Apocrine breast cancer, *ESR1* mutations at center of tumor board review

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February 2019—Two breast cases—one of apocrine carcinoma and androgen receptor overexpression and another of metastatic ER-positive cancer and an *ESR1* mutation—were the focus of a molecular oncology tumor board session at CAP18.

Aditya Bardia, MD (MBBS), MPH, breast medical oncologist at Massachusetts General Hospital and assistant professor of medicine, Harvard Medical School, opened the presentation last fall with the first case: a 58-year-old woman with a history of stage I triple-negative breast cancer. Dr. Bardia's co-presenter was Deborah A. Dillon, MD, breast pathologist, Brigham and Women's Hospital, and assistant professor of pathology, Harvard Medical School.

Routine screening mammography detected a 1.4-cm tumor in the patient in 2013. There was no noted presence of cancer in nearby lymph nodes. The patient underwent right breast conservation surgery, which revealed a 1.4-cm invasive ductal carcinoma, grade two to three, which was moderately to poorly differentiated. It was "essentially triple-negative breast cancer," Dr. Bardia said, noting the estrogen and progesterone receptors were zero and the HER2 was 2+—borderline but with a negative FISH result.

"Given that she had triple-negative breast cancer of more than one centimeter, the plan was made to give adjuvant chemotherapy to reduce the probability of recurrence," Dr. Bardia said.

During a routine follow-up exam in 2015, it was discovered that the patient had a right palpable supraclavicular lymph node associated with a sore throat, both of which the patient attributed to a viral illness.

"It was large, 2.5 centimeters, non-tender, firm, and somewhat fixed," Dr. Bardia said. The exam did not find any other palpable lymph nodes in the cervical or axillary region bilaterally, and the rest of the exam was negative. The woman's lab results were within normal ranges. An ultrasound of the lymph node revealed a highly suspicious 2-cm supraclavicular lymph node, and a CT scan of the neck, chest, and pelvis revealed a 2.6-cm supraclavicular lymph node as well as a lytic lesion in the pelvis and several approximately 1-cm bilateral pulmonary masses.

So what would be the best site to biopsy—lymph node, lung, or bone? While one could consider a bone biopsy, Dr. Bardia said, "from a molecular diagnostic perspective, there's potential concern with decalcification. From a patient perspective, it's more difficult to do. And there's always a concern about missing the lesion. If you get a negative bone biopsy, you cannot completely rely on it being truly negative." The preference, then, would be to perform a lymph node biopsy (core needle), which is what the patient had.

Does it matter to the oncologist, Dr. Dillon asked, whether distant disease is confirmed as opposed to supraclavicular disease?

"If this was an axillary lymph node," Dr. Bardia said, "then one could consider a biopsy of a more distant site. But a supraclavicular lymph node would be considered metastatic disease."

The patient also had *BRCA* testing because of the likely metastatic disease, for which "one would consider a PARP inhibitor, particularly for germline *BRCA* mutant triple-negative breast cancer." A maternal aunt had been diagnosed with postmenopausal breast cancer at about age 60, but there was no family history of ovarian cancer or any other malignancy, Dr. Bardia added. The germline *BRCA* test result was negative.

Tumor genomic profiling to identify additional actionable alterations was the third consideration. The woman declined out of concern that a discovery of hereditary risk could have family and insurance implications.

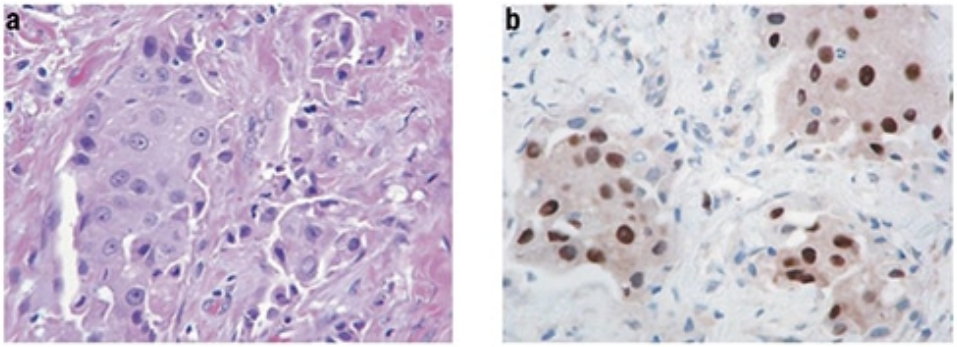
The lymph node biopsy results revealed a poorly differentiated invasive carcinoma with apocrine features that was

ER/PR-negative and HER2-negative. “You can appreciate the apocrine differentiation, the enlarged nuclei, prominent nucleoli, and granular eosinophilic or foamy cytoplasm,” Dr. Dillon said. “This is an appearance we can see in invasive carcinoma of no special type, but we also can see this apocrine appearance in a number of the special-type cancers, including lobular and micropapillary.”

