BREAST CANCER IN FOCUS

Current Developments in the Management of Breast Cancer

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Circulating Tumor DNA in Early-Stage Breast Cancer: Ready for the Clinic?

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H&O What are the different techniques or technologies that are used to evaluate circulating tumor DNA (ctDNA) in breast cancer?

HP Currently, 3 general categories of testing are available. The first is comprehensive genomic profiling, in which a cancer-specific panel test is used to look at genes that may be altered in that type of cancer. For example, this test can be used in patients with advanced breast cancer by identifying *PIK3CA* or *ESR1* mutations. This type of profiling is not used in early-stage breast cancer because the level of ctDNA is not high enough to detect. The second type of testing is a tumor-informed ctDNA assay, in which whole exome sequencing (WES) or whole genome sequencing of a primary tumor is used to detect the tumor's fingerprint. Following this test, a personalized assay is designed to look specifically for the tumor's fingerprint in cell-free DNA to identify ctDNA. The idea is that if you know what you are looking for and if you look for many tumor-specific alterations, you are more likely to be able to find them. This second type of testing appears to be the most sensitive approach right now for evaluating ctDNA in early breast cancer. The third type of testing is the tumor-agnostic approach, also called the tumor-uninformed approach. Assays based on this approach in breast cancer include looking at the methylation status, other epigenetic features, and/or fragment positioning and size of the ctDNA. This approach has been used successfully in other tumors, but in breast cancer, we have fewer available data, and the approach has not been shown to be more sensitive.

H&O What are the impediments to using ctDNA testing in early-stage breast cancer?

HP As exciting as this technology is, all the results we have so far in early-stage breast cancer are from small, retrospective studies. We still need to see results from prospective studies showing that this technology can make a difference for patients with early-stage breast cancer. For example, we now have some data showing that ctDNA detection could be useful in deciding whether to administer chemotherapy after surgery for colon cancer.[1] Although other studies do not support this finding,[2] some physicians are using the data to help guide treatment in some types of colon cancer. We do not have similar data for breast cancer. One of the dangers in using this technique to dictate treatment is the potential for false negatives, particularly when one is looking at a single time point for decision making. Someone who tests negative for ctDNA 2 weeks after surgery may have a positive test result in the future, so we do not want to omit treatment on the basis of a deceptive early result.

H&O What are some of the other potential uses for ctDNA in early-stage breast cancer?

HP When early-stage breast cancer is first diagnosed, the presence of, absence of, or changes in ctDNA could potentially help to tailor neoadjuvant therapy for patients with human epidermal growth factor receptor 2–positive (HER2+) or triple-negative breast cancer. Studies are currently looking at this use. Next, the presence or absence of ctDNA could potentially be used to guide treatment in

the adjuvant setting. One can imagine taking a series of samples over time to see whether a patient has acquired ctDNA and adjusting treatment accordingly. Previous studies of postoperative monitoring in breast cancer with older techniques, such as ultrasound and radiography, have not shown that they can improve patient outcomes. Even though we have more sensitive techniques now, we still need to be skeptical of the idea that fancy new technologies are automatically effective. Just as we would not use a drug if evidence did not support its use, we should not be using tests to guide treatment if we do not have evidence that they will help patients have better outcomes.

H&O What factors have led to an increased interest in the use of ctDNA in early-stage breast cancer?

HP Multiple advances have led to an increased interest in the use of ctDNA. When Dr Bert Vogelstein's laboratory at Johns Hopkins first did its work sequencing the genome in the early aughts, gene sequencing was far more expensive than it is now and error suppression methods were limited, making it difficult to detect very low levels of ctDNA (eg, ≤1%). Thanks to the much lower cost of sequencing and to continued research by many groups, we can now detect levels of ctDNA in the range of parts per million. Another important advance is the ability to assess epigenetic features in ctDNA, which could add to the sensitivity of ctDNA assays in early cancer. In more advanced disease, it might allow us to achieve a real-time understanding of what is going on with a particular person's cancer. Another technique that is being developed is fragmentomics, in which the size and position of ctDNA molecules provide information about a cancer. Groups are also working to integrate information from different features of ctDNA to increase sensitivity. We are currently seeing rapid advances across the field of ctDNA.

