Detection of Tumor PIK3CA Status in Metastatic Breast Cancer Using Peripheral Blood

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Abstract

Purpose: We sought to evaluate the feasibility of detecting PIK3CA mutations in circulating tumor DNA (ctDNA) from plasma of patients with metastatic breast cancer using a novel technique called BEAMing.

Experimental Design: In a retrospective analysis, 49 tumor and temporally matched plasma samples from patients with breast cancer were screened for PIK3CA mutations by BEAMing. We then prospectively screened the ctDNA of 60 patients with metastatic breast cancer for PIK3CA mutations by BEAMing and compared the findings with results obtained by screening corresponding archival tumor tissue DNA using both sequencing and BEAMing.

Results: The overall frequency of PIK3CA mutations by BEAMing was similar in both patient cohorts (29% and 28.3%, respectively). In the retrospective cohort, the concordance of PIK3CA mutation status by BEAMing between formalin-fixed, paraffin-embedded (FFPE) samples and ctDNA from temporally matched plasma was 100% (34 of 34). In the prospective cohort, the concordance rate among 51 evaluable cases was 72.5% between BEAMing of ctDNA and sequencing of archival tumor tissue DNA. When the same archival tissue DNA was screened by both sequencing and BEAMing for PIK3CA mutations (n = 41 tissue samples), there was 100% concordance in the obtained results.

Conclusions: Analysis of plasma-derived ctDNA for the detection of PIK3CA mutations in patients with metastatic breast cancer is feasible. Our results suggest that PIK3CA mutational status can change upon disease recurrence, emphasizing the importance of reassessing PIK3CA status on contemporary (not archival) biospecimens. These results have implications for the development of predictive biomarkers of response to targeted therapies. Clin Cancer Res; 18(12); 3462–9. ©2012 AACR.

Introduction

Ablant phosphoinositide 3-kinase (PI3K) pathway signaling is being studied as a prognostic marker in breast cancer and as a predictive marker for targeted-specific therapies (1–5). The gene encoding the p110α catalytic domain of PI3K, PIK3CA, is the most commonly mutated oncogene in breast cancer, and more than 80% of somatic PIK3CA mutations occur in one of 3 recurrent "hotspot" locations (6–8). Key to the interpretation and success of clinical trials targeting the PI3K pathway is the accurate identification of tumors with sensitizing or desensitizing mutations to specific drugs, as observed with EGFR mutations in non–small cell lung cancer, somatic KRAS mutations in colorectal cancers, and BRAF mutations in metastatic melanoma (9–13). Determination of somatic mutational status traditionally requires freshly obtained or archival biopsy specimens for sequencing. However, the contamination of tumor samples with normal tissue, tumor heterogeneity, and variable quality of extracted and stored DNA can interfere with accurate analyses (14). Therefore, current methods routinely used to detect mutations in DNA from formalin-fixed, paraffin-embedded (FFPE) samples are limited, and more accurate and less invasive detection methods are needed. The ability to detect tumor DNA mutations in a blood sample (i.e., a liquid biopsy) would allow an easy to obtain,
Translational Relevance

We report a prospective study on the feasibility and accuracy of screening for the presence of tumor PIK3CA mutations in patients with metastatic breast cancer using blood as a tissue source by way of a novel and robust assay called BEAMing. We observed significant discordance between testing of primary early stage tumors compared with testing at a later time point after cancer recurrence. We describe that loss or gain of PIK3CA mutations can occur with metastatic disease in up to 20% of cases. Because multiple inhibitors of phosphoinositide 3-kinases are currently in development, an accurate determination of current PIK3CA mutation status has clear implications for trial design and future clinical practice. Our results suggest that BEAMing provides an accurate and relatively non-invasive method for current ascertainment of PIK3CA mutational status in patients with metastatic breast cancer.

noninvasive, and quantifiable method for use in the clinical setting to identify candidates for specific therapies and monitoring of disease status over time. It would also provide real-time assessment of mutational status without having to rely on archival specimens from the original primary tumor (if available) or the need for invasive biopsy procedures of a metastatic site.

