Tumor Tissue- versus Plasma-based Genotyping for Selection of Matched Therapy and Impact on Clinical Outcomes in Patients with Metastatic Breast Cancer

Neelima Vidula, Andrzej Niemierko, Giuliana Malvarosa, Megan Yuen, Jochen Lennerz, A. John Iafrate, Seth A. Wander, Laura Spring, Dejan Juric, Steven Isakoff, Jerry Younger, Beverly Moy, Leif W. Ellisen, and Aditya Bardia

ABSTRACT

Purpose: Actionable mutations can guide genotype-directed matched therapy. We evaluated the utility of tissue-based and plasma-based genotyping for the identification of actionable mutations and selection of matched therapy in patients with metastatic breast cancer (MBC).

Experimental Design: Patients with MBC who underwent tissue genotyping (institutional platform, 91-gene assay) or plasma-based cell-free DNA (cfDNA, Guardant360, 73-gene assay) between January 2016 and December 2017 were included. A chart review of records to identify subtype, demographics, treatment, outcomes, and tissue genotyping or cfDNA results was performed. The incidence of actionable mutations and the selection of matched therapy in tissue genotyping or cfDNA cohorts was determined. The impact of matched therapy status on overall survival (OS) in tissue genotyping or cfDNA subgroups was determined with Cox regression analysis.

Results: Of 252 patients who underwent cfDNA testing, 232 (92%) had detectable mutations, 196 (78%) had actionable mutations, and 86 (34%) received matched therapy. Of 118 patients who underwent tissue genotyping, 90 (76%) had detectable mutations, 59 (50%) had actionable mutations, and 13 (11%) received matched therapy. For cfDNA patients with actionable mutations, matched versus nonmatched therapy was associated with better OS [HR 0.41, 95% confidence interval (CI): 0.23–0.73, $P = 0.002$], and this remained significant in a multivariable analysis correcting for age, subtype, visceral metastases, and brain metastases (HR = 0.46, 95% CI: 0.26–0.83, $P = 0.010$).

Conclusions: Plasma-based genotyping identified high rates of actionable mutations, which was associated with significant application of matched therapy and better OS in patients with MBC.

See related commentary by Rugo and Huppert, p. 3275

Introduction

In recent years, genomic analyses of breast tumors have demonstrated that breast cancer is a heterogeneous disease entity with varying genomic alterations (1, 2). Furthermore, metastatic breast cancer (MBC) may harbor a number of oncogenic mutations that are acquired from tumor evolution and prior treatment (3), which are attractive targets for genotype-directed matched therapy targeted to the actionable mutation (4–6).

Tumor tissue genotyping and plasma-based genotyping via cell-free DNA (cfDNA) analysis are two broad methods for the assessment of oncogenic mutations (7), with cfDNA having the advantage of being less invasive than a tissue biopsy (8–11). Genotyping of tumor tissue can occur via next-generation sequencing (NGS; ref. 12). cfDNA genotyping analyses can utilize NGS or digital droplet PCR on cell-free fragments of DNA released by cancer cells (13).

However, the clinical impact of targeting mutations identified by routine tumor tissue genotyping or cfDNA in patients with MBC is unclear. In some settings, it is clearly beneficial such as the identification of *PIK3CA* mutations present in hormone receptor–positive (HR^+) MBC, for which the PI3K inhibitor, alpelisib, is now approved (5), but the utility of widespread routine tumor genotyping and the optimal technique is yet to be determined.

We evaluated the utility of tumor tissue genotyping and cfDNA in patients with MBC for the identification of actionable mutations, as well as the impact of testing on the selection of matched therapy targeted to an actionable mutation and clinical outcomes. Furthermore, we evaluated the relative utility of these tests in the selection of matched therapy in patients undergoing both forms of testing.

Materials and Methods

Study population

In the first set of analyses, consecutive patients with MBC who underwent tumor tissue genotyping (NGS, institutional platform, 91-gene assay) or cfDNA testing (NGS/Guardant360, 73-gene assay) as part of routine clinical care at Massachusetts General Hospital (Boston, MA) at any time after their diagnosis of MBC from January 2016 to December 2017 were identified. All patients with MBC seen at the center during this period were offered testing, either at the time of

Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, Massachusetts.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Vidula N. and colleagues. Comparison of tumor genotyping and cell-free circulating tumor DNA sequencing in metastatic breast cancer patients and their utility in the selection of matched therapy [abstract]. In: Proceedings of the 2018 San Antonio Breast Cancer Symposium; 2018 Dec 4–8; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2018;79(4 Suppl):Abstract nr P4-01-06.

Corresponding Author: Neelima Vidula, Department of Hematology/Oncology, Massachusetts General Hospital Cancer Center, Boston, MA 02114. Phone: 617-726-6500; E-mail: nvidula@mgh.harvard.edu

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Translational Relevance

Actionable mutations can guide genotype-directed matched therapy. We conducted a comprehensive analysis of patients with metastatic breast cancer (MBC) to determine the utility of tissue-based [next-generation sequencing (NGS), 91-gene assay] and plasma-based genotyping [Guardant360, 73-gene NGS assay, cell-free DNA (cfDNA)] for the identification of actionable mutations, the selection of matched therapy, and impact on overall survival. Both forms of testing demonstrated high rates of detection of actionable mutations (cfDNA: 78% actionable mutations, tissue genotyping: 50% actionable mutations), and relevance for matched therapy (cfDNA: 34% matched therapy, tissue genotyping: 11% matched therapy). For cfDNA patients with actionable mutations, matched versus nonmatched therapy was associated with better overall survival. On the basis of these findings, plasma-based genotyping may identify high rates of actionable mutations, with relevance for matched therapy in MBC, and a potential improvement in outcomes, but further prospective research is needed for validation.