The differential diagnosis sometimes will include, for example, squamous cell carcinoma, histiocytoid carcinoma, and granular cell tumors. “You may want to do a couple of stains in order to rule those out before calling it a carcinoma with apocrine differentiation,” she said.

Whether the apocrine carcinoma is a specific subtype and whether it matters is another consideration, Dr. Dillon said.

A 2008 gene expression study showed that the morphologic special subtypes, including mucinous, classic, invasive lobular, and micropapillary, could each be classified within a single intrinsic subtype, except for apocrine carcinomas (Weigelt B, et al. *J Pathol.* 2008;216[2]:141–150). “It is interesting because the apocrine carcinomas live with the pleomorphic lobular carcinomas in a group that has been called molecular apocrine, a subtype of triple-negative cancer,” Dr. Dillon said.



A triple-negative (ER negative, PR negative, and HER2 negative) apocrine tumor (a; × 400, H&E) showing strong AR reactivity (**b;** × 400, anti-AR)
Reprinted by permission from Springer Nature: Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM, Bhargava R. Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. *Mod Pathol.* 2010;23(2):205–212. doi: 10.1038/modpathol.2009.159

Because of this overlap in the gene expression studies, the World Health Organization has not classified invasive carcinoma with apocrine differentiation as a special subtype. Survival for the non-apocrine invasive cancers in most studies is similar to that of apocrine carcinomas, which supports the idea that it is not a special subtype, Dr. Dillon said.

“However, it does have a very characteristic biologic profile that is ER/PR negative, androgen receptor positive, and GCDFP15 positive. So we think this is best considered at this time to be a distinct morphologic variant of invasive cancer that has a characteristic gene expression profile. But this unique profile may represent a distinct biologic entity, and we expect to see more research activity in this area moving forward.”

Most important is that it suggests an alternative treatment strategy. “This is not just pathologist navel-gazing,” Dr. Dillon said, noting that the patient’s cancer was strongly androgen receptor positive at 80 percent. “We are talking about possibly doing androgen receptor blockade in these cancers.”

Does androgen receptor positivity influence the choice of first-line chemotherapy, and given the AR positivity, are there other targeted therapies to consider? Dr. Bardia introduced data from the TNT trial in which patients with metastatic triple-negative breast cancer were randomized to receive a taxane-based chemotherapy, docetaxel,

versus a platinum-based chemotherapy, carboplatin (Tutt A, et al. *Nat Med.* 2018;24[5]:628–637).

“Overall there was no difference in progression-free survival between carboplatin and docetaxel,” Dr. Bardia said.

The study also included genomic sequencing to look at gene expression based on the PAM50 test (Prosigna). “That is where you could really see a difference,” he said. In basal-like tumors, carboplatin was associated with better progression-free survival as compared with docetaxel, while in non-basal tumors, carboplatin had much less activity. Based on those results, one would consider docetaxel over carboplatin for a patient with an androgen receptor-positive, likely non-basal tumor, he added.

“In terms of targeted therapy, the drug enzalutamide blocks the androgen receptor.” It is FDA approved for prostate cancer, Dr. Bardia said, and was also evaluated in a phase two, single-arm trial for patients with triple-negative breast cancer and demonstrated a modest progression-free survival of about three to four months in patients who had received prior treatment.

“The authors then looked at the androgen receptor by a signature that was developed by the sponsor [Medivation],” he said. The androgen receptor-positive signature was associated with better outcomes with enzalutamide compared with the androgen receptor-negative signature. Medivation was sold to Pfizer and it’s unclear, he said, if Pfizer will proceed with the plan for a confirmatory phase three trial.

The patient in the case enrolled in a clinical trial with an oral androgen receptor antagonist and remained on treatment for six months. “If you go back to whether the androgen receptor findings were actionable, in this case it helped triage the patient into a clinical trial with an oral androgen receptor antagonist,” he said.