DNA containing somatic mutations is highly tumor specific and thus can potentially be used as a biomarker. A novel technique for identifying cell free, plasma-derived circulating mutant DNA termed "BEAMing" has recently been developed. BEAMing is named after the 4 key components of the method (Beads, Emulsification, Amplification, and Magnetics; refs. 15, 16). Assessment of circulating tumor DNA (ctDNA) by BEAMing can provide the mutational status of a patient's cancer (17, 18). BEAMing can be carried out on virtually any tissue source without enriching for tumor cells, and thus the risk of "masking" mutations due to tumor heterogeneity and/or contamination of normal cells is greatly reduced. Use of plasma (peripheral blood) offers many advantages such as ease of access and the ability to repeat tests over time as the source of DNA is continuous.

We designed this study to test the feasibility of using BEAMing on ctDNA to determine PIK3CA mutational status in peripheral blood of patients with breast cancer. An initial retrospective cohort analysis used 49 paired patient samples of tumor tissue and blood obtained at the same time. We then conducted a separate confirmatory, prospective study to determine the feasibility of using BEAMing to detect PIK3CA mutations in patients with recurrent metastatic breast cancer (blood and tissue) and compared it to standard DNA sequencing methods currently used. We describe rates of observed mutation detection and concordance, and the resulting critical implications for clinical practice and research studies.

Materials and Methods

Retrospective contemporaneous tissue and blood collection cohort

Paired samples of breast cancer tumor tissue and blood samples taken from the same patients on the same day were obtained by Indivumed GmbH as part of their tissue repository service. In 45 of these cases, the tumor specimen collected was the primary breast tumor and a biopsy of a metastatic breast cancer lesion was the tissue obtained for the remaining 4. The collection of biospecimens and clinical data within the Federal republic of Germany is not regulated by national law; these samples were collected ethically within the framework of the "Hamburger Krankenhausgesetz 12a". Genomic DNA was extracted from 49 tissue samples and BEAMing used to identify one of 3 mutations in the PIK3CA gene: (Ex 9 1633G>A E545K; Ex 20 3140A>G H1047R; Ex 20 3140A>T H1047L). ctDNA derived from plasma from all patients in whom a PIK3CA mutation was identified in the tissue samples, and also from 20 randomly selected patients whose tumors were PIK3CA wild-type, were subsequently analyzed by BEAMing.

Prospective feasibility study patient cohort

Women with metastatic breast cancer were prospectively enrolled at The Johns Hopkins Sidney Kimmel Comprehensive Cancer Center (Baltimore, MD). Patient characteristics are shown in Supplementary Table S1. All subjects were required to have a prior breast cancer tissue sample available and be willing to provide a 10 mL peripheral venous blood sample. Review of medical records was conducted to confirm sites of metastatic disease and histopathologic features of the primary breast tumor. The protocol was approved by the Johns Hopkins Institutional Review Board and informed consent was obtained from all patients. Of note, blood for ctDNA was obtained at the time of trial entry, and then archival tumor tissue was retrieved from pathologists, however these tissue specimens had been acquired months to years before study entry.

Tissue sequencing

A hematoxylin and eosin–stained slide of each patient’s tumor (primary or metastatic) was used for identification of tumor by the study pathologist. Areas of tumor tissue were removed from serial unstained slides (10μ) using the Zymo pen and Pinpoint solution (Zymo Research) as per the manufacturer’s protocol. The percentage of tumor cells in the resultant samples was approximately 80% to 90%. DNA was purified using QIAamp DNA spin columns (Qiagen). PIK3CA exons 9 and 20 were PCR amplified using one biotinylated primer in each reaction. Following amplification, the biotinylated PCR products were purified and sequenced using the PyroMark Q24 (Qiagen) with sequencing primers designed to identify the following mutations: PIK3CA Ex 9 1624G>A E542K; PIK3CA Ex 9 1633G>A
E545K; and PIK3CA Ex 20 3140A>G, H1047R. Two known PIK3CA mutation-positive cell lines, HCT-15 (E545K) and HCT-116 (H1047R), were run as controls (19). Investigators conducting the sequencing assays were blinded to the BEAMing results.