MBC diagnosis or on progression, prior to starting a new therapy, and consecutive patients who chose to have this testing done were included. No patients undergoing either of these tests during this time period were excluded.

In the second set of analyses, we went on to analyze a subset of patients who underwent both tumor tissue genotyping and cfDNA analyses after MBC diagnosis between January 2016 and October 2017.

A retrospective review of medical and pathology records [Institutional Review Board (IRB)-approved institutional protocol] to identify tumor subtype, patient demographics, treatment, outcomes, and tumor tissue genotyping or cfDNA results was performed. Tumor subtype was known for all cases, and was determined from pathology reports of the metastatic specimen, or if unavailable, the primary tumor. Tumor subtype was designated as HR^+ (estrogen receptor and/or progesterone receptor positivity >1%), HER2 positive ($HER2^+$; based on HER2 IHC and/or FISH results), or triple-negative breast cancer (TNBC, $HR^-/HER2^-$). The presence of detectable mutations in patients with tumor tissue genotyping or cfDNA testing was determined and characterized by subtype. The incidence of actionable mutations and the selection of matched therapy targeted to an actionable mutation in tumor tissue genotyping or cfDNA cohorts was determined. Actionable mutations in this study were defined as genomic alterations detected in the tumor tissue or cfDNA, which are variants associated with cancer that are linked with a potential increased or decreased treatment response to a genotype-directed treatment (defined by Guardant360 guidelines described in greater detail below), either as an FDA-approved drug or a clinical trial of a targeted treatment in any related pathway.

The most common matched therapies in tumor tissue genotyping or cfDNA cohorts were analyzed. The most common reasons for not pursuing a matched therapy in tumor tissue genotyping or cfDNA cohorts were identified. The impact of matched therapy status on overall survival (OS) in tumor tissue genotyping and cfDNA subgroups was determined.

cfDNA analysis

cfDNA analysis was conducted via Guardant360, an NGS assay examining 73 genes using massively parallel and deep sequencing (Supplementary Table S1). This test has an analytic sensitivity of 0.1% mutant allele fraction, specificity above 99.9%, clinical sensitivity of 85.0%, average single read depth of 15,000 molecules, and average molecule count of 8,000 molecules (10). Guardant360 has been validated with orthogonal tumor tissue-based genotyping (14). The analytic reports provided by Guardant360 classify detected mutations as pathogenic or unlikely to be pathogenic, and also provide recommendations for matched therapies. This assay was ordered as a clinical assay for patients, and likewise covered either by insurance and/or support from Guardant360.

Tumor tissue genotyping

A chart review of tumor tissue genotyping results from archival samples was conducted. An institutional NGS assay that evaluates up to 91 genes for copy-number changes and mutations (SNaPshot) was utilized (Supplementary Table S1; ref. 7). This anchored multiplex PCR assay detects insertions and deletions, gene rearrangements, single-nucleotide variants, and copy-number changes at allelic frequencies of 5% or higher. This platform has 100% analytical specificity and 100% analytic sensitivity (7).

Designation of matched therapy

For the analyses described in this study, we primarily utilized the classifications provided by Guardant360 (master list from January 2018 obtained from Guardant360 for assessment) to determine whether the mutations identified were actionable, and if so, whether the patient's subsequent treatment (at any time after the identification of an actionable mutation) was matched to the actionable mutation, for both the tissue and cfDNA genotyping cohorts. A few modifications were made to the list provided by Guardant360 to accommodate targeted clinical trials, selective estrogen receptor degraders (SERD) for ESR1 mutations, and DNA-damaging chemotherapy (in addition to PARP inhibition) for mutations in the DNA damage repair pathway. Supplementary Table S1A summarizes the alterations and corresponding related genotype-directed treatment. An important caveat is that the Guardant360 designations change with time as new data emerge, and for this study we used the related matched therapies at the time of data cutoff. Clinical trials were included if the targeted therapy on-trial was relevant to the actionable mutation, as indicated in Supplementary Table S1A. The additional alterations included in the institutional tumor tissue NGS assay that are not included in Guardant360 are not particularly relevant to MBC and/or do not have any obvious matched therapies. Only a few mutations included in Guardant360 were not found in the institutional tumor tissue NGS assay, and were generally not particularly relevant for MBC and/or do not have any designated matched therapy (Supplementary Table S1).

