had pain. The lymphadenopathy, particularly in her cervical chain,

was very tender and limited her mobility — it was quite large. So, fortunately, she was randomized to gemcitabine/carboplatin with the BSI-201.

Now, she had a minor response. And I had to do a lot of pep talking, because she really still had — her pain got better, but she never really reached partial response criteria, but she wasn't progressing either, and that's kind of remarkable. Because in triple-negative breast cancer, if you get any kind of a response, the problem with the responses is they are generally not durable, particularly in patients whose breast cancer has manifested itself to be pretty drug refractory.

So she really had stable disease for 15 months. She developed some cumulative thrombocytopenia. We had to dose reduce her gem/carbo per protocol. Eventually, I felt that she progressed by RECIST criteria. It wasn't a massive progression, however. It wasn't one of these explosive progressions. It was just one of these slow, breaking through progressions.

So we changed her over at that time to vinorelbine and bevacizumab. Now there, interestingly, she had a very rapid partial response after one cycle. So she had dramatic response. The problem is, it wasn't terribly durable. She got about six months and then, unfortunately, she developed bilateral pleural effusions and significant brain metastases, and she died.

So that was an interesting example to me. She got a minor response but prolonged stable disease with the gem/carbo and the BSI-201.

DR LOVE: And we want to hear about the data, again, that you presented. But just one more question back to Jenny. One of the things that came up last week that we were talking about with Cliff and Lisa is the data suggesting maybe more brain mets, maybe more pulmonary mets in triple-negative breast cancer. Is that a reality? And if so, any speculations about what's going on biologically?

DR CHANG: I think that the data is possible. I think, like HER2 breast cancer, triple-negative breast cancer, if they relapse, tend to relapse in the CNS and in maybe viscera.

I don't know. Is there something that is intrinsic within the tumor? Can you predict who would develop eventual brain metastases? I think that's a very interesting point. And can we select those patients for maybe up-front cranial radiation if we can actually pick up those patients?

I think that's an area of great interest. And I think a number of people are working on that.

Role of the PARP protein in the mechanism of DNA repair

DR LOVE: So, Joyce, if you could talk about, not just the data, but I know you're going to talk a little bit more about the biology and how it's integrated into the rationale for this study.

DR O'SHAUGHNESSY: Sure. No, I think it's a very, very interesting topic and Jenny has really very nicely laid out some major DNA repair pathways. And I sort of simplistically — my understanding takes me as far as the double-strand breaks and the single-strand breaks, and the single-strand breaks are being repaired by base excision repair. The PARP protein, I was interested to find out, is the single most plentiful protein in the nucleus.

So really, really important protein to repair single-strand breaks. And then BRCA1, BRCA2 and the associated proteins repair double-strand breaks. And one of the things that happens is, if you're not able to repair your single-strand breaks — and I'll just back that up here — if you're not able to repair your single-strand breaks, at cell division the single-strand breaks deteriorate into a double-strand break. And you've got to have your BRCA1/2 pathway intact to repair the double-strand break now. If you don't, the cell is going to commit mitotic catastrophe.

So if you inhibit PARP in the setting of a loss of the homologous recombination BRCA1/BRCA2 function, then you're going to have the ability to kill the cell.

Inhibiting PARP alone in the setting of intact repair of double-strand breaks doesn't hurt the cell at all, and that's actually really, really good for the host and for our patient's normal tissue. Because their normal tissues have the ability to repair a double-stranded break. And so you can inhibit PARP and it's not going hurt the patient's normal tissue.

So this PARP is very highly conserved. It's in every cell, and shown here is the normal tissues, all the normal tissues, and you see low levels of PARP and they're all pretty uniform. But then if you assay different cancers to the level of PARP expression, you see that in breast cancer, endometrial cancer, lung cancer — both small cell and non-small cell — as well as ovarian cancer, there's marked upregulation. There's just more of the PARP protein, suggesting that these cells are very dependent on this enzyme. There must be a lot of single-strand breaks going on in these cells, or these cells may have lost their ability to repair double-strand breaks. And the PARP enzymes can kind of moonlight a little bit as a poor-man's repair enzyme for double-strand breaks when the BRCA1/2 pathway is gone.

So, though it's predominantly a single-strand break enzyme, it can moonlight as a double-strand repair pathway.

Downregulation of BRCA1/BRCA2 expression in nonmutated germlines

So it turns out, if you look at the table on the right, about 80 percent of triple-negative breast cancers have PARP-1 upregulation. So these triple-negatives really have a lot of PARP — not all of them, but most of them have a high level of PARP — suggesting that they really need this, maybe because they're not so able to repair double-strand breaks in their breast cancer.