As far as clinical reasons for the increased interest in early-stage disease, it is hard to ignore the association between the presence of ctDNA and poor outcomes. We do not have another blood biomarker that has such a strong association with outcomes, with hazard ratios in the range of 5 to 20.

H&O What are the most important studies that have looked at ctDNA in early-stage breast cancer?

HP Many studies have looked at ctDNA in patients with early-stage breast cancer, either in clinical trials or in cohort studies. In 2019, Dr Nicholas Turner's group at The Royal Marsden Hospital in London published results from a cohort study in *JAMA Oncology* that found an association between clinical outcomes and the presence of ctDNA across various subtypes of early breast cancer.[3] This was a very important finding, and the group presented updated work at the 2024 American Society of Clinical Oncology (ASCO) annual meeting that found improved detection rates with the use of a more sensitive assay.[4] Our group, in collaboration with Dr Viktor Adalsteinsson's laboratory at the Broad Institute in Cambridge, Massachusetts, showed that a highly sensitive, whole genome sequencing–informed assay could detect ctDNA at baseline in all patients with triple-negative breast cancer who were receiving neoadjuvant therapy.[5] In the prospective CHiRP study, our group at Dana-Farber looked at the presence of ctDNA in 83 patients with high-risk hormone receptor–positive (HR+), HER2– breast cancer in the late adjuvant setting.[6] These patients have a high risk of distant recurrence after 5 years. After a median follow-up of 10.4 years from diagnosis, our research found that ctDNA was identified at a median of 1 year before distant metastasis.

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Also important is the I-SPY 2 study from the University of California, San Francisco, which made use of a WES-informed assay.[7] I-SPY 2 showed that ctDNA changes during treatment were associated with response to neoadjuvant therapy for early breast cancer. Finally, in a translational analysis of the monarchE study that was presented at the most recent ASCO annual meeting, a WES-informed assay was applied to serial samples in patients with high-risk HR+, HER2– breast cancer in the adjuvant setting.[8] The researchers saw that patients who persistently tested positive for ctDNA had a very high risk of recurrence, whereas those who cleared ctDNA or never had detectable ctDNA were at a lower risk of recurrence.

H&O What ongoing studies are especially important?

HP Two of the prospective interventional studies that are farthest along are the phase 2 LEADER study (NCT03285412) and the phase 2 DARE trial (NCT04567420). Both studies are evaluating the efficacy of adding a CDK4/6 inhibitor to treatment in patients

who have detectable ctDNA and a history of high-risk HR+, HER2– breast cancer without evidence of distant metastatic disease. A large study from the European Organisation for Research and Treatment of Cancer (EORTC) Breast Cancer Group, called TREAT ctDNA (EORTC 2129-BCG), is looking at the use of adjuvant elacestrant (Orserdu, Stemline Therapeutics) in patients who have early-stage HR+, HER2– breast cancer and are ctDNA+. Also ongoing is the phase 2 ASPRIA study, which is looking at whether the use of sacituzumab govitecan (Trodelvy, Gilead) and atezolizumab (Tecentriq, Genentech) can improve outcomes in patients with triple-negative breast cancer who have residual disease after preoperative therapy and detectable ctDNA (NCT04434040). These are just a sampling of the many studies that are ongoing.

H&O Is this technology ready for the clinic in early-stage breast cancer?