Guardant360 relies upon variant interpretation performed by the QCI Precision Insights team. Variants are classified as actionable or variants of unknown significance based on assessment of the literature and public databases. Curation resources include, but are not limited to, the following: COSMIC, cBioPortal for Cancer Genomics, UniProt, Integrative Genomics Viewer, the scientific and medical literature as cataloged by PubMed (RRID: SCR_004846), IARC Knowledgebase (for TP53), Clinicaltrials.gov, NCI Drug dictionary, Rxlist.com, Drugs@FDA, Therapeutic Target Knowledgebase, NCCN.org, and Cancer.gov. Linkage of variants with potential therapies are performed on the basis of available drug agency approvals, practice guidelines, and analysis of the literature. Levels of evidence are assigned to each gene–drug–disease linkage based on the guidelines published by Li and colleagues (2017; ref. 15).

Statistical analyses

The incidence rates of actionable mutations in tumor tissue genotyping and cfDNA cohorts in patients undergoing either test were compared using the Pearson χ^2 test. Notably, this was an exploratory analysis as this study was not a prospective randomized trial. Univariate and multivariable Cox regression analyses were used to determine the HR of matched therapy status on progression-free survival on the first therapy after testing and OS in tumor tissue genotyping and cfDNA subgroups. Tumor subtype was included in multivariable Cox regression as a nominal confounder (HR^+, $HER2^+$, and TNBC), as well as age, presence of visceral metastases at the time of genotyping, and brain metastases. For all analyses, $P < 0.05$ was considered to be statistically significant. All analyses were done using Stata (StataCorp. 2019. Stata Statistical Software: Release 16. StataCorp LLC; RRID: SCR_012763).

This research was conducted in accordance with recognized ethical guidelines, including the Declaration of Helsinki, and the retrospective review was conducted on the basis of an IRB-approved institutional protocol. Patients provided written informed consent prior to sending tumor tissue and/or plasma-based genotyping.

Results

Patients undergoing either cfDNA or tumor tissue genotyping

Patient demographics

Between January 2016 and December 2017, 252 patients with MBC underwent cfDNA testing and 118 patients had tumor tissue genotyping performed on a tumor specimen (Supplementary Fig. S1A). The results for 30 patients who received both forms of testing are described in a later section. **Table 1** summarizes the demographics of these cohorts. Patients who had cfDNA testing versus tumor tissue genotyping had similar median age, subtype distribution, type of metastatic disease (mainly recurrent disease), and ECOG performance status. Patients undergoing cfDNA testing had received a median of 0 prior chemotherapy regimens or 1 prior hormone therapy, whereas patients undergoing tumor tissue genotyping had a median of 0 prior chemotherapies or hormone therapies, and these numbers remained the same for the matched and nonmatched subgroups of both cohorts. Those receiving matched therapy in both cohorts had a similar median number of prior therapies in the metastatic setting preceding the initiation of matched therapy.

Actionable mutations and matched therapy in cfDNA and tumor tissue genotyping cohorts

Of 252 patients who underwent cfDNA testing, 232 (92%) had detectable mutations (20 patients had no detectable mutations), and 196 (78%) had actionable mutations (**Fig. 1A**). Of 118 patients who underwent tumor tissue genotyping, 90 (76%) had detectable mutations (18 patients had no detectable mutations), and 59 (50%) had actionable mutations (**Fig. 1A**). A significantly higher proportion of patients undergoing cfDNA testing had actionable mutations compared with those undergoing tumor tissue genotyping ($P < 0.0005$). The median number of actionable mutations by cfDNA analysis was 2 (range, 0–10), and by tumor tissue genotyping was 1 (range, 0–5). Notably, failure of the tumor tissue genotyping assay was seen in 12 cases (10%).

Ultimately, 86 patients (34%) received matched therapy based on cfDNA results (**Fig. 1B**), while 13 patients (11%) received matched therapy based on tissue genotyping results (**Fig. 1B**). On the basis of cfDNA analysis, 36 of 252 patients (14.2%) received the matched therapy in a clinical trial. Matched therapies based on cfDNA analysis (collectively, on-trial and off-trial, breakdown in **Fig. 2A**) included CDK4/6 inhibitors, SERDs, PI3K inhibitors, HER2-directed therapy, mTOR inhibitors, DNA-damaging chemotherapy, AKT inhibitors, PARP inhibitors, AR antagonists, and an FGFR inhibitor. When evaluated by disease subtype, receipt of matched therapy in patients with actionable mutations in cfDNA occurred in 48% (65/136) of HR^+ MBC, 75% (12/16) of $HER2^+$ MBC, and 28% (9/32) of TNBC. Notably, 3 patients without known $HER2^+$ disease were treated with HER2-directed therapies based on the presence of *ERBB2* mutations in cfDNA. The median number of therapies for MBC prior to the receipt of matched therapy based on cfDNA actionable mutations was 2 (range, 0–11), but about half of the patients had 0–1 prior lines of therapy.

Overall, 9 of 118 patients (8%) received the matched therapy on a clinical trial based on tumor tissue genotyping. Matched therapies based on tissue genotyping (collectively, on-trial and off-trial, breakdown in **Fig. 2B**) included mTOR inhibitors, SERDs, PI3K inhibitors, DNA-damaging chemotherapy, and AKT inhibitors. When evaluated by disease subtype, receipt of matched therapy in patients with actionable tissue mutations occurred in 30% of HR^+ MBC (11/37), one of three $HER2^+$ MBC cases, and one of seven TNBC cases. The median number of therapies for MBC prior to the receipt of matched therapy based on the presence of actionable mutations in tissue genotyping was 2 (range, 0–9), but about half of the patients had 0–1 prior therapies.

