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Genotyping cell-free tumor DNA in the blood to detect residual disease and drug resistance.
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Minimal residual disease in breast cancer: in blood veritas

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SUMMARY

A blood based molecular test might direct recommendations for systemic therapies in early stage breast cancer patients receiving surgery with curative intent. A new study suggests that droplet digital polymerase chain reaction (ddPCR) can be used to detect cancer-specific DNA alterations in plasma with sensitivity suitable for monitoring minimal residual disease.

MAIN TEXT

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 plasma tumor DNA (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 early stage breast cancer patients. 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 drugs will benefit relatively few, thus resulting in over treatment (3).

Beaver and colleagues exploited somatic mutations in the *PIK3CA* gene as a blood based tumor specific markers for women with breast cancer. *PIK3CA* 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 (Figure 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 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 droplet digital PCR (ddPCR), a technology capable of detecting genetic alterations with high specificity and up to 0.001% sensitivity (7). In ddPCR, individual DNA fragment 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 very 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 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 (1 false negative) and 100% specificity (no false positives) compared to ddPCR results of the tumor tissues. While 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 study reported ptDNA in patients with early stage cancer (8, 9)

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

Approximately 90% of all breast cancer cases are diagnosed with 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).

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). In spite of 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.

While data by 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 studies in large cohorts of patients. In some instances, such as

women with ER/PR positive breast cancer which typically relapse many years after surgery, studies assessing whether liquid biopsies predict recurrence will require decades to complete. In addition, *PIK3CA* mutations are found in less the 40% of breast cancer patients. To perform far-reaching longitudinal studies, blood based markers capable of capturing the entire patient population will need to be developed. This issue should be fairly easy to address; for example by tracking 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 5-10 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 regarding adjuvant systemic therapies and to recommend surveillance of patients with a high risk for recurrence.

Legend to Figure 1

PtDNA analysis in blood for PIK3CA mutations in early stage breast cancers. Three clinical scenarios in which surgery is indicated are shown. Blood is collected pre and post-resection, ptDNA isolated and PIK3CA mutations are identified. In the first two scenarios, mutant PIK3CA molecules (red double helices) are still present in the blood following surgery, indicating residual disease. In these instances further adjuvant therapies are recommended. In the third case only normal circulating DNA (green double helices) is present after surgery. This indicates that the procedure was curative and further adjuvant treatment is not required.

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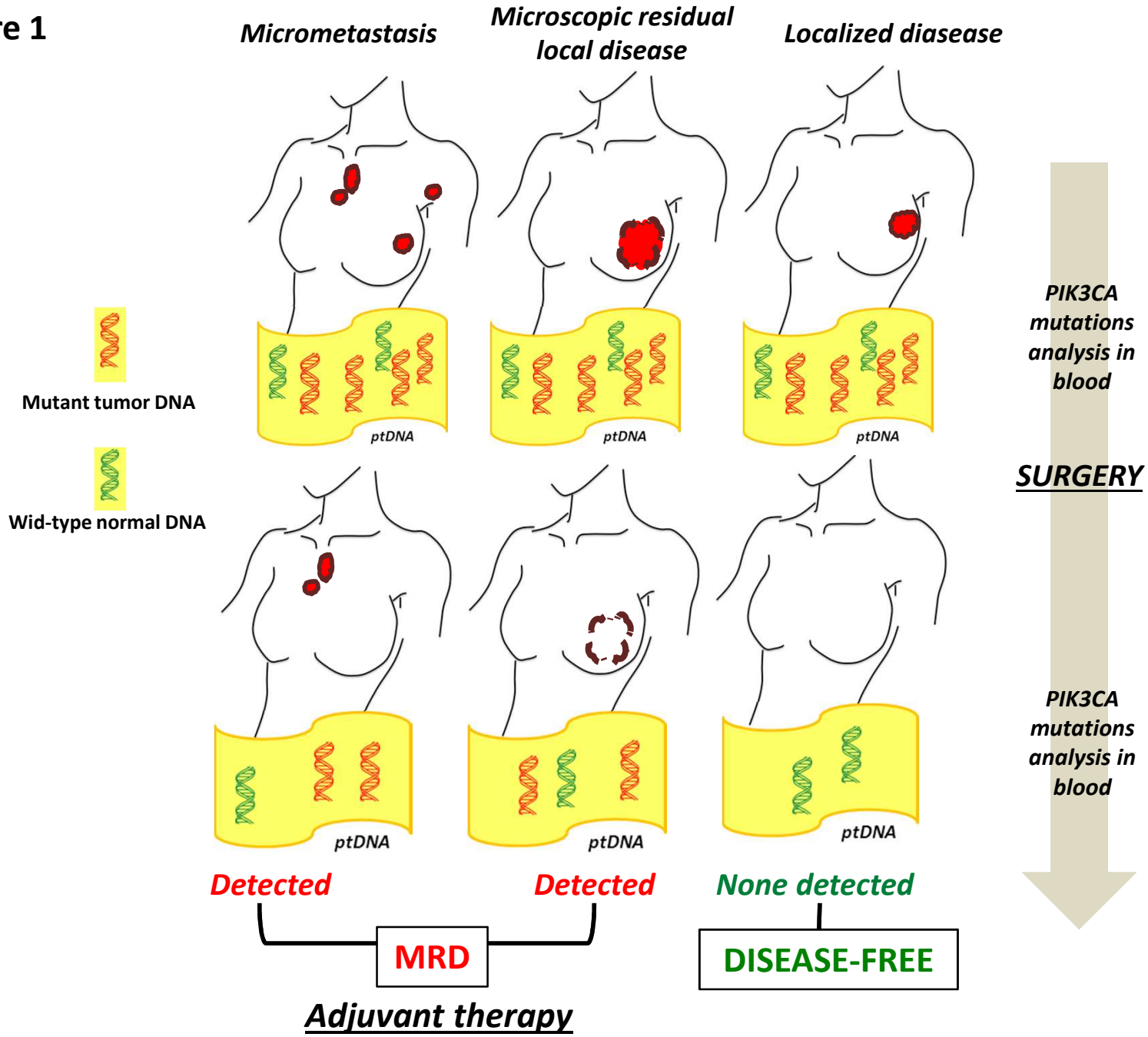
Figure 1


Micrometastasis
Microscopic residual local disease
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Mutant tumor DNA
Wid-type normal DNA
ptDNA
ptDNA
ptDNA
PIK3CA mutations analysis in blood
SURGERY
ptDNA
ptDNA
ptDNA
PIK3CA mutations analysis in blood
Detected
Detected
None detected
MRD
DISEASE-FREE
Adjuvant therapy