So, like, what do we really know about that? Most triple-negatives, of course, do not have germline BRCA1 mutation. So is there something else the matter with their pathway? We just mentioned the PTEN story and how there's some speculation that if you lose PTEN, your RAD51 doesn't work very well. And RAD51 is an important part of the BRCA1/2 complex.

PTEN is lost in most cancers. So that's a very common problem in triple-negative. So there's an example of where the pathway may be messed up in triple-negative.

But there's also data, shown on this slide here, that you downregulate BRCA1 expression because of the loss of a protein — oh, sorry, overexpression of a protein — called ID4. ID4 negatively regulates BRCA1, and when there's too much of it, you get downregulation of BRCA1 and so you lose the functionality of BRCA1, though you don't have a germline mutation.

And then there's some evidence that in metaplastic breast cancers and in medullary breast cancers that you can have some BRCA1 gene-promoter methylation turning off BRCA1 expression.

So there's a whole ton of work going on to really dissect out how it might be that basal-like triple-negative breast cancers have problems with their BRCA1 pathway.

So Jenny very nicely laid this out for us, that if you inhibit PARP and you don't have your BRCA1/BRCA2 pathway intact, that's synthetic lethality and the cell is going to die. And particularly if you give a DNA-damaging agent and you create lots of single-strand breaks and now you're inhibiting PARP, the cell is basically in crisis and these single-strand breaks deteriorate into double-strand breaks, and if you don't have BRCA1/BRCA2, the cell is going to die.

Potentiation of platinum and anthracycline agents with the PARP inhibitor BSI-201

And so the olaparib, which is the oral PARP inhibitor by AstraZeneca — these data were at ASCO by Dr Andrew Tutt. These are BRCA1/BRCA2 germline mutation patients with metastatic breast cancer pretreated with prior therapy, given single-agent olaparib, no chemotherapy, and at the higher-dose olaparib, 41 percent objective response rate. In the waterfall plot, we see that most patients responded, as Jenny said. But the few patients who didn't respond, interestingly, had had prior platinum. So those cancers were resistant to platinum. They had — though they were BRCA1/2 germline mutation-positive — they had found their way around the platinum. And so, therefore, they weren't somehow — they were resistant as well, to the PARP inhibitor, which is quite interesting.

So we had the opportunity to study a small molecule intravenous PARP inhibitor called BSI-201. And it directly binds to the NAD binding site of the PARP enzyme, and it inhibits DNA repair very clearly, and it potentiates DNA-damaging agents, such as the anthracycline and the platinums, and it also potentiates gamma irradiation. And it also penetrates the blood-brain barrier.

And it had been shown in preclinical models that it was basically synergistic with the platinum and with gemcitabine. And we thought that we would really go into a triple-negative model, because we kind of were hoping that most triple-negatives had enough problems repairing double-strand breaks that we might be able to really potentiate the effectiveness of DNA-damaging chemotherapy by inhibiting PARP.

Interestingly, as a single agent as well as in combination with chemotherapy, the BSI-01 has — 201 — has no dose-limiting toxicities at all. There was no maximal tolerated dose that could be found, even with escalating it way, way up in the Phase I trial.

So we did a proof-of-concept trial in the US Oncology network with triple-negative patients. They had zero to two prior chemotherapy regimens for metastatic disease — they did have measurable disease. And they were randomized to gemcitabine/carboplatin on a day one, day eight schedule with or without the BSI-201 IV day one, day four, day eight, day 11. We decided to give the gem/carbo day one, day eight because we were going to be giving the BSI-201 over a two-week period. Women whose disease progressed on the gemcitabine/carboplatin had the opportunity to cross over to have the BSI-201 added to the chemotherapy.

Phase II randomized study of carboplatin/gemcitabine in combination with BSI-201 in mTNBC

And the data that I presented at ASCO, at the plenary session, was actually quite early. And I only had the objective response data you see here on 86 patients, so we didn't even have all the patients to report on. So these are early data for sure, but we felt tripling of the response rate, tripling of the clinical benefit rate — which of course we don't see very often in breast cancer, particularly not in triple-negative disease. And then the median progression-free survival — now we'll have to take this with a grain of salt because this was not a blinded trial, there was no placebo control — but the PFS went from 3.3 months up to 6.9 months, and that's a big, big difference you can see here on these curves.