Table 1. Characteristics of patients undergoing cfDNA or tumor tissue genotyping testing.

Characteristic	cfDNA ($n = 252$)	Tumor tissue genotyping ($n = 118$)
Median age at MBC diagnosis	55	58
Subtype		
HR^+	186 (74%)	83 (70%)
$HER2^+$	22 (8.7%)	10 (8.5%)
TNBC	44 (17%)	25 (21%)
Type of MBC		
De novo	36 (14%)	23 (19%)
Recurrence	216 (86%)	95 (81%)
Baseline performance status (ECOG)		
	0: 113 (45%)	0: 59 (50%)
	1: 109 (43%)	1: 46 (39%)
	2: 12 (5%)	2: 8 (6.8%)
	3: 1 (0.4%)	3: 1 (0.8%)
	4: 0 (0%)	4: 0 (0%)
Detectable mutation	232 (92%)	90 (76%)
HR^+	171	58
$HER2^+$	19	10
TNBC	42	22
Actionable mutation	196 (78%)	59 (50%)
Median number of actionable mutations	2	1
Matched therapy	86 (34%)	13 (11%)
HR^+	65	11
$HER2^+$	12	1
TNBC	9	1
Median number of prior lines of therapy in patients receiving matched therapy	2 (Range: 0–11)	2 (Range: 0–9)
Matched therapy		
Trial	36 (14.2%)	9 (8%)
Off-trial	50 (19.8%)	4 (3%)

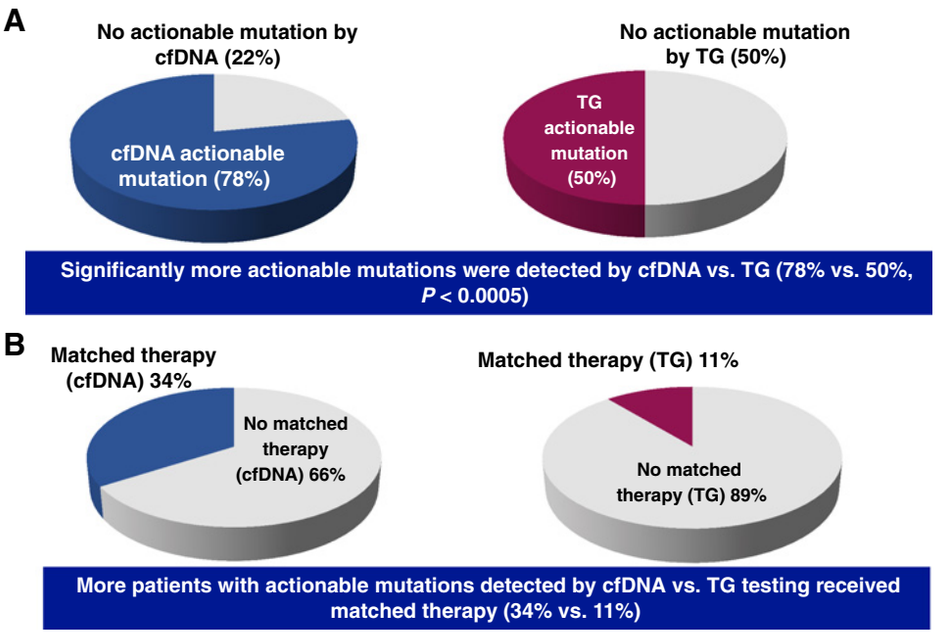

Figure 1.

A, Actionable mutations detected by cfDNA versus tumor tissue genotyping (TG). **B,** Matched therapy in patients with actionable mutations detected by cfDNA or TG.

As a subset analysis, we analyzed the impact of tumor genotyping on the selection of matched therapies by excluding FDA-approved standard therapies at the time of testing. Of note, at the time of conducting this study, PI3K inhibitors were not approved by the FDA, and because the approval of the PI3K inhibitor, alpelisib, is based on a *PIK3CA* mutation detected by tumor genotyping (5, 16), we did not exclude PI3K inhibitors in these analyses, but as shown in **Fig. 2**, PI3K inhibition did not account for a significant proportion of treatment in either cohort. After excluding patients who received CDK 4/6 inhibitors, HER2-directed therapy (if known $HER2^+$ by standard HER2 IHC and FISH testing of tumor) and DNA-damaging chemotherapy, 35.5% (54/152) of patients with cfDNA actionable mutations received matched therapy and 26.1% (12/46) of patients with actionable mutations by tumor tissue genotyping received matched therapy.

While many patients received matched therapy, some patients with an actionable mutation did not receive matched therapy. **Table 2** summarizes common reasons why patients did not receive matched therapy, which often included receiving alternate treatments, prior matched therapy, undergoing standard-of-care first-line therapy, absence of targeted therapy trials, advanced disease, heavily pretreated disease, and consideration of hospice.