BEAMing
Plasma was derived from blood samples by centrifugation within 2 hours of collection and were spun twice for each sample to ensure the absence of cellular contamination. Free circulating DNA was isolated from plasma samples by the QIAamp DNA purification kit (Qiagen). After sequencing had been conducted on the genomic DNA from the archival tissue samples, the remaining DNA if available, was also used for BEAMing. ctDNA isolated from plasma and BEAMing assays were conducted on each sample by Inostics GmbH. BEAMing is a technique in which individual DNA molecules are attached to magnetic beads in water-in-oil emulsions and then subjected to compartmentalized PCR amplification. The mutational status of DNA bound to beads is then determined by hybridization to fluorescent allele-specific probes for mutant or wild-type PIK3CA. Flow cytometry is then used to quantify the level of mutant DNA present in the plasma (15, 17). For the retrospective contemporaneous tissue and blood collection cohort analysis, Ex 9 1633G>A E545K, Ex 20 3140A>G H1047R, Ex 20 3140A>T H1047L mutations were queried by BEAMing (at the time of the retrospective study, BEAMing for the Ex 9 E542K mutation was not available). For the prospective study, PIK3CA Ex 9 1624G>A E545K, PIK3CA Ex 9 1633G>A E545K, and PIK3CA Ex 20 3140A>G H1047R were analyzed by BEAMing. To contain costs, the Ex 20 3140A>T H1047L was not conducted in the prospective study. Investigators conducting the BEAMing assays were blinded to the sequencing results.

Statistical analysis
In the statistical analyses, each BEAMing test result was treated as an independent variable under the assumption that inaccuracies in detecting a single base pair substitution with either method would not influence the test result using the other method. The mechanical ability of BEAMing to detect specific mutations in tumor tissue identified by standard sequencing methods was tested by directly comparing results obtained by the 2 assay methods in the tumors from the prospective patient cohort (same tumor, different assay). The ability of BEAMing to identify the same specific mutations previously detected by standard sequencing of tumor tissue in circulating DNA was done by using the blood that was collected at the time of surgery (i.e., the retrospective patient cohort). Finally, the hypothesis that the mutational status of patients with breast cancer would not change over time was tested by comparing the ctDNA by BEAMing to surgical specimens that had been previously collected (i.e., the prospective study cohort). Where appropriate, the concordance between platforms and tissues was tested with Kappa statistics, which is a conservative measure of relative agreement between 2 categorical items (in this case mutant vs. wild-type) that takes into account agreement by chance. Kappa ranges from $\kappa = 1$ (perfect agreement) to $\kappa = 0$ (no agreement other than would be expected by chance). Calculations of chance overlap with confidence intervals (CI) were used to estimate significance of each calculation based on the observed data. Expected concordance rates were calculated using a collection of the mutation frequencies of PIK3CA detected in previous studies of breast cancers (19). On the basis of these data, the expected frequencies of mutations in the prospective cohort were estimated at 13.3%, 4.4%, and 2.5% for variants 3140A>G, 1633G>A, and 1624G>A, respectively (collectively 20.1%), whereas in the retrospective cohort the expected frequencies were estimated at 13.3%, 4.4%, and 1.1% for mutations 3140A>G, 1633G>A, and 3140A>T, respectively (collectively 18.7%). Because of the anticipated large fraction of ‘wild-type’ scores, expected concordance rates were tested against the hypothesis that either assay would detect mutations at the expected frequency.

Results
Patients
Between January 2004 and 2009, samples from 49 patients with metastatic breast cancer (median age, 62; range, 39–84) were collected in the retrospective cohort from Germany. Thirty-five (71.4%) patients had estrogen receptor–positive disease. HER2 status was unavailable for some patients as shown in Table 1. In 45 of 49 cases, tumor tissue was collected from the primary breast tumor on the same day as the blood sample for BEAMing. Four tissue samples came from a metastatic breast tumor site with blood samples also collected on the same day of biopsy.

Between February 2010 and May 2010, 60 patients (median age, 56; range, 36–85) were enrolled in the prospective feasibility study at Johns Hopkins. All had histologically confirmed breast cancer with radiological and/or pathologic evidence of distant recurrent metastatic disease. Most patients (65%) had estrogen receptor–positive breast cancer, 6 (10%) had triple-negative disease, and 20 (33.3%) tumors showed overexpression of HER2. The source of archival tissue sample was primary tumor for 42 (80.8%) participants and biopsy of a distant metastatic tumor site for 10 (19.2%).

