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Decoding Co-Mutations in HR+, HER2- Metastatic Breast Cancer

Announcer:
You're listening to *On the Frontlines of Metastatic Breast Cancer* on ReachMD. And now, here's your host, Dr. Charles Turck.

Dr. Turck:
This is *On the Frontlines of Metastatic Breast Cancer* on ReachMD, and I'm Dr. Charles Turck. Today I'm joined by Dr. Abirami Sivapiragasam to discuss her recent analysis, which she presented at the 2025 San Antonio Breast Cancer Symposium. The analysis compared gene expression and immune cell infiltration in patients with *PIK3CA*- and/or *ESR1*-mutant versus wild-type HR+, HER2- metastatic breast cancer.

Dr. Sivapiragasam is an Associate Professor of Medicine at the Medical University of South Carolina in Charleston and the Director of Medical Oncology and Fellowship Director at the MUSC Hollings Cancer Center. Dr. Siva, welcome to the program.

Dr. Sivapiragasam:
Thank you, Dr. Turck. Thanks for the opportunity.

Dr. Turck:
So if you would, Dr. Siva, talk to me about the rationale behind this study. What made the comparison between *ESR1*, *PIK3CA*, and wild-type tumors, especially timely or clinically relevant?

Dr. Sivapiragasam:
So the rationale really stems from how treatment resistance emerges in hormone positive metastatic breast cancer. So we usually treat these patients with first-line endocrine therapy options—with a CDK4/6 inhibitor. But over time, many of these patients develop resistance by multiple mechanisms, including an *ESR1* mutation. *ESR1* mutations are typically acquired under the pressure of therapies like aromatase inhibitors, whereas *PIK3CA* mutation is usually something that's often present from early on in the cancerous history. So both mutations are common and clinically important. And we also have targeted options for these mutations.

However, when both mutations occur, we call this group a co-mutated population, which we see in roughly about 10 to 15 percent of our patients, so it's not very uncommon. These tumors have been poorly understood until now. So it's very timely to compare these groups because with the advent of targeted therapies, clinicians are encountering issues like sequencing; when you have two different options, how do you best sequence treating these patients? And that's how this idea came to me to look into this specific population.

Dr. Turck:
Give us a bit more background. Would you walk us through the methodology in the patient cohort you studied?

Dr. Sivapiragasam:
Yeah, certainly. So we conducted a retrospective study using a large real-world database with the NGS platform of these hormone positive, HER2- metastatic breast cancer patients. So we identified a pretty good size of patients—about 8,626 patients—who had comprehensive genomic profiling with tempus assays. It could be either the tissue sequencing, liquid sequencing, or both. Then, to ensure good quality data, we only included samples with at least 30 percent tumor cellularity. And then for each patient, we extracted all the pathogenic or likely pathogenic mutations in *ESR1*and *PIK3CA*. We also looked at the subgroup of TP53 mutation as well. And based on the results, we stratified these patients into four major groups: the *ESR1* mutant, *PIK3* mutant, the co-mutated patients with both alterations, or wild type with neither mutation present. And then we compared their clinical and genomic data and looked at the

clinical outcomes.

Out of all these 8,626 patients, they were divided into about 53 percent wild type, 32 percent *PIK3*, 9 percent *ESR1*, and 6 percent comutation. Although the *ESR1* and co-mutated numbers appear smaller in the overall cohort, the snapshot underestimates their relevance because in real-world practice, you'll see much higher numbers.

So we looked at the groups very carefully and we found out that the mutation frequency by time from first line of therapy initiation was actually very different. So the *ESR1* mutations were emerging in up to 24 percent of patients—often two or more years on therapy—and the co-mutated patients actually went up to 15 percent when you look at those patients who received treatment at the two-plus year mark from this first line of therapy. We also examined their real-world overall survival time to next therapy. We looked at their tumor mutational burden, polyclonal status, and also RNA sequencing data for about 4,500 patients. That's where we looked at the gene expression profiling, and the tumor immune microenvironment was also assessed in these patients using a quantity seeq method.

Dr. Turck:
For those just tuning, you're listening to *On the Frontlines of Metastatic Breast Cancer* on ReachMD. I'm Dr. Charles Turck, and I'm speaking with Dr. Abirami Sivapiragasam about her recent research on the impact of *ESR1* and *PIK3CA* in HR+, HER2- metastatic breast cancer.

Dr. Siva, now that we have some context around your analysis, let's dig a little bit more into the results. What else stood out to you in terms of differential gene expression across mutation subtypes?

Dr. Sivapiragasam:
So we found some very intriguing gene expression differences. In particular, tumors with an *ESR1* mutation, either alone or in combination with *PIK3CA*, showed a distinct gene signature compared to the wild-type patients. They had significantly higher expression of a set of genes, including TFF1, FGA, and SCGB2A2. So I'll talk a little bit about some of those genes, especially the SCGB2A2 gene. This codes for the protein mammaglobin A, a protein associated with differentiated breast epithelium, and it's highly expressed in these *ESR1* mutant, *PIK3* mutant, and co-mutated patients compared to the wild-type patients.