Selection of matched therapy based on cfDNA or tumor tissue genotyping in patients undergoing both tests

We evaluated the relative utility of cfDNA and tumor tissue genotyping in the selection of matched therapy in patients undergoing both platforms of testing (Supplementary Fig. S1B). Thirty patients who underwent both types of testing after MBC diagnosis between January 2016 and October 2017 were identified. The demographics of these patients are summarized in Supplementary Table S2. Notably, 83.3% of patients underwent simultaneous cfDNA and tumor tissue genotyping, and 16.7% underwent sequential testing. More patients had actionable mutations detected by cfDNA (80%) versus tumor tissue genotyping (63.3%; **Fig. 3A**), and this finding was upheld when confined only to the patients undergoing simultaneous cfDNA and tumor tissue genotyping. In addition, 36.7% of patients had ≥1 concordant mutation detected by cfDNA and tumor tissue genotyping. Failure of the tumor tissue genotyping assay occurred in 2 patients (6.7%). **Figure 3D** depicts the spectrum of actionable mutations detected by cfDNA versus tumor tissue genotyping in this cohort. Twelve of 24 (50%) of patients with actionable cfDNA mutations went on to receive matched therapy compared with 7 of 19 (36.8%) of patients with actionable tumor tissue genotyping results (**Fig. 3B**). Altogether, 12 of 30 (40%) of patients received matched therapy, 7 based on cfDNA and tumor tissue genotyping results (common mutations seen in both assays in five cases, differing actionable mutations in the same pathway enabling matched therapy in one case, and differing mutations enabling varying matched therapies in one case), and five based on cfDNA results independently (Supplementary Table S3 summarizes actionable mutations and matched therapy received in these cases). Tumor tissue genotyping results alone did not inform the selection of matched therapy (**Fig. 3C**). Advanced disease burden and preference for standard-of-care therapy again dissuaded the use of matched therapy.

Clinical outcomes of patients receiving matched therapy

The impact of matched therapy on OS is illustrated by Kaplan–Meier curves in **Fig. 4A** for patients who underwent cfDNA testing and in **Fig. 4B** for patients who underwent tumor tissue genotyping. In the cfDNA testing cohort with actionable mutations, OS was significantly better for patients who received matched therapy compared with those who received nonmatched therapy (log-rank test $P = 0.002$). The 6-month and 12-month OS was 93% and 73%, respectively, for the matched therapy subgroup versus 72% and 53%, respectively, for the nonmatched therapy subgroup. The advantage of matched therapy was confirmed after adjusting for confounding effects of age, disease subtype, presence of visceral metastases at cfDNA testing, and brain metastases at the time of cfDNA testing, by multivariable Cox regression (HR = 0.46, 95% CI: 0.26–0.83, $P = 0.010$). Within the cohort of patients with actionable mutations on cfDNA testing who received matched therapy, the number of prior lines of treatment before receipt of the matched therapy did not impact OS. The OS in the cohort of patients who underwent tumor tissue genotyping and were found to

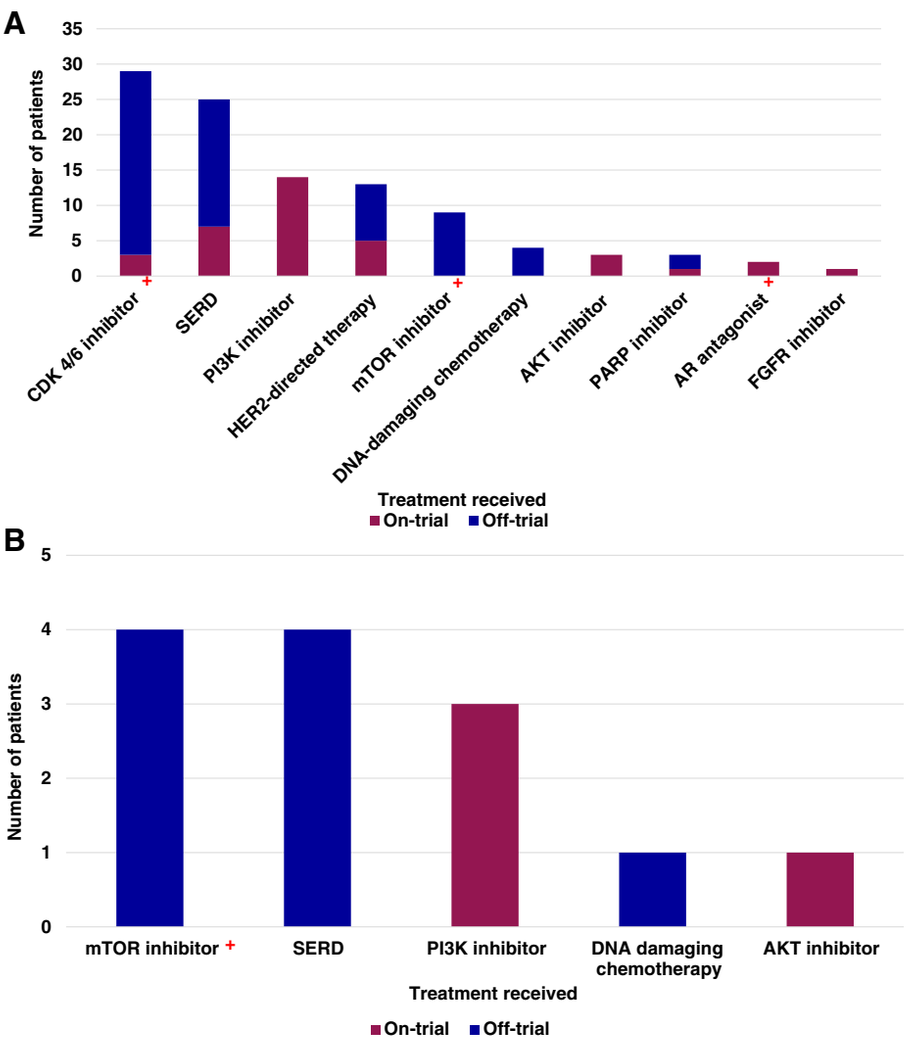

Figure 2.