Now we also looked in our triple-negative breast cancer patients' primary breast cancer tissue in the paraffin block. We looked at PARP expression. These are now orange dots — you can see that there is, on average, a higher level of PARP expression in these patients, triple-negative breast cancers, and one of the things we're interested in is to see whether you did better with a PARP inhibitor if you did have a higher level of the PARP. We don't have those data yet. So we're going to see if that's a biomarker for a responsiveness and benefit from the PARP inhibitor.

We did update our survival data at San Antonio in '09, and these are still not yet the final survival, but they are more mature than I presented at ASCO '09. The median survival with gem/carbo, 7.7 months and 12.2 months with the addition of the BSI-201, in spite of the fact that about 50 percent of the patients randomized to gem/carbo crossed over to receive the BSI-201 at the time of disease progression. So even with the crossover, a 50 percent reduction in the risk of death. That's a hazard ratio of 0.5, and that was highly statistically significant. We should have the final data analyses over the next couple of months.

Another very good thing about this drug was that there was no discernible toxicity above and beyond the gemcitabine/carboplatin alone with regard to hematologic or nonhematologic toxicities — an exceptionally well-tolerated agent. So we were very happy about these data and launched the Phase III trials in July '09, with — enrolled in six months and is now closed — 420 patients. And the schema for the Phase III is really, essentially, identical to the randomized Phase II, again allowing for crossover at the time of disease progression for those randomized to gemcitabine/carboplatin alone. So an extremely fast-accruing study. So we'll have those data later in the year.

Planned clinical trials designed to evaluate BSI-201 in combination with cytotoxic therapies

So other key questions about BSI-201: Can we give it weekly? There's an ongoing randomized Phase II looking at the twice-a-week versus the weekly schedule with gem/carbo, so we'll have that answer. Can it potentiate other DNA-damaging agents, such as cyclophosphamide/doxorubicin? Studies are planned there. What about adding it to paclitaxel — paclitaxel plus/minus 201? Does that lead to potentiation of paclitaxel? I think that's a question worth asking. What about brain irradiation — brain irradiation with or without BSI-201? And what about other subtypes? And a big study has just been launched in non-small cell lung cancer: gem/carbo with or without BSI-201.

So, just to finish off, I just want to mention that the BRCA1/BRCA2s are very genomically unstable, shown here. And if you look at other breast cancers on the right, we see that some other breast cancers are also genomically unstable. They don't have germline mutations, and so those are the ones we want to go after with the PARP inhibitors?

So, Neil, we'll visit about that a little bit more. But that's just a little bit of an update on some of the clinical data.

DR LOVE: And it's such fascinating work. And one thing — you mentioned squamous cell of lung? I'm not — what's the BRCA-ness of that?

DR O'SHAUGHNESSY: I've actually not seen the data yet, Neil, but the lung cancer investigators, along with some of the BSI-201 investigators, took our paraffin blocks from our Phase II study and did some molecular microarray profiling. And we're finding microarray profiles, as Jenny had done, that were really seeming to essentially predict for benefit from 201, and interestingly, we're really picking out a basal-like molecular microarray.

And apparently there are data that the squamous non-small cell lung cancers have a molecular array pathway profile that looks like the basal breast cancers. So the closest of the non-small cells to the basal breast cancers apparently were the squamous cells. Now I've not actually seen those data, but when I inquired why squamous, that's what I have been told.

Role of PARP inhibitors as single-agent therapy

DR LOVE: That's really fascinating. Definitely could use some help in squamous, that's for sure.

What about the issue, Joyce, of combining with chemo? You mentioned the olaparib was looked at

without the chemo. Can all these agents be easily combined, in terms of toxicity, with chemo? How important do you think the chemo is in using these agents, Joyce?

DR O'SHAUGHNESSY: I think, Neil, that chemo's going to be very important outside of the BRCA1/2 setting. Because in the BRCA1/2 setting, that homologous recombination is gone. But my suspicion is that in triple-negative or basals that have BRCA-like problems, then it may not be so complete. And I say that because I don't think in a standard triple-negative breast cancer that we're going to see high levels of activity of olaparib or BSI-201 by itself. And so I think you really have to stress the cell and put that DNA-damaging chemotherapy on there, cause a lot of breaks and then inhibit the repair pathway.

Now I've not seen the data, but I'm told that it has been difficult to combine some of the other PARP inhibitors, like olaparib and the ABT-888, the Abbott compound, with chemotherapy, although the ABT-888 is being combined with temozolomide successfully. But the olaparib — I'm told that it's more difficult to combine it because of myelosuppression.

