A blood-based molecular test might direct recommendations for systemic therapies in patients with early-stage breast cancer undergoing surgery with curative intent. A new study suggests that droplet digital PCR (ddPCR) can be used to detect cancer-specific DNA alterations in plasma with sensitivity suitable for monitoring minimal residual disease. *Clin Cancer Res;* 20(10); 2505–7. ©2014 AACR.

In this issue of *Clinical Cancer Research,* Beaver and colleagues studied plasma tumor DNA (ptDNA) to detect PIK3CA mutations in early-stage breast cancer patients’ circulation (1). Fragmented DNA is found in the circulation within the cell-free component of whole blood. In the field of oncology, studies of cell-free DNA derived from tumors, usually termed circulating tumor DNA (ctDNA) or ptDNA, have flourished in the recent years (2). Notably, however, clinically relevant applications for ptDNA have yet to emerge. This is because most reports have been descriptive, based on retrospective analyses, and often did not tackle questions of immediate clinical applicability.

The study by Beaver and colleagues (1) represents a significant step forward. The authors performed a ptDNA prospective analysis to begin addressing a clinically relevant issue: whether plasma-derived tumor DNA can be used to monitor residual disease after surgery in patients with early-stage breast cancer. The question is significant as reliable methods for detecting residual disease after surgery are presently not available for solid tumors such as breast or colorectal cancers. As a result, oncologists typically prescribe adjuvant therapy to the majority of patients even though the disease is detected in up to 40% of samples (4). Somatic mutations are the most common oncogenic event in breast tumors, being detected in up to 40% of samples (4). Somatic mutations such as those occurring in PIK3CA occur in the genome of cancer cells but are absent in normal cells of the same individual (Fig. 1). This juxtaposition makes somatic DNA changes extremely attractive as a tumor-specific biomarker.

Detection of genetic alterations in the blood is, however, challenging, largely because ptDNA often represents a very small fraction (<1.0%) of the total circulating free DNA (5). Technological advancements such as droplet digital PCR (ddPCR) and next-generation sequencing have allowed for unprecedented progress in this field (6).

The study published in this issue of *Clinical Cancer Research* is based on ddPCR, a technology capable of detecting genetic alterations with high specificity and up to 0.001% sensitivity (7). In ddPCR, individual DNA fragments are first partitioned into microscopic (nanoliter- or picoliter-sized) emulsion-droplet reactions, amplified, and then queried for a given mutation. Such requirements are fundamental when rare variants need to be identified, or when they are diluted in a large amount of normal DNA, as often happens when working with plasma DNA from early-stage disease.

As a first step, the authors analyzed primary tumor tissue samples by Sanger sequencing and identified 10 out of 30 patients (33.3%) carrying PIK3CA mutations. Blood was collected before and after surgery and ddPCR analysis was performed on tumor tissues and plasma-derived DNA. Five additional PIK3CA mutations were detected in tumor tissues using ddPCR, reflecting the low sensitivity of Sanger sequencing. In samples collected before surgery, PIK3CA mutations were detected with 93.3% sensitivity (one false negative) and 100% specificity (no false positives) compared with ddPCR results of the tumor tissues. Although several studies also have shown that ptDNA can be used as a proxy to assess the genetic status of solid tumors, most investigators focused on patients with extensive disease and relatively few studies reported ptDNA in patients with early-stage cancer (8, 9).

The most remarkable findings of the present article stem from the analysis of plasma collected postsurgery. Despite having no other clinical evidence of disease, 5 patients continued to have mutant ptDNA detected in their postsurgery blood draw.

Approximately 90% of all breast cancer cases are diagnosed at an early stage, when neoplastic cells are thought to be confined to the breast and/or extend locally into the axillary lymph nodes. Unfortunately, nearly 30% of women with localized disease and 75% of women with nodal...
involvement eventually relapse, most likely due to undetectable micro-metastases (10). Accordingly, additional treatments are administered after surgery to eradicate the undetectable residual cancer cells. Subsequent adjuvant treatments typically involve radiotherapy and/or chemotherapy that can be associated with localized or systemic toxicity (11).

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As stated above, a large proportion of women with early-stage breast cancer will never relapse and do not need adjuvant treatment and its consequent side effects. Distinguishing those patients who can be spared from chemotherapy is, therefore, central. To tackle this, hundreds of randomized clinical trials have been performed (3, 12). Despite these efforts, reliable methods to detect microscopic residual disease after surgery remain undefined. Accordingly, oncologists will typically recommend adjuvant therapy to the vast majority of women to benefit relatively few. In this regard, if confirmed in large prospective studies, the approach described in this issue of Clinical Cancer Research could transform clinical practice.

Although data from Beaver and colleagues are clearly inspiring, the present report remains, in essence, a “feasibility” study limited by the low number of cases and the relatively short follow-up period. Considering the conceivable clinical impact of the approach, there is no question this work will lead to follow-up analyses in large cohorts of patients. In some instances, such as women with estrogen receptor/progesterone receptor (ER/PR)–positive breast cancer, which typically relapses many years after surgery, studies assessing whether liquid biopsies predict recurrence will require decades to complete. In addition, \textit{PIK3CA} mutations are found in less than 40% of patients with breast cancer. To perform far-reaching longitudinal studies, blood-based markers capable of capturing the entire patient population must be developed. This issue should be fairly easy to address, for example, by tracking \textit{TP53} mutations.
that are prevalent in this tumor type. HER2 amplification could also prove valuable in 10% patients and ddPCR technique has already shown some promising results (13). Alternatively, one can envision the identification of patient-specific genetic markers such as translocation events, which are known to occur in nearly every solid tumor (6).

Even with these limitations, the present study is noteworthy as it sets the stage for the use of ptDNA detection for minimal residual disease in solid cancers. It may well be possible that in 5 to 10 years oncologists will recommend liquid biopsies as a routine test for patients with solid cancers such as those of breast, colorectal, prostate, or lung origin. The results could be used to individualize decisions about adjuvant systemic therapies and to recommend surveillance of patients at high risk for recurrence.

References
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Minimal Residual Disease in Breast Cancer: In Blood Veritas

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