A, Matched therapies received on the basis of cfDNA actionable mutation results. **B,** Matched therapies received on the basis of tissue genotyping (TG) actionable mutation results. + denotes treatment for which current evidence in 2020 does not support genomic biomarker predictors of response to therapy. However, these patients were included in this study based on the designation of treatment matching described in the methodology.

have actionable mutations was not different between the matched and nonmatched subgroups (**Fig. 4B**, log-rank test $P = 0.71$). A small number of cases (35) and events (13) in this cohort did not allow for adjustment of confounders.

Table 2. Common reasons for not receiving matched therapy.

cfDNA (# of patients)	Tumor tissue genotyping (# of patients)
Receiving alternate treatment (6)	Receiving alternate treatment (6)
Prior matched therapy (10)	Prior matched therapy (1)
No targeted trial available (1)	On first line therapy (10)
Advanced disease (29)	Advanced disease (9)
Multiple prior therapies (12)	Multiple prior therapies (2)
Hospice (5)	Hospice (2)
Test failure (0)	Tumor tissue genotyping failed (12)

Note: Reasons for not receiving matched therapy in cfDNA and tumor tissue genotyping groups in patients with MBC undergoing either test found to have an actionable mutation (for those patients for whom data were available).

After exclusion of CDK4/6 inhibitors, DNA-damaging chemotherapy, and HER2-directed therapy (if $HER2^{+}$), receipt of matched therapy versus nonmatched therapy for patients with actionable mutations in cfDNA was still statistically significantly associated with an improvement in OS (HR 0.43, 95% CI: 0.22–0.83, $P = 0.012$) as shown in **Fig. 4C** (median OS for matched group not reached, nonmatched median OS 11.3 months, log-rank $P = 0.0047$), and this finding remained statistically significant on multivariable Cox regression analysis after adjusting for visceral metastases, brain metastases, age, and disease subtype (HR 0.49, 95% CI: 0.25–0.99, $P = 0.046$).

Discussion

In this comprehensive analysis of patients with MBC, we observed that both cfDNA and tumor tissue genotyping may identify a significant proportion of patients with actionable mutations, across breast cancer subtypes, for consideration of matched therapy. We observed a higher proportion of actionable mutations and application of matched therapy in patients undergoing cfDNA testing than those undergoing tumor tissue genotyping, although this was not a randomized controlled prospective trial, and was an exploratory analysis. Patients who