Retrospective cohort
For the retrospective cohort, where blood was collected at the time of tumor biopsy, the goal was to determine whether BEAMing could reliably detect mutations in ctDNA that were identified by direct BEAMing analysis of tumor tissue. Mutations were identified by BEAMing of FFPE-derived genomic DNA in 14 of 49 patients (29%). BEAMing of plasma ctDNA from the same patients identified the same 14 mutations as observed by BEAMing the FFPE-derived tissue samples (Table 1). BEAMing of 20 FFPE-derived genomic DNA wild-type PIK3CA samples was 100%
concordant with BEAMing of corresponding ctDNA. These results suggest that ctDNA BEAMing accurately detects the PIK3CA mutational status in patients with breast cancer when testing a blood sample collected at the time of tissue biopsy ($\kappa = 1.0$). The actual agreement did not overlap with what would be expected by chance for the retrospective study ($\kappa = 0.070; 95\%$ CI, 0.0–0.57).

Prospective cohort

In a prospective setting, a 10-mL blood sample was obtained from patients with recurrent metastatic breast cancer ($n = 60$) at the time of study entry. For each patient, consent to retrieve archival tumor tissue for DNA analysis was also obtained. The median time between blood draw at study entry and time that the tumor specimen was removed from the patient was 5.0 years (range, 0.26–23.9 years). ctDNA was successfully extracted from all patients. Archival tumor tissue with adequate DNA for standard sequencing was obtained in 51 cases, whereas tumor from 9 patient cases had insufficient tissue or no archival tissue available.

Oncogenic mutations were detected in 28.3% of plasma samples (Table 2) and most were in exon 20. PIK3CA

Table 1. Spectrum of PIK3CA mutations identified retrospectively in peripheral blood of patients with breast cancer by BEAMing of ctDNA and also by BEAMing of breast tumor tissue collected simultaneously from the same patients

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Amino acid change</th>
<th>Observed frequency in both ctDNA and tumor tissue/expected frequency* $N = 49$ (%)/ (%)</th>
<th>Tumor hormone receptor positive $N (%)$</th>
<th>HER2 status of tumor $N (%)$</th>
<th>&quot;Triple-negative&quot; tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1633G&gt;A</td>
<td>545E&gt;K</td>
<td>3 (6.1)/(4.4)</td>
<td>3 (100)</td>
<td>2 (75) negative 1 (25) unknown</td>
<td></td>
</tr>
<tr>
<td>3140A&gt;G</td>
<td>1047H&gt;R</td>
<td>10 (20.4)/(13.3)</td>
<td>7 (70)</td>
<td>4 (40) positive 1 (10) unknown</td>
<td></td>
</tr>
<tr>
<td>3140A&gt;T</td>
<td>1047H&gt;L</td>
<td>1 (2)/(1.1)</td>
<td>1 (100)</td>
<td>1 (100) positive 0</td>
<td></td>
</tr>
<tr>
<td>Total number of mutations</td>
<td>14 (28.6)/(18.7)</td>
<td>11 (78.6)</td>
<td></td>
<td>7 (50) negative 2 (14.3) unknown</td>
<td></td>
</tr>
</tbody>
</table>

*Distribution of somatic mutations in PIK3CA, Catalogue Of Somatic Mutations in Cancer (COSMIC).

Table 2. Distribution of PIK3CA mutations detected prospectively in plasma of patients with metastatic breast cancer by BEAMing and correlation with hormone receptor and HER2 status of primary tumor

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Amino acid change</th>
<th>Observed frequency/expected frequency* $N = 49$ (%)/%</th>
<th>Primary tumor hormone receptor positive $N (%)$</th>
<th>Primary tumor HER2 status $N (%)$</th>
<th>Primary tumor &quot;triple negative&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1624G&gt;A</td>
<td>542E&gt;K</td>
<td>3 (5)/(2.5)</td>
<td>2 (66.6)</td>
<td>1 (33.3) positive 2 (66.6) negative</td>
<td></td>
</tr>
<tr>
<td>1633G&gt;A</td>
<td>545E&gt;K</td>
<td>4 (6.7)/(4.4)</td>
<td>3 (75)</td>
<td>3 (75) positive 1 (25) negative</td>
<td></td>
</tr>
<tr>
<td>3140A&gt;G</td>
<td>1047H&gt;R</td>
<td>12 (20)/(13.3)</td>
<td>11 (81.7)</td>
<td>4 (33.3) positive 8 (66.6) negative</td>
<td></td>
</tr>
<tr>
<td>Total number of mutations</td>
<td>17 (28.3)/(20.1)</td>
<td>Among 17 samples with at least 1 mutation detected: 14 (82.4)</td>
<td></td>
<td>Among 17 samples with at least 1 mutation detected: 7 (41.2) positive 10 (58.8) negative</td>
<td></td>
</tr>
</tbody>
</table>

*Distribution of somatic mutations in PIK3CA, Catalogue Of Somatic Mutations in Cancer (COSMIC).