HP I am super-excited about the technology, and I am very interested in getting it into the clinic. Having said that, ctDNA in early-stage breast cancer is not ready for use in the clinic outside clinical trials. In the COBRA study,[2] we saw that ctDNA-guided treatment did not improve patient outcomes in colorectal cancer, and we need to learn why this result is different from that of the DYNAMIC study. Historically, the routine use of blood-based biomarkers or imaging studies to detect recurrence early has not proved useful for patients. I am optimistic that we now have highly sensitive tests to pair with our highly effective therapies, but we need to show that this paradigm works. It is possible that by starting treatment earlier, we could cause more harm than good by adding toxicity without improving outcomes. I worry about the possibility of people not receiving care that would benefit them. In my experience, this test appeals to patients mostly because they like the idea of being reassured by a negative test result, which is understandable. Unfortunately, a single negative test result is currently the least powerful aspect of ctDNA testing. A positive test result is very powerful and can be worth factoring into treatment decisions, but the same cannot be said for a negative test result.

H&O What questions remain to be answered?

HP We need to figure out which subsets of patients are most likely to benefit from ctDNA testing because it has the potential to be useful in so many different settings. We also need to learn which test is best. Do we need to use a super-sensitive, whole genome–informed personalized assay for each patient? I think that we will in some settings, but in others we may be able to use an off-the-shelf assay that will provide answers far more quickly and easily. Also, we need to learn exactly when we should be conducting ctDNA testing. Should we be testing postoperatively, before adjuvant therapy? Or will we need to do serial testing over time? I am incredibly excited about this technology and how useful it has the potential to be, but we need to do a lot of work to prove its value.

H&O Will we someday be able to use this technology for breast cancer screening?

HP The technology is very promising for use as a screening test in cancer types for which we do not have screening options, such as pancreatic and ovarian cancers. We already have mammography screening for breast cancer, which is not perfect but is widely used. We would need to prove that ctDNA testing is better than mammography before we replace it. It may also be possible to improve the specificity or sensitivity of mammography by using the techniques in combination. Proving this would require lengthy studies at the population level, so it would be a long time before we could have answers about using this combination. We are much closer to using ctDNA in early-stage breast cancer than we are to using it as a tool for breast cancer screening.

Disclosures

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References

1. Tie J, Cohen JD, Lahouel K; DYNAMIC Investigators. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. *N Engl J Med.* 2022;386(24):2261-2272.
2. Morris VK, Yothers G, Kopetz S, et al. Phase II results of circulating tumor DNA as a predictive biomarker in adjuvant chemotherapy in patients with stage II colon cancer: NRG-GI005 (COBRA) phase II/III study [ASCO GI abstract 5]. *J Clin Oncol.* 2024;42(3)(suppl).
3. Garcia-Murillas I, Chopra N, Comino-Méndez I, et al. Assessment of molecular relapse detection in early-stage breast cancer. *JAMA Oncol.* 2019;5(10):1473-1478.
4. Garcia-Murillas I, Cutts R, Abbott C, et al. Ultra-sensitive ctDNA mutation tracking to identify molecular residual disease and predict relapse in patients with early breast cancer [ASCO abstract 1010]. *J Clin Oncol.* 2024;42(16)(suppl).
5. Parsons HA, Blewett T, Chu X, et al. Circulating tumor DNA association with residual cancer burden after neoadjuvant chemotherapy in triple-negative breast cancer in TBCRC 030. *Ann Oncol.* 2023;34(10):899-906.
6. Lipsyc-Sharf M, de Bruin EC, Santos K, et al. Circulating tumor DNA and late recurrence in high-risk hormone receptor-positive, human epidermal growth factor receptor 2-negative breast cancer. *J Clin Oncol.* 2022;40(22):2408-2419.
7. Magbanua MJM, Swigart LB, Wu HT, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. *Ann Oncol.* 2021;32(2):229-239.
8. Loi S, Johnston ST, Arteaga CL, et al. Prognostic utility of ctDNA detection in the monarchE trial of adjuvant abemaciclib plus endocrine therapy (ET) in HR+, HER2-, node-positive, high-risk early breast cancer (EBC) [ASCO abstract LBA407]. *J Clin Oncol.* 2024;42(17)(suppl).