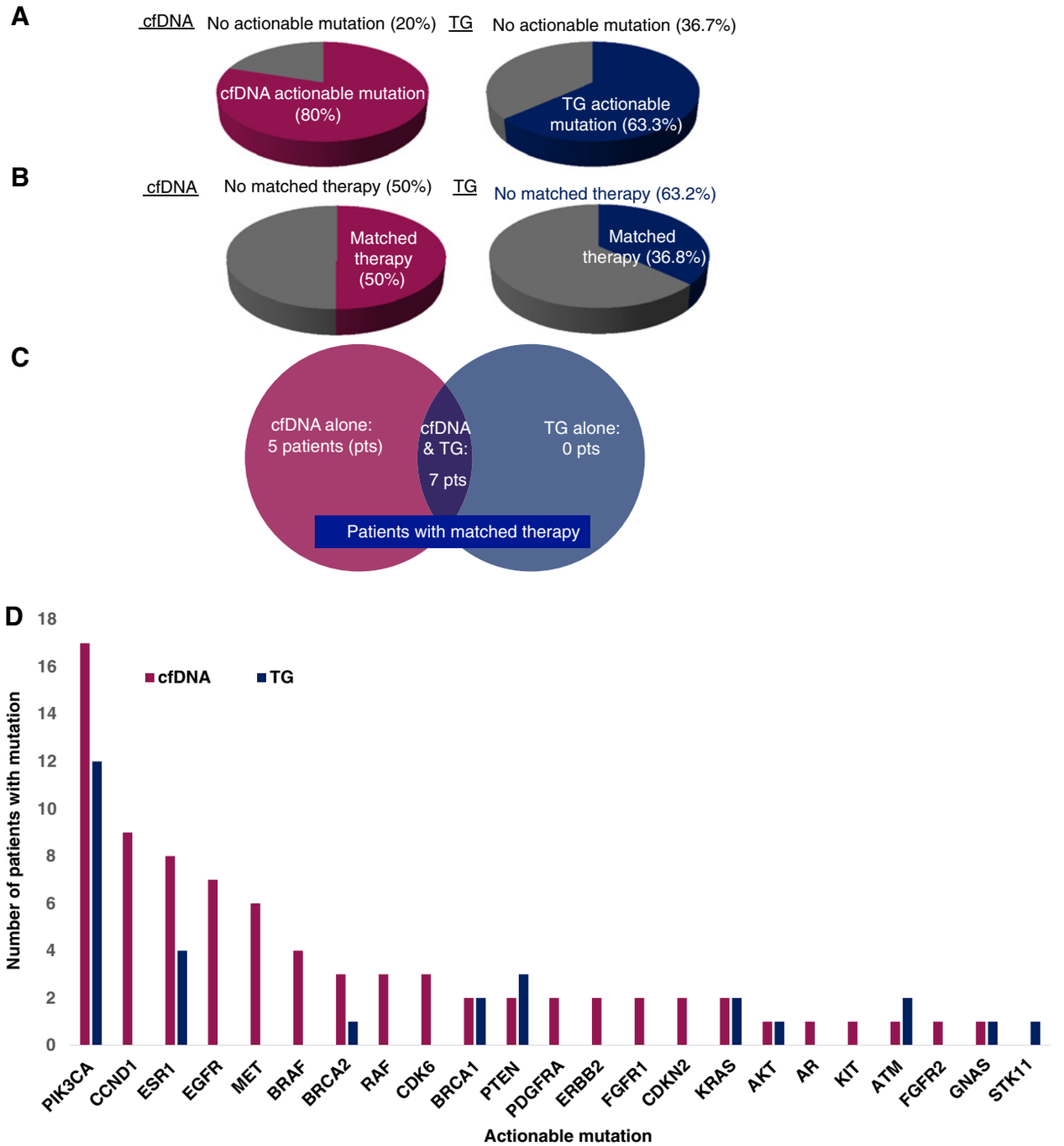

Figure 3.

A, Actionable mutations detected by cfDNA versus tissue genotyping (TG) testing in patients undergoing both tests. **B,** Matched therapy based on cfDNA or TG actionable mutation results in patients undergoing both tests. **C,** Relative utility of cfDNA versus TG in the selection of matched therapy in patients undergoing both tests. **D,** Spectrum of actionable mutations detected by cfDNA or TG in patients undergoing both tests.

had actionable mutations detected by cfDNA who went on to receive matched therapy had a better OS than those who received nonmatched therapy post cfDNA testing, and this finding remained significant on correction for receptor subtype, age, presence of visceral metastases, and brain metastases.

Our findings that cfDNA identified high rates of actionable mutations and led to significant application of matched therapy may be explained in part by increased sensitivity of blood-based assays, similar to what has been observed in lung cancer (11). Prior work at our institution in patients with MBC has also demonstrated that cfDNA