Dr. Dillon pointed out that there is no standard test for predicting response to androgen receptor targeting: “It often seems to be the case that clinicians are getting ahead of us in terms of treating patients on clinical trials before we have even established our optimal cut points and figured out the best way to do this.”

Data from breast cancers that developed in women enrolled in the Nurses’ Health Study found more than 75 percent of breast cancers were positive for androgen receptor by immunohistochemistry (Collins LC, et al. *Mod Pathol.* 2011;24[7]:924–931). “This includes the large majority of estrogen receptor-positive tumors, more than half of *HER2*-positive tumors, and about a third of triple-negative breast cancers,” Dr. Dillon said, noting that most of the interest in androgen receptor targeting is in the triple-negative breast cancers.

Numbers will vary based on antibody, dilutions, and cutoff points, she said. The Nurses’ Health Study used the Dako AR441 antibody at 1:200 with a one percent cutoff.

Two recent phase two clinical trials used a cutoff point of at least 10 percent to try to enrich for patients who were more likely to respond, she said. “This includes the TBCRC011 trial of bicalutamide. When they defined positivity as greater than or equal to 10 percent, 12 percent of triple-negative breast cancer patients screened were positive for androgen receptor.”

A recently published phase two enzalutamide trial also defined AR positivity as greater than or equal to 10 percent. Fifty-five percent of the patients screened were positive for AR by that definition (Traina TA, et al. *J Clin Oncol.* 2018;36[9]:884–890). Two antibodies were used in the trial, Dako AR441 and Ventana SP107, and concordance was high.

“The best cutoff point to use in the selection of patients for treatment with an androgen receptor inhibitor is not known,” Dr. Dillon said. “We also don’t know what the significance is of other androgen receptor alterations, such as amplification and mutation, which of course we are starting to see now that we’re sequencing tumors.”

Amplification would be expected to lead to overexpression of the proteins, so that is a “pretty straightforward lead that amplification is going to predict response,” she said.

Mutations, in some cases, abrogate the function of the androgen receptor. “There’s a suggestion, at least in the

literature, for prostate. There's report of a missense mutation in androgen receptor that conferred enzalutamide resistance, so there is certainly more work to be done here."

Dr. Dillon

At Brigham and Women's Hospital, pathologists report the apocrine morphologic features in apocrine cancers. "We think this is helpful in the recognition of recurrences and metastases. It's also a tipoff to our oncology colleagues if they want to consider the patient for androgen receptor testing." They don't routinely do androgen receptor testing but will do so at the oncologist's request if a trial is being considered. "When we do androgen receptor testing," Dr. Dillon said, "we don't report it as positive or negative; we just report the percent positive tumor cells and leave the trial eligibility decision to the oncologist."

In summing up, Dr. Bardia said triple-negative breast cancer is a heterogeneous disease in molecular profile as well as drivers. About 55 percent of patients with triple-negative breast cancer had an androgen receptor of more than 10 percent staining by IHC in the phase two enzalutamide trial, "but it should also be noted that there was a lot of patient selection in the trial. You do have some sense that this could be a patient who has an androgen receptor-positive tumor based on their prior history and age as well as response to a prior chemotherapy agent. I suspect that is why 50 percent of patients had an androgen receptor of more than 10 percent in this clinical trial."

Dr. Bardia said that if he has a patient who has an androgen receptor-positive triple-negative breast cancer, the patient would be considered for an androgen receptor antagonist clinical trial. "It's not something that can be used in routine clinical practice, but if the institution does have a clinical trial with an androgen receptor antagonist, I think this information could be helpful."

Dr. Dillon estimates that the breast pathologists at Brigham and Women's receive requests for androgen receptor testing once or twice a month. "We run the immunohistochemistry in our regular CLIA lab," she said. "We're not using the sequencing results for that, although the sequencing results do feed back to the oncologists."

In case No. 2, a 70-year-old woman was first diagnosed with ER-positive breast cancer in 2000. She had surgery followed by adjuvant tamoxifen, which was discontinued after two years because of weight gain and bloating. In 2003, a pleural biopsy was performed that revealed the presence of an ER-positive and *HER2*-negative tumor, for which the patient received letrozole. It was discontinued in 2012 due to disease progression and the patient was started on fulvestrant. After two years, further disease progression prompted a switch to vinorelbine ("one of the few chemotherapy agents that does not cause alopecia," which the patient wanted to avoid, Dr. Bardia said).

