Gene Mutation Profiling of Breast Cancers for Clinical Decision Making
Drivers and Passengers in the Cart Before the Horse

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Recent advances in molecular profiling allow for a rapid and relatively inexpensive assessment of multiple altered genes or gene products from small amounts of tumor tissues or blood. The challenge ahead is how to properly incorporate these results into clinical practice, and how to provide patients with the best possible yet evidence-based care. Herein, we provide a brief overview of genetic mutation profiling with a focus on next-generation sequencing (NGS) and possible clinical utility.

Mutation Detection Techniques
Highly sensitive techniques are now available to detect mutations quantitatively and qualitatively. Typically, NGS involves creating “libraries” of short DNA fragments that are then sequenced massively in parallel to identify genetic alterations in a tumor. Since there are only a few known “driver” genes (ie, genes implicated in carcinogenesis and/or drug resistance), most tests do not query the entire genome but instead incorporate select gene panels, generally consisting of 50 to 400 cancer-related genes. An assay may include all coding exons or only known “hotspot” regions within a given set of genes.

Clinical Utility
Tumor mutation profiling may ultimately have a role in the continuum of breast cancer care. Genetic mutation testing for breast cancer is currently limited to assessing germline mutation status, that is, heritable genetic alterations such as deleterious mutations in BRCA1 or BRCA2. Testing today is reserved for individuals with strong family histories of breast and other cancers or other high-risk characteristics.

Unlike germline mutations, most cancers are characterized by somatic mutations that are present only in the tumor. Data to support the use of somatic mutation profiling in breast cancer are not currently available. Nonetheless, several gene sequencing panels are commercially offered, and their use is increasing for patients with breast cancer. Some of these tests compare tumor alterations against germline DNA obtained from the same patient, reporting only somatic variants. Other tests “filter” out germline variants from public databases. The latter format has the disadvantage of not knowing whether a reported variant is germline or somatic, yet it can also reveal germline variants that may be of clinical significance (eg, BRCA1 mutations). Most test results mirror the known mutational landscape of primary breast cancers, including an 80% to 90% frequency of TP53 mutations in triple-negative breast cancers, and approximately 40% frequency of PIK3CA mutations in estrogen receptor (ER) and HER2-positive disease. Mutation status may change with cancer progression. For example, recent studies show that ESR1 mutations occur in metastatic sites of disease from ER-positive breast cancers, suggesting a causative role for ESR1 mutations in endocrine-resistance but not carcinogenesis of the primary tumor. In fact, clonal evolution leading to mutationally distinct and heterogeneous tumor populations is a well-described phenomenon.

Whether targeting one specific driver mutation in the metastatic setting will afford clinical benefit is unknown. Several large studies will investigate response and other outcomes in individuals who receive targeted therapies based on mutation profiling. For example, the National Cancer Institute’s Molecular Analysis for Therapy Choice Program (MATCH) umbrella protocol offers multiple single-arm phase II trials in which molecular subgroups are matched to targeted agents.

A difficulty with these trials is the need for fresh biopsy specimens of metastatic lesions. Metastatic sites are not always accessible, and even when they are, biopsies are invasive, costly, and may lead to treatment delays. An alternative may be the use of blood to assay for circulating plasma tumor DNA (ptDNA). All cells, normal and cancerous, shed or secrete DNA into the circulation, and, because DNA somatic mutations are tumor specific, ptDNA serves as a source for assessing the metastatic mutational landscape within an individual. ptDNA therefore theoretically contains the mutations found in all sites of metastatic disease, blood, or other circulating body fluids and may be suitable as a “liquid biopsy specimen” for mutation profiling. This approach may help overcome tumor heterogeneity because different disease sites may harbor diverse mutation profiles, as reported for ESR1 mutations. PtDNA could also serve as a useful adjunct to measure response to treatment, especially with targeted agents in clinical trials. One can envision that patients with a partial or mixed response to a targeted therapy may still show a molecular response as evidenced by decreasing amounts of the targeted mutant gene in ptDNA.

Studies validating mutations in ptDNA with metastatic disease are emerging. We retrospectively queried PIK3CA mutations from a metastasis and plasma obtained simultaneously from 49 women with metastatic breast cancer. The concordance of PIK3CA mutation status between tissue samples and ptDNA from temporally matched plasma was 100%. We then
enrolled 60 patients with metastatic breast cancer in a prospective validation study comparing PIK3CA hotspot mutations in plasma vs archival tumor tissues and demonstrated a 72.5% concordance. Discordant results were seen only in patients whose archival tumor tissues were obtained 3 or more years prior to blood draw for ptDNA analysis. We are currently evaluating the feasibility of real-time mutational profiling via NGS in women with metastatic breast cancer using a fresh tissue biopsy specimen and correlating mutational status with ptDNA from blood obtained simultaneously. Other investigators have recently reported high concordance between tissue and blood mutation profiles in metastatic breast cancers.

ptDNA may also influence treatment decisions for early-stage breast cancers. Mutations can be detected with high sensitivity and specificity in the plasma of women with stages I to III breast cancer prior to surgery and may persist postoperatively in a small proportion.7 If validated, ptDNA could be used to differentiate women with residual micrometastatic disease who should be offered adjuvant systemic therapy from those who may be cured of their disease with local therapy alone.

Molecular Profiling Tumor Board

Until evidence-based data are available to support treatment selection according to somatic mutation analysis, test results must be carefully interpreted for individual patients who are not enrolled in a trial. As part of a personalized medicine effort the Johns Hopkins Kimmel Cancer Center, we have initiated a Genetic Alterations In Tumors With Actionable Yields (GAITWAY) tumor board to interpret genetic alterations found in a patient’s tumor sample. The mission of the GAITWAY tumor board is not only to review a patient’s molecular tumor profile to identify “actionable” genes and/or proteins but also to review the relevant literature on the evidence that such actionable genetic alterations are of functional consequence and to provide treatment recommendations. An “actionable” alteration includes a mutation that (1) has an US Food and Drug Administration (FDA)-approved therapy for the given tumor type, (2) has an FDA-approved therapy for a different tumor type, (3) may provide rationale for participation in a clinical trial, or (4) may lead to recommendations for genetic counseling and germline mutation testing.

There is much excitement and enthusiasm for using cutting-edge technologies to uncover the molecular underpinnings of each patient’s tumor. However, great care and consideration must be taken to ensure that physicians and care providers do no harm—a tenant of our profession. Prior to ordering a genomic profiling test, we would urge clinicians to review available data and ask whether the results from these tests would affect management and how to best reach that conclusion. For most of these tests, clinical validation and utility have yet to be proven. Ultimately, further research and validation and a multidisciplinary approach are needed to prove the clinical utility of mutation profiling for breast cancers as a future standard of care.

REFERENCES