What the blood knows: interrogating circulating tumor DNA to predict progression of minimal residual disease in early breast cancer

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Breast cancer is the most common cancer diagnosis for women in the developed world, with an estimated 246,660 new cases in the United States in 2016 (1). Surgical excision is the primary treatment for early stage breast cancer (ESBC), leading to a cure in the majority of patients. However, approximately 25% of patients with ESBC will eventually develop a metastatic recurrence that is incurable in virtually all cases. The risk for recurrence is greatest during the first 5 years after diagnosis, but in some subtypes extends to 20 years. This timeframe suggests an extended period of dormancy during which the growth of micrometastases is restricted, and remains undetectable with current imaging technologies. In a previous issue of *Science Translational Medicine*, Garcia-Murillas *et al.* reported a study in which minimal residual disease (MRD) was followed serially using detection of tumor associated variants in circulating tumor DNA (ctDNA) to predict recurrence in patients with ESBC treated with neoadjuvant chemotherapy (NACT) followed by methodologies, as described below, could, with further refinement, represent a significant advance in the field and hopefully portend a future in which clinicians are able to tailor therapies to reduce or eliminate the risk of relapse in patients with ESBC.

Currently, oncologists recommend adjuvant (i.e., post-surgical) systemic therapies such as chemotherapy or anti-estrogen agents to eliminate micrometastatic disease. Until recently, selection of an individual's systemic regimen was limited to clinicopathologic characteristics, such as age, menopausal status, tumor size and grade, lymph node status, and biomarkers (estrogen receptor, progesterone receptor, and HER-2). Commercially available multigene assays such as Oncotype DX®, MammaPrint®, and Prosigna® now assess the mutational profile of patient tumors and provide additional risk assessment, in some cases predicting benefit from adjuvant therapy (2,3). Unfortunately, oncologists do not yet have access to a tool that demonstrates the effectiveness of a systemic therapy on an individualized basis. Guidelines by the American Society of Clinical Oncology and the National Comprehensive Cancer Network recommend that after completing primary therapy, patients with ESBC undergo symptom review, physical exam, and breast imaging at regular intervals (4,5). These guidelines recommend against regular blood tests or systemic imaging in the absence of symptoms, as historic data have demonstrated no increase in overall survival with these tests. New technologies that detect MRD prior to the development of metastatic disease are greatly needed. Assessment of MRD in ESBC would ideally allow clinicians to tailor adjuvant therapy, affecting both under- and overtreatment, while reducing recurrence.

In 1977 Leon *et al.* reported the use of a radioimmunoassay to detect free DNA in serum, with higher levels observed in patients with cancer in comparison to healthy volunteers (6).

Patients with metastatic cancer had the highest levels, consisting of a background of cell-free DNA from normal cells plus ctDNA associated with their metastatic tumor burden. It was quickly recognized that more refined methods were necessary to track the low level of residual ctDNA in the setting of early stage disease.

Shaw *et al*. hypothesized that genomic analysis of ctDNA isolated from plasma would provide a method to monitor MRD from ESBC (7). With a cohort of 50 patients with a history of ESBC, they demonstrated a method using ctDNA to follow specific copy number variations (CNV) similar to an individual's respective primary tumor. In some cases, these CNV were detected 12 years after a patient's original diagnosis. Dawson *et al*. demonstrated higher sensitivity for breast cancer MRD in ctDNA quantified using digital PCR (dPCR) or tagged amplicon deep sequencing as compared with CA 15-3 biomarker (P<0.001) and circulating tumor cell quantification (P<0.001) (8). And a prospective study by Beaver *et al*. utilized standard Sanger sequencing and droplet digital polymerase chain reaction (ddPCR) to identify common PIK3CA mutations in primary tumor DNA (9). Serum samples both before and after primary tumor surgery as well as the primary tumor were then assessed for PIK3CA mutations with ddPCR. They demonstrated the ability to detect mutations in tumor tissue and ctDNA, before and after surgery.

In further advancement from the above studies, Garcia-Murillas *et al*. demonstrated the ability to predict recurrence many months before the development of symptoms due to metastatic disease, a result with multiple implications (10). They enrolled a cohort of 55 women with higher risk ESBC who intended to undergo NACT. A majority (96%) of the patients were treated with standard anthracycline/taxane-containing regimens. All patients underwent surgery and 13% achieved a pathologic complete response, with no evidence of residual disease at the time of surgery.