She remained on vinorelbine for about a year before her disease progressed. She was then seen in the metastatic breast cancer clinic at Massachusetts General Hospital for a discussion of treatment options.

One option is whether, in this patient with disease progression on various regimens, there is a role for tumor genotyping and, if so, what the potential actionable alterations would be. In general, for hormone receptor-positive breast cancer, *PIK3CA* and *HER2* mutations would potentially be actionable, Dr. Bardia said. A third consideration would be *ESR1* mutations.

PI3K is a heterodimer composed of two subunits: an 85 kDa regulatory subunit (p85) and a 110 kDa catalytic subunit (p110), Dr. Dillon said. "The *PIK3CA* gene encodes the p110α, one of the catalytic subunits."

PI3K works when growth factor binds to the RTK and activates signaling along two pathways: the MAPK (RAS-RAF-MEK-ERK) pathway and the PI3K (PI3K-AKT-mTOR) pathway.

“When we see mutations in *PIK3CA* in breast cancer, typically these are missense mutations. They occur at hotspots,” Dr. Dillon said. The large majority of missense mutations occur at hotspots in exon 9, the helical domain, and exon 2, the kinase domain, and are present in 35 percent of estrogen receptor-positive/HER2-negative breast cancers. So this is “a high frequency event in breast cancer.”

The Sandpiper trial, presented at the 2018 American Society of Clinical Oncology annual meeting, looked at targeting *PIK3CA* with taselisib (Roche), a PIK3CA inhibitor. “This was a somewhat disappointing trial,” she said. “However, this still remains a target of considerable interest with other agents.”

Dr. Bardia compared the results with the positive results of the Solar-1 trial of Novartis’ alpelisib (presented at the 2018 European Society for Medical Oncology), a PI3K inhibitor expected to be approved for *PIK3CA*-mutant metastatic breast cancer.

“Why was one trial positive and the other negative?” he said. “Some of the answers would lie in the specific type of PI3K inhibitor that was utilized for therapy selection, and management of adverse events in the clinical trials.”

HER2 mutations in breast cancer are rare gain-of-function missense mutations clustered in the kinase domain, Dr. Dillon said. They are capable of activating *HER2* signaling, even in cases that have normal *HER2* copy number.

Fifteen percent of all high-grade, *HER2*-negative, invasive lobular cancers will have a *HER2* mutation, and 50 percent of *HER2*-mutated breast cancers are high-grade solid variant lobular cancers.

In preclinical models, many *HER2* mutations are activating mutations that significantly increase tumor cell growth and thus represent an alternative mechanism of *HER2* activation in tumors. Many of these show less sensitivity to trastuzumab and lapatinib than do *HER2* amplified cancers, but most are sensitive to the tyrosine kinase inhibitor neratinib (Nerlynx, Puma Biotechnology). Knowing which tumor had a *HER2* mutation versus *HER2* amplification might change the choice of drug.

“In fact, we are beginning to see data coming out using neratinib in tumors that have *HER2* mutations,” Dr. Dillon said. The Summit trial was a multi-histology, phase two basket trial, a molecularly driven trial of solid tumors with *HER2* or *HER3* mutations. The study, presented at the 2017 American Association for Cancer Research annual meeting, showed an objective response rate of 21 percent for neratinib plus fulvestrant in *ERBB2*-mutant, *HER2*-non-amplified, ER-positive metastatic breast cancer.

Dr. Dillon further pointed out that the study’s breast data showed response rates to be considerably better in breast as opposed to other organs, such as bladder, where these mutations are also present.

Mutations in the gene coding for the estrogen receptor, *ESR1*, are extremely rare in primary tumors but are present in a significant portion of patients who have metastatic ER-positive disease and who have received endocrine therapy, Dr. Dillon said. “These mutations are clustered in the ligand-binding domain. They lead to constitutive ER activity, and they are associated with acquired endocrine resistance.”

When Dr. Bardia received the sequencing report for his patient, he saw that she had one of the most common *ESR1* mutations, *D538G*.

Dr. Bardia

“The estrogen receptor mutations have been a game changer in the field of estrogen receptor-positive breast cancers,” he said. *ESR1* mutations were not even included in The Cancer Genome Atlas network’s comprehensive

genomic analysis of hundreds of primary tumors published in *Nature* in 2012.

Then in 2015, three papers published in *Nature Genetics* and *Clinical Cancer Research* reported the presence of *ESR1* mutations in the sequencing of metastatic tumors. “That was the key,” Dr. Bardia said. “These mutations were not seen in the primary tumor.”