**Nineteen mutations were identified in 17 patient samples.**
mutations were most commonly observed in patients with hormone receptor–positive breast cancer and in HER2-positive breast cancers. Of interest, BEAMing of plasma samples from 2 patients showed 2 separate PIK3CA mutations in each of exons 9 and 20. PIK3CA mutations were detected by sequencing of archival tissue in 14 samples (27.5%, see Table 3). While the ctDNA mutation profile in the retrospective cohort (tissue and blood collected at the same time) mimicked that of the tumor tissues, discordant results (pos/neg and neg/pos) were seen in 14 of 51 samples (blood sample compared with archival tumor). Six patients had mutant PIK3CA in archival tissues with no mutation detected in blood whereas 8 other patients had the opposite profile with normal archival tissue but mutated PIK3CA in blood ($\kappa = 0.4829$; CI, 0.22–0.74). Although the agreement is better than what would have been expected by chance ($\kappa = 0.0366$; CI, 0.0–0.45), the agreement fell significantly below the real-time estimates of PIK3CA mutations from ctDNA when blood was collected at the time of tissue biopsy (where $\kappa = 1.0$).

In view of the observed tissue/blood discordance observed in the prospective cohort, we decided to also test these tissues by BEAMing as was done in blood. Of the 51 prospective tissue samples initially tested by standard sequencing, enough DNA remained in 41 of them for BEAMing allowing cross-platform comparisons to assess PIK3CA status (same tissue, different assay). As shown in Table 4, there was 100% concordance between BEAMing and standard sequencing when assessing PIK3CA mutation status in these 41 tissue samples ($\kappa = 1.0$). The calculated $\kappa = 0.036$ (95% CI, 0.0–0.53) for chance agreement of this direct comparison of platforms.

### Discussion

We report the feasibility of screening for the presence of common oncogenic PIK3CA mutations in patients with breast cancer by a simple blood test using BEAMing. Overall, ctDNA was isolated from 109 patient blood samples and a PIK3CA mutation was identified in 28.4%. Of critical importance, testing done in the prospective cohort clearly exemplified the current challenges of locating quality archival tissue samples for biomarker testing. In this case, sufficient tissue was available for only 51 of 60 prospectively enrolled patients (85%). In contrast, BEAMing on ctDNA

### Table 3. Results of standard sequencing of archival tissue tumor specimens and mutations detected by BEAMing of ctDNA in prospective study

<table>
<thead>
<tr>
<th>Description of tissue tumor DNA and plasma-derived ctDNA for mutation analysis</th>
<th>Number of samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples with adequate archival tissue available for sequencing</td>
<td>51/60 (85)</td>
</tr>
<tr>
<td>Number of samples with adequate plasma available for ctDNA extraction and BEAMing</td>
<td>60/60 (100)</td>
</tr>
<tr>
<td>Number of PIK3CA mutations identified by sequencing archival tissue</td>
<td>14/51 (27.4)</td>
</tr>
<tr>
<td>Number of PIK3CA mutations identified by BEAMing of plasma-derived ctDNA</td>
<td>17/60 (28.3)</td>
</tr>
<tr>
<td>Number of samples that were PIK3CA wild-type by sequencing of archival tissue and PIK3CA wild-type by BEAMing of plasma-derived ctDNA</td>
<td>37/51 (72.5)</td>
</tr>
<tr>
<td>Number of samples that were PIK3CA mutant by sequencing of archival tissue and PIK3CA mutant by BEAMing of plasma-derived ctDNA</td>
<td>8/51 (15.6)</td>
</tr>
<tr>
<td>Number of samples that were PIK3CA wild-type on sequencing of archival tissue and PIK3CA mutant by BEAMing of plasma-derived ctDNA</td>
<td>8/51 (15.6)</td>
</tr>
<tr>
<td>Number of samples that were PIK3CA mutant on sequencing of archival tissue and PIK3CA wild-type by BEAMing of plasma-derived ctDNA</td>
<td>6/51 (11.7)</td>
</tr>
</tbody>
</table>

**NOTE:** N = 60 samples available for ctDNA extraction and BEAMing. N = 51 samples available for sequencing.

aNineteen mutations were identified by BEAMing in 17 patient samples.