Patients underwent a core biopsy prior to starting NACT, at the time of surgery, and at first recurrence. They additionally had serum samples collected prior to NACT, 2 to 4 weeks after surgery, and every 6 months while on the study. In order to assess somatic mutations associated with the breast primary, tumor DNA was extracted from the initial core biopsy and subjected to massive parallel sequencing (MPS). At least one tumor specific somatic mutation was identified in 78% of tumors [43 of 55; 95% confidence interval (CI), 65–88%]. Next, personalized dPCR was designed for each somatic mutation, allowing for quantification of mutant DNA with single molecule specificity. A high level of agreement was demonstrated between MPS and dPCR in assessment of mutant allele fractions in the primary tumor DNA. The serial plasma samples were assessed with personalized dPCR assays to track mutations overtime.

ctDNA was detected in 69% (95% CI, 53–82%) of baseline plasma samples. Higher baseline levels were associated with higher tumor grade and hormone receptor negative disease, but baseline ctDNA levels did not predict disease-free survival (DFS). ctDNA was detected in 19% (95% CI, 8–35%) of post-surgical serum samples and was a significant predictor of early relapse in a multivariable model. Patients for whom ctDNA was detected in serial samples experienced early relapse in comparison to those remaining MRD negative by ctDNA assessment, with a median DFS of 13.6 months versus median not reached, respectively [hazard ratio (HR), 12.0; 95% CI, 3.36–43.07]. Of those patients who relapsed, 50% had ctDNA detected in a solitary sample obtained within 4 weeks after surgery, while 80% had ctDNA detected with serial sampling, with a median lead time of 7.9 months (0.03 to 13.6 months). In contrast, 96% of those who remained without relapse during the 2-year period of follow up had no evidence of ctDNA in post-surgical serum samples. In a subset of those cases with evidence of ctDNA (or MRD), targeted capture sequencing analysis was utilized to assess changes in tumor mutations overtime and effectively predict genetic differences between the primary tumor and the subsequent metastatic relapse.

The tools developed by Garcia-Murillas will require further refinement and testing in larger, prospective clinical trials before they could become a part of clinical practice. The lack of adequate sensitivity of this approach is exhibited by the fact that only 69% of patients had detectable ctDNA at baseline (i.e., when tumor burden was high), and only 50% of those experiencing relapse had detectable MRD in the sample obtained shortly after tumor surgery. Additionally, the amount of ctDNA detected was generally low—only 19.2 copies/mL (range, 1.8–6,284 copies/mL)—raising questions about the robustness of variant allele detection. As stated by the authors, additional enhancements in sensitivity may be possible with refinements to the target capture MPS approach and use of newer barcoding techniques to facilitate interpretation of sequencing data and minimize the impact that sequencing errors have on the ability to call low abundance mutations within the repertoire of an amplified gene (11).

With such enhancements, and after appropriate validation, detection of breast cancer MRD via MPS or targeted dPCR, has the potential to significantly impact clinical practice. For instance, the detection of MRD following surgery may help select patients for intensification of therapy via the addition of consolidative adjuvant treatment. Furthermore, it is tantalizing to speculate whether serial sampling could permit the implementation of preemptive therapy at the time of molecular progression to alter the natural history of disease progression prior to overt clinical relapse. Lastly, the detection of specific mutations present in MRD within each individual patient could help personalize therapy by directing the selection of specific agents to use for either consolidation or preemptive therapy.

Genomic technologies such as those developed by Garcia-Murillas *et al*. represent an important advance in achieving more personalized treatment and management decisions for patients with ESBC and other solid tumor malignancies, as well as a better understanding of the biology of metastasis. As sequencing techniques improve and become more affordable, non-invasive and longitudinal surveillance may become an important tool at the disposal of clinical oncologists.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7-30.
2. Harris LN, Ismaila N, McShane LM, et al. Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol 2016;34:1134-50.
3. Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. N Engl J Med 2016;375:717-29.
4. Runowicz CD, Leach CR, Henry NL, et al. American Cancer Society/American Society of Clinical Oncology Breast Cancer Survivorship Care Guideline. J Clin Oncol 2016;34:611-35.
5. Gradishar WJ, Anderson BO, Balassanian R, et al. Breast Cancer Version 2.2015. J Natl Compr Canc Netw 2015;13:448-75.
6. Leon SA, Shapiro B, Sklaroff DM, et al. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977;37:646-50.
7. Shaw JA, Page K, Blighe K, et al. Genomic analysis of circulating cell-free DNA infers breast cancer dormancy. Genome Res 2012;22:220-31.
8. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199-209.
9. Beaver JA, Jelovac D, Balukrishna S, et al. Detection of cancer DNA in plasma of patients with early-stage breast cancer. Clin Cancer Res 2014;20:2643-50.
10. Garcia-Murillas I, Schiavon G, Weigelt B, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 2015;7:302ra133.
11. Kukita Y, Matoba R, Uchida J, et al. High-fidelity target sequencing of individual molecules identified using barcode sequences: de novo detection and absolute quantitation of mutations in plasma cell-free DNA from cancer patients. DNA Res 2015;22:269-77.

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