On the flip side, there was one gene that caught our eye, which was SFRP2. It's an inhibitor of Wnt signaling. And this was significantly downregulated, especially in the *ESR1*-mutant tumors. The wild-type tumors did not show these extremes. And also interestingly, the *PIK3*-mutated tumors were actually quite similar to the wild-type tumors in overall gene expression profiling. Aside from an increase in the SCGB2A2—the mammaglobin protein I mentioned—the *PIK3CA*-only group didn't have many genes that were dramatically different from the wild-type group. So essentially, the presence of the *ESR1* mutation was associated with some genes' response to the estrogen transcriptional program and also the Wnt pathway regulator SFRP2, suggesting a possible tilt towards an active Wnt signaling in those tumors.

But in contrast, the *PIK3CA* mutants alone did not rewire the global transcriptome nearly as much. So those tumors maintained a more normal luminal gene-like expression pattern compared to the *ESR1* or co-mutated patients. It also suggests that the *ESR1* tumors may have a distinct biology, especially an estrogen pathway activation signature, whereas the *PIK3*-mutant tumors are remaining closer to the baseline tumors at the mRNA level. And this is all in the mRNA level, not proteomic, level.

Dr. Turck:
And what else can you tell us about how the mutation status correlated with differences in immune cell infiltration?

Dr. Sivapiragasam:
So from the existing literature, we don't have a clear understanding of the immune microenvironment in these tumors, particularly in the co-mutated patients. So this is why I found some of these findings from our poster especially interesting. Overall, immune infiltration was low across all subgroups, which is consistent with the well-established view of HR+ breast cancer as an immunologically cold disease.

That said, we did observe meaningful differences between mutation subtypes, so the wild-type tumors had the highest level of B-cell infiltration whereas the co-mutant tumors had the lowest. And we know that B-cell can contribute to anti-tumor immunity, including through the formation of tertiary lymphoid structures. So the elective depletion of B-cells in the co-mutated tumors suggest an even more immune suppressed or cold tumor microenvironment with reduced immune surveillance.

But when we looked at other immune cells, we found some surprising dichotomy, I would say, between the *PIK3* and *ESR1* tumors. For example, the *PIK3* tumors showed the greatest enrichment of macrophages and Tregs. Both M2 macrophages and Tregs are cell types that we know can cause a lot of immune suppressive effects—we noted that as an interesting finding from this analysis—whereas on the *ESR1*-mutant tumors, they had the lowest level of those immunosuppressive cells in the microenvironment. And then we also saw

that if you combine these findings and look at the co-mutated tumors, they had the poor features of both the *ESR1* and *PIK3*-mutated patients. So they had low B-cell presence, but they also had high levels of Tregs and macrophages.

Dr. Turck:
And how could the distinct immune pathophysiology between *ESR1* only and *PIK3CA*-driven tumors influence treatment resistance or therapeutic design?

Dr. Sivapiragasam:
I think that's a critical question: how do we use these insights to improve clinical decisioning or improve the treatment landscape? The differences in these immune landscapes may give us some clues, especially in the *ESR1*-mutant and *PIK3*-mutant tumors, as to why there is resistance happening with the therapeutic approaches that we currently have.

So for *ESR1*-mutant tumors, since they had fewer immunosuppressive cells, their primary mode of resistance is through altered hormone signaling, especially the estrogen receptor pathway. So these tumors remain very much hormone driven as evidenced by the estrogen receptor genes that we saw initially. So the logical approach is to double down on the estrogen receptor targeted therapy. In practice, this means we have to use more effective endocrine therapies. For example, selective estrogen receptor degraders, or SERDs, or other stronger novel down-regulators may be necessary for these patients. So our findings reinforce that for an *ESR1*-mutant tumor, targeting the ER pathway is the key because these tumors are still addicted to estrogen signaling despite the mutation.

And in the co-mutated patients, these are the tricky ones, I think, because they have the resistance from both pathways. So on one hand, they have the active estrogen receptor signaling, and on the other hand, they have the immunosuppressive environment. So I think a multi-pronged combination therapy strategy would be the best way to approach those co-mutated patients. So in practice, the question is, is there a role to combine the SERDs with a *PIK3* inhibitor so you can hit both oncogenic drivers simultaneously? Again, we don't have any data there, but that's something I'm questioning in my mind. More importantly, how do we even use our single agents in a more strategic way to sequence them? We currently don't know if we should go with an *ESR1* blocker first followed by a *PIK3* inhibitor or vice versa. So I think that those are other practical questions that we need to think about.

Dr. Turck:
Before we wrap up our discussion, Dr. Siva, what do you see as the next steps for this research, and how else do you think it could impact clinical practice down the line?

Dr. Sivapiragasam:
I want to look at some very practical clinical questions from here. So like I said before, sequencing is a very important question that we are all struggling with in our clinics when we see a co-mutated patient. So we are actually planning to do a multi-institutional retrospective study where we look at real-world treatment patterns in these co-mutated patients and compare the outcomes based on different sequencing approaches. But ultimately, a prospective study looking at a combination strategy would also be very intriguing.

Dr. Turck:
Great comment for us to thank on as we come to the end of today's program. And I want to thank my guest, Dr. Abirami Sivapiragasam, for joining me to discuss how *ESR1* and *PIK3CA* mutations shape resistance and immune features in HR+,HER 2- metastatic breast cancer. Dr. Siva, it was great having you on the program.

Dr. Sivapiragasam:
Thank you for the opportunity.

Announcer:
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