So I think we — I'll have to wait and see the data, but that's what I'm hearing, that it's not turning out to be so easy to combine all of them with the DNA-damaging agent.

PARP and PTEN protein expression level as predictors of response to various chemotherapeutic agents

DR LOVE: So we're going to spend the rest of our time addressing some of the questions that have come in from the audience across the world, but one more thing back to Jenny. Joyce mentioned measuring PARP levels, and that kind of makes sense, but I'm not sure I've heard about that before. Did you look at the actual levels of PARP? And are there any other things we could be looking at to predict response to these agents?

DR CHANG: Yes, we did look at the levels of PARP expression and that does distinguish triple-negative breast cancers — there are responses to anthracyclines or not. But our signature work did a little bit better.

I think PTEN is going to be a very important assay in predicting who may respond to DNA-damaging agents. But PTEN, by immunohistochemistry today, is a difficult assay to do because it's a dirty assay. A lot of the stain — the cells stain positive for PTEN. So I know there are some companies out there trying to make immunohistochemistry for PTEN a little bit better. But I think those are the two things that we need to look at — but PARP expression itself is very important.

And you're right, I think, Joyce, in looking at non-small cell lung cancer and ovarian cancer. They have very high expression of PARP and may, therefore, benefit from PARP inhibitors.

Exploring the correlation between BRCA1 mutations and TNBC

DR LOVE: There's that old PTEN coming back to haunt again. Maybe, Jenny, you can take a shot at the first of the two questions from Steve there in Los Angeles. People often ask the unanswerable, but let's see if you can come up with an answer to this one.

Why is it that most of the tumors that occur in BRCA patients are triple-negative — BRCA1 actually —– are triple-negative? Any speculations?

DR CHANG: It's a very difficult question. It's interesting though. There was a paper that came out from Australia not that long ago, that looked — it's fascinating about BRCA1 mutation carriers that even though most of the tumors, when you think about it, are triple-negative, yet, for prevention, and estrogen deprivation works — tamoxifen works, ovarian ablation works in preventing cancers in BRCA1 mutation carriers, but the cancers that come out are triple-negative.

So these are totally unknown — we can't really explain why. So I can't. Maybe Joyce can help me out and I'll be —

DR LOVE: So that Joyce won't — we'll just pass on that one, Joyce.

Ascertaining clinical benefit with "compassionate use" of BSI-201

Let's go on to the other — I think the other end of this question, and really it's a tough one. You see it — I was actually flashing on — you remember the TC study, docetaxel/capecitabine. I think you reported that, like in 2001 — survival advantage, and people criticized it because there wasn't cross over.

Well you did cross over here. And actually, you did the other study, which is trastuzumab/lapatinib, showing survival advantage in metastatic disease. But it's so tough: Patients and physicians see exciting data and actually, it can create a lot of anxiety. People try to get patients on this study, but it's closed in six months.

Steve wants to know about compassionate use of PARP inhibitor or BSI-201. And if patients aren't able to access this in a trial right now, based on what we know, I mean what are they losing? What is — what do we know right now in terms of actual patient benefit?

DR O'SHAUGHNESSY: The compassionate use protocol is going through IRB review now. It's finalized. And so it should be open by April or early May. And all the — over 100 practices around the country who participated in a Phase III trial, and it's really kind of geographically spread out on purpose all around the United States. So it's just single-arm treatment gemcitabine/carboplatin to BSI-201, measurable or evaluable disease. It's okay to have had prior gemcitabine and/or prior carbo or both. And so that's good, and it's one to three prior regimens — not zero prior regimens, but one to three. And I think it's because, until we have the results of the Phase III, we don't really, honestly know the level of patient benefit.

I'm very encouraged about the Phase II. We have to be clear that we have seen over and over again, unfortunately, in oncology, randomized Phase IIs do not pan out in the Phase III. So we don't actually have a real, real high level of proof about the benefit yet. So it's not open to zero first line, but it's one to three prior regimens.

So it's quite flexible for patients and will open here very soon.

DR LOVE: So there was another question that came in, Joyce, and I think it kind of touches into the question from Dr Ge, who just sent this question in a few minutes ago, if we can bring that up. And it kind of gets into the issue of new trials that are going to be looked at. Of course, there's a lot of question about adjuvant therapy, but this physician brings up the issue of a patient who actually has a BRCA mutation, who had neoadjuvant therapy for locally advanced disease and then has significant residual tumor. And the question of what about a PARP inhibitor? Are there trials where you can get PARP inhibitors for a patient like this? Is this going to be looked at? And what about adjuvant therapy?