### Table 4. Concordance of PIK3CA mutational status detected by sequencing or by BEAMing of DNA derived from the same tissue specimen (N = 41 matched samples, %)

<table>
<thead>
<tr>
<th>Description</th>
<th>Number of samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples with adequate archival tissue available for BEAMing</td>
<td>41 (68.3)</td>
</tr>
<tr>
<td>Number of samples with adequate plasma available for ctDNA extraction and BEAMing</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Number of samples that were PIK3CA wild-type by sequencing of archival tissue and PIK3CA wild-type by BEAMing of archival tissue</td>
<td>30 (73.1)</td>
</tr>
<tr>
<td>Number of samples that contained the same PIK3CA mutation by sequencing of archival tissue and by BEAMing of archival tissue</td>
<td>11 (26.8)</td>
</tr>
<tr>
<td>Number of discordant results between sequencing of archival tissue and BEAMing of archival tissue</td>
<td>0</td>
</tr>
</tbody>
</table>
was successful in the blood samples from all 60 enrolled patients, with a PIK3CA mutation frequency similar to that previously reported (Table 2; refs. 6, 7, 20, 21). Of interest, BEAMing of plasma samples from 2 patients showed 2 separate PIK3CA mutations in each of exons 9 and 20; this is a rare phenomenon but has been previously described (22).

Most critical and with implications for both clinical trial design and clinical practice, we observed a significant discordance between mutation analysis using current versus archival tissue. In the retrospective cohort, the concordance between PIK3CA mutational status by BEAMing in blood and by standard sequencing in tissue was 100%. However, a 27.5% discordance was observed among 51 patients with recurrent metastatic disease prospectively tested by BEAMing in blood compared with standard sequencing of archival tissue obtained months to years before study entry. There are several important implications of this observation. It is theoretically possible that BEAMing failed to detect a PIK3CA mutation and provided a “false-negative” result in the cases that were mutation positive by sequencing of tissue yet wild-type by BEAMing of ctDNA. We feel this is unlikely given the previously reported, extremely high sensitivity of the BEAMing technique (23), and the fact that all PIK3CA mutations in tissue samples were detected in ctDNA by BEAMing in the retrospective study. That said, we cannot formally exclude this as a possibility with our current samples and data. As a potential explanation, it is possible that some patients had very little disease burden at the time of blood draw, therefore, plausibly BEAMing may not have been sensitive enough to identify PIK3CA mutations in this setting. However, reexamination of our patients’ recurrent disease status did not show this in cases where discordant results occurred. Notably, we did observe that discordant results were only seen in patients whose archival tumor specimen was at least 3 years before blood draw for ctDNA (Supplementary Table S2). An ongoing study will establish the lower threshold of PIK3CA mutation detection using early, operable patients with breast cancer who will have BEAMing conducted on ctDNA both before and after surgical resection of their tumors.

Currently, many trials testing PI3K inhibitors are enrolling only patients with mutant PIK3CA tumors. Our findings suggest that in patients whose archival tumor specimen was obtained 3 or more years before blood draw for ctDNA, PIK3CA mutational status may change, as paired samples from 8 patients (15.6%) showed wild-type PIK3CA in their archival tissue sample and mutant PIK3CA in their peripheral blood when recurrent metastatic disease was present, whereas paired samples from 6 other patients (11.7%) were discordant in the opposite direction [mutant to wild-type]. Our findings support recent reports by others that PIK3CA mutational status in breast cancer differs approximately 18% of the time between primary tumors and corresponding metastatic disease with changes in both directions (wild-type to mutant and mutant to wild-type) being observed (24, 25). Furthermore, our data suggest that this discordance is reflective of tumor evolution and not due to technical issues or platform selection as tissue DNA assessed for PIK3CA mutations by both standard sequencing and BEAMing showed 100% concordance. It has been assumed that clonal evolution in progressing advanced disease results from a gain of mutations for instance as a result of treatment selection, though spontaneous loss and gains of mutations have also been reported (26, 27). Tumor heterogeneity and sampling issues may also account for some of the previously reported findings, as a single biopsy using only micron thin sections for DNA analysis may not be representative of the whole tumor within a particular site or across metastatic sites (28–30). There is also evidence to suggest that multiple genetically diverse clonal subpopulations exist within primary breast cancers, in contrast to previously accepted models of tumor progression and metastatic dissemination punctuated by clonal expansions (31). It is therefore tempting to speculate that the finding of PIK3CA mutations in an original primary archival tumor that then “converts” to wild-type PIK3CA status at the time of recurrence or metastasis might represent the emergence of a new population of drug resistant clones and/or clones with increased metastatic potential.