Dr. Bardia’s group saw the same *ESR1* mutations when they isolated circulating tumor cells in patients with metastatic breast cancer (Yu M, et al. *Science.* 2014;345[6193]:216–220). Three of six patients had the *ESR1* mutations in the circulating tumor cells but not in the primary tumor, suggesting the acquired nature of the mutations.

“That is potentially a role for circulating tumor cells or circulating tumor DNA, to look at acquired mutations that would not be present in the primary tumor,” he said. “Based on circulating tumor DNA, up to 50 percent of patients with hormone receptor-positive breast cancer who have received a prior endocrine agent would have *ESR1* mutations.”

In the normal ER pathway, estrogen binds to the estrogen receptor and sends signals to the estrogen response element, which causes proliferation, Dr. Bardia said. *ESR1* mutations cause a conformational change in the estrogen receptor.

“The switch for the estrogen receptor is constantly on and it becomes ligand independent,” he said. “You can have the best aromatase inhibitor in the world, you can have the estrogen levels to zero, but the tumor would still signal because it’s estrogen independent.”

While the tumors are resistant to aromatase inhibitors, they can still be targeted with estrogen receptor degraders. “If we have a drug that targets the estrogen receptor, those drugs could still work because while the tumor is estrogen independent, it is still estrogen receptor dependent.”

In preclinical models, a number of selective estrogen receptor degraders have shown activity, much more than fulvestrant or tamoxifen, which also blocks the estrogen receptor for *ESR1*-mutant cancers.

In the SoFEA trial, published in 2013, patients with hormone receptor-positive metastatic breast cancer were randomized to receive the aromatase inhibitor exemestane versus fulvestrant. Since the trial took place before *ESR1* mutations were discovered, “the authors recently went back and looked at the banked plasma specimens to look at *ESR1* mutations and they could really see the separation of the curves,” Dr. Bardia said. “Tumors that were *ESR1* mutant did not have any benefit with exemestane but still derived some benefit with fulvestrant.”

“The next question is, can we do better than fulvestrant?” Dr. Bardia said.

A number of clinical trials investigating selective estrogen receptor degraders, or SERDs, are ongoing in metastatic breast cancer. Among the various SERDs, probably the one that is clinically most advanced is elacestrant (Radius Health). Elacestrant, an oral SERD, is moving into a phase three trial after previous clinical trial results revealed better progression-free survival than what one would historically anticipate with fulvestrant, Dr. Bardia said. “The activity was seen in this agent even in the tumors that had received fulvestrant as well as a prior CDK4/6 inhibitor.”

Fifty percent of the elacestrant trial participants (20/40) had tumors with *ESR1* mutations, and based on plasma sequencing, Dr. Bardia said, the mutant-allelic fraction of *ESR1* mutation decreased. However, not all mutations were the same: While *L536R* decreased, *D538G* was found to increase after initially showing a decrease. “There might be some difference in the response to the selective estrogen receptor degrader based on the type of *ESR1* mutation,” he said.

Dr. Bardia said the index patient, the 70-year-old woman with *ESR1*-mutant metastatic breast cancer, enrolled in a clinical trial with an oral SERD and remained on treatment for 14 months, deriving some benefit from the agent.

Dr. Dillon summed up: Acquired mutations can develop under selective pressure from aromatase inhibitor

treatment and result in estrogen-independent activation of the estrogen receptor. “The selection of tissue for testing matters, because if you test just the primary tumor, you’re going to miss it,” she said. “And if you test one metastatic site, you might miss it as well, which is why Dr. Bardia’s point about profiling the circulating tumor DNA or circulating tumor cells is important, because if you sequence different metastatic sites, not all of them may have resistance mutations.”

The advantage of enrolling patients who have *ESR1* mutations in SERD trials, Dr. Bardia said, is “not only would you potentially get disease control but you can also monitor the *ESR1* mutant allele fraction, so it can serve as a pharmacodynamic marker. There might be patients who do not have a drop in the *ESR1* mutant allele fraction for whatever reason, and maybe for that patient it’s not a good idea to use the SERD,” and better to switch to a different therapy. “So you use the *ESR1* mutation both for therapy selection and as a pharmacodynamic marker.”

Amy Carpenter Aquino is CAP TODAY senior editor. This session was an ASCO-CAP collaboration. Case No. 1 had been discussed online as part of the ASCO/CAP/AMP molecular oncology tumor board series.