Potential role of platinum agents in the adjuvant setting in TNBC

You were in a workshop we did on this a couple of months ago, when the question of, if we did an adjuvant trial, what's the control arm? Is it going to be gem/carbo? What about postneoadjuvant therapy, Joyce, and adjuvant?

DR O'SHAUGHNESSY: So I have actually submitted a concept to BiPar Sciences to look at precisely that patient population, because there is no standard of care. And even in the BRCA1 germline mutation carriers, where I would be most inclined to consider a platinum in somebody who did not have a pathologic complete response, I have to admit I don't have any data to use a platinum in that setting.

I think I would not use the platinum if the patient had been totally refractory to chemotherapy. However, if she had had an excellent response to the ACT-like therapy but had small-volume residual disease left over, because of the data with preoperative platinum looking so good in BRCA1 germline mutation carriers, I personally would be inclined to give her four cycles of platinum outside of a clinical trial.

And we put in a concept in the no-PCR triple-negative patient to do a randomized trial of no treatment, which is the standard, versus gemcitabine/carboplatin versus gem/carbo/BSI-201. So we're waiting to hear whether or not that study will be able to be supported by the company. Because I do think it's an excellent place to look at the question, if the Phase III data, in particular, of course, relate — really pan out, which we certainly hope they will.

Again, with regard to adjuvant. My opinion is that if the Phase III study shows a survival advantage for sure, or even a large progression-free survival advantage, I think we need to get the BSI-201 into triple-negative adjuvant patients ASAP. And we don't really know — I think I'd look at an AC followed by T, with or without the 201. Because certainly AC or DNA-damaging agents, and I'd like to see what the 201 does with that, whether or not there should be a third arm, for example, of a docetaxel/carboplatin with the BSI-201, kind of taking a page out of Denny Slamon's book in the HER2-positive setting — ACT plus/minus trastuzumab versus the TCH — is a thought.

But I would like to see a triple-negative adjuvant study launch if the Phase III trial is positive.

Current perspectives and future directions of TNBC

DR LOVE: So next Tuesday, when Hope and Kathy join us, we'll pick up on some of these things, but why don't we see if we can just kind of finish out in the last couple of minutes, addressing some of the questions. One of our favorite oncologists from the West Coast of Florida, Bill Harwin, asks us, Jenny, "What about delayed recurrence in triple-negative breast cancer? Does that occur?" He asks about monotherapy, which I think we've already talked about. And also, is there a difference between triple-negative that's BRCA-positive and BRCA-negative?

Do you want to take a crack at those three, Jenny?

DR CHANG: Sure. I think it is very infrequent to see late recurrences in triple-negative breast cancer patients. I mean the most recurrences are seen — or recur — within the first couple of years.

Of the promising PARP inhibitors — I think Joyce touched on this briefly — the primary problem, from

what I hear, is myelosuppression — thrombocytopenia — which is even harder to manage than neutropenia. So the other PARP inhibitors may be more effective in decreasing PARP levels, but they may be more difficult to combine to chemotherapy. And like what Joyce said, I do think that PARP inhibitors will be used with DNA-damaging agents, with chemotherapy, because you do need to hit the homologous recombination repair mechanism.

DR LOVE: So, we're going to close —

DR CHANG: And the final question is, there's a difference if you are triple-negative or not in terms of — if you're a BRCA mutation carrier? I think we do not know the answer to that. My gut feeling is that if the BRCA pathway is not intact — it's defective — then you will respond as well to PARP inhibitors.

DR LOVE: So, final question, back to you, Joyce. Anything hot coming out at ASCO in this area or being published in this area that we might want to look forward to in the next few months?

DR O'SHAUGHNESSY: Not that I am aware of, Neil. I think that the next data that I'm aware of that are going to come will be the final data from the randomized Phase II. We've not submitted to ASCO. I think it'll be published as soon as we have the data. Phase III data will be coming out later this year, maybe by San Antonio — probably.

There are no other BSI-201 data, that I'm aware of, coming out, and I'm not aware of olaparib, although there may be some Phase I data on olaparib — and I know there's some interesting endometrial cell line — there are a lot of endometrial cancers that have lost PTEN and apparently are exquisitely sensitive to PARP inhibition.

So there will be other cancers, probably preclinical studies and gearing up for a clinical trial.

DR LOVE: It sounds like it's going to be a pretty interesting San Antonio meeting — it always is, but particularly related to that Phase III study.

I want to thank Jenny and Joyce for participating tonight and thank our audience for joining us. And please come back on Tuesday night.

DR CHANG: Bye, Neil.