A strength of the BEAMing technology is the ability to detect rare mutant molecules present in any source of DNA. A notable limitation is that BEAMing can detect only known mutations that have been optimized for the assay before sample analysis, that is, BEAMing currently cannot be used for mutation discovery. BEAMing is therefore ideally suited for genes with common recurrent “driver” mutations such as the PIK3CA mutations described here, as well as BRAF V600E and KRAS codon 12 and 13 mutations, with potential use in current clinical practice for targeted cancer therapies where these mutations have positive and negative predictive value (11–13). Other available assays can screen for higher numbers of somatic mutations such as the “SNuPshot” platform, which offers the possibility of screening more than 100 mutations. However, the limits of sensitivity of this technique for PIK3CA mutation detection is reported to be approximately 5% (5 mutant PIK3CA molecules per 100; ref. 32) compared with BEAMing which is capable of detecting at least one mutant molecule in 10,000 (15). Similarly, Board and colleagues have also described the detection of PIK3CA mutations in circulating free DNA using the Amplification Refractory Mutation System (ARMS; ref. 33). While their study showed in patients with metastatic breast cancer a high sensitivity and specificity for detecting PIK3CA mutations, no mutations were detected in PIK3CA-positive patients with operable disease. This may be due to the limits of detection of this technique, which have been reported to be 0.1% to 1% (34). In contrast, BEAMing is at least an order of magnitude more sensitive (0.01%) and this sensitivity can be improved with higher fidelity DNA polymerases (15, 18). In addition, though not a focus of the current study, BEAMing is quantitative unlike most current technologies. This aspect of BEAMing may allow for its use as a surrogate marker of disease burden. As example, we have recently found that
PIK3CA mutations can be detected in early, patients with nonmetastatic breast cancer before surgery (unpublished observations). Following surgery, we would expect that ctDNA levels would dramatically decrease unless there is the presence of occult disease. These studies are currently ongoing and the basis for future analysis on the use of BEAMing for early-stage disease.

To our knowledge, this is the first prospective study evaluating the feasibility of BEAMing to identify oncogenic PIK3CA mutations from plasma-derived ctDNA. Our results suggest that the characterization of PIK3CA mutational status by testing a blood sample using BEAMing in patients with metastatic breast cancer is highly feasible. We have shown that BEAMing of plasma ctDNA correlates 100% with mutational status of a metastatic tumor specimen, when both samples are collected synchronously. If indeed PI3K inhibitors are shown to be the greatest benefit in patients whose tumors harbor a PIK3CA mutation, our results and those of others suggest that patients should optimally be selected for these trials based on PIK3CA mutational status at the time of enrollment, rather than on mutational status of archival tissue. BEAMing offers a reliable, noninvasive blood test to assess PIK3CA mutational status that could be theoretically conducted in lieu of a biopsy making it highly attractive to patients and health care providers.

Disclosure of Potential Conflicts of Interest

F. Diehl and P. Angenendt are employees and stakeholders of Inostics GmbH who conducted BEAMing analyses. K.E. Bachman and J. Greshock are employees of GlaxoSmithKline. L.A. Emens has a commercial research grant for Genentech Incorporation and is a consultant/advisory board member for Genentech Incorporation, Bristol Myers Squibb, and Roche. B.H. Park is a consultant/advisory board member for GlaxoSmithKline and Horizon Discovery, Ltd. No potential conflicts of interest were disclosed by the other authors.

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