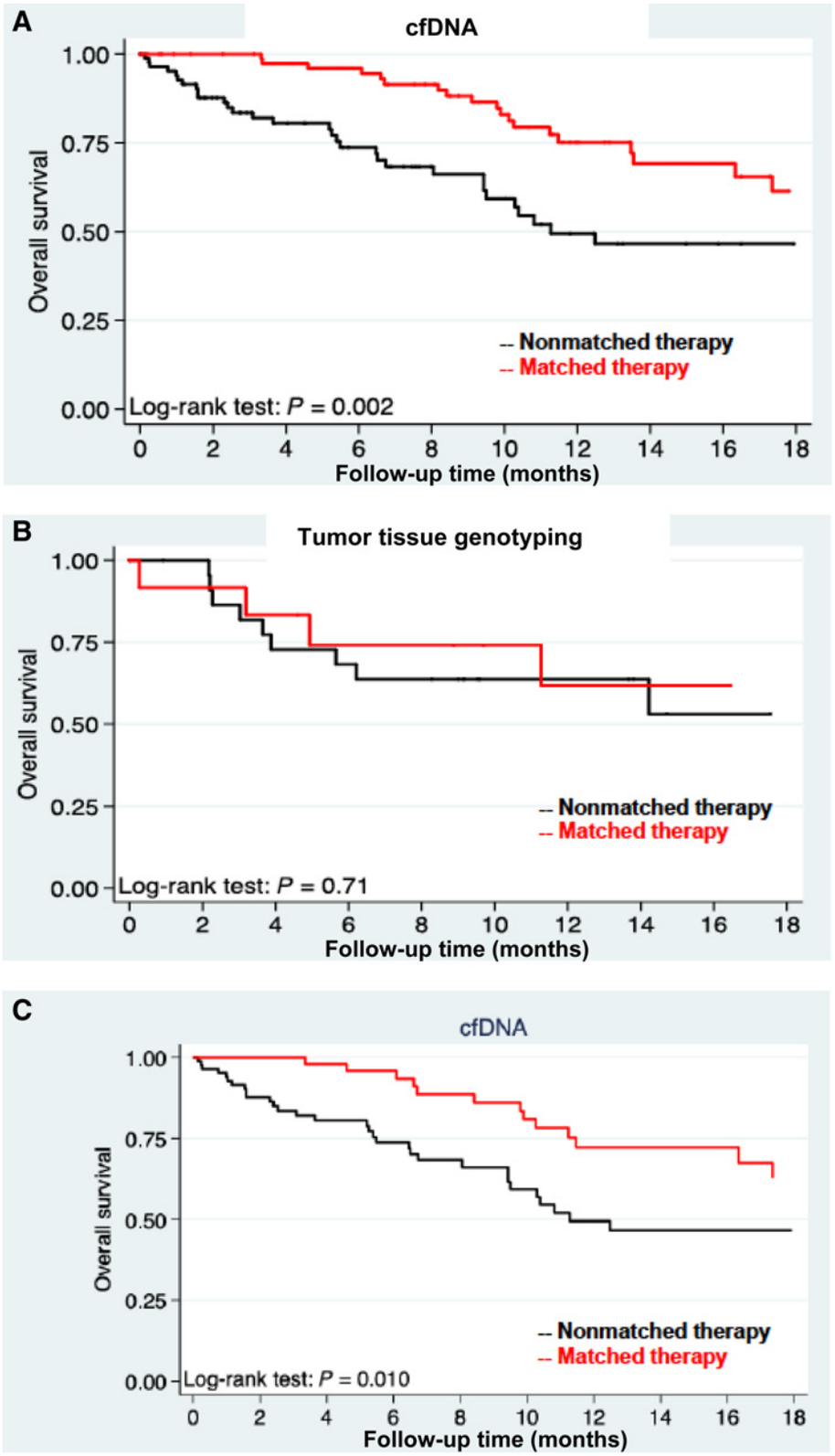

Figure 4.

A, OS in patients with matched therapy versus nonmatched therapy based on cfDNA actionable mutation results. Matched therapy was associated with better OS in patients undergoing cfDNA testing with actionable mutations (HR 0.41, 95% CI: 0.23–0.73, $P = 0.002$). **B,** OS in patients with matched therapy versus nonmatched therapy based on tumor tissue genotyping actionable mutations. In patients undergoing tumor tissue genotyping, OS was not significantly different in matched versus nonmatched therapy cohorts, which may be due in part to the small sample size of patients undergoing tumor tissue genotyping with actionable mutations receiving matched therapy. **C,** OS in patients with matched therapy versus nonmatched therapy based on cfDNA actionable mutation results, after exclusion of patients receiving CDK4/6 inhibitors, DNA damaging chemotherapy and HER2-directed therapy (if known $HER2^{+}$) as matched therapies. Matched therapy was associated with better OS in patients undergoing cfDNA testing with actionable mutations (after exclusion of standard therapies; HR 0.43, 95% CI: 0.22–0.83, $P = 0.012$).

identified more actionable alterations than tumor tissue genotyping (17). In addition, timing of testing in a patient's disease course with MBC, and the failure of the tumor genotyping assay in about 10% of cases, a similar failure rate to what has been observed in other studies (18), may have contributed. However, it is intriguing that in a cohort of patients who had both cfDNA and tumor tissue genotyping results available, the selection of matched therapy was more often determined by the cfDNA results, and cfDNA results also independently informed the selection of matched therapy unlike tumor tissue genotyping. This finding is worth exploring further in future clinical studies, given that cfDNA testing avoids the risks and burdens associated with biopsy of tumor tissue. A limitation of this analysis is that the gene coverage and depth of the Guardant360 and institutional tissue NGS assays differ somewhat. However, on closer evaluation of discrepant genes between our institutional tissue NGS assay and the Guardant360 assay, these appear to be genes that do not play a significant role in MBC and/or for which no actionable therapies are available (Supplementary Table S1).

Another limitation of this work is that the assessment of therapy matching was primarily made based on Guardant360's designations, which are supported by varying levels of evidence. Consequently, in certain instances, the utilization of the assay and linkage to a genotype-directed therapy may not be clearly associated with prediction of benefit to the therapy. Furthermore, as the treatment landscape and FDA approvals are a moving target, over the course of this retrospective study, the availability of genotype-directed therapies may have varied, with more FDA approvals accrued over time. However, given that this study was an analysis conducted over a 2-year time period, this effect is mitigated. With the advent of newer targeted treatments, the results of this type of study might vary in a future assessment.

We observed relatively high rates of selection of matched therapy (34% of patients who had cfDNA testing, and 11% of patients who had tumor tissue genotyping testing). On exclusion of patients who received therapies that are standard of care and might have been considered without the use of a genotyping assay, 35.5% of patients with cfDNA actionable mutations received matched therapy and 26.1% of patients with actionable mutations on tumor tissue genotyping received matched therapy. Notably, in this latter analysis, we excluded CDK 4/6 inhibitors from the designation of matched therapy because this class of drugs is often used as a standard-of-care option for $HR^+/HER2^-$ MBC, and because the suggestion of a CDK 4/6 inhibitor as matched therapy based on Guardant360's classification was established by the presence of amplifications or alterations in *CDK 4, CDK 6, CCND1, CCND2*, or *CDKN2A*. Recent research has suggested that there are not clear biomarkers to predict response to CDK 4/6 inhibition, as this class of therapy appears to be beneficial across biomarker subtypes (19). While we excluded FDA-approved therapies in this cohort analysis, a limitation of this work is that some therapies such as SERDs might potentially have been considered by clinicians even in the absence of the utilization of genotype-directed treatments.

A significant proportion of patients in both cohorts received matched treatment on clinical trials (14.2% of patients undergoing cfDNA testing and 8% of patients undergoing tumor tissue genotyping), which may help explain the relatively high rates of treatment matching. A known barrier to genotype-directed therapy is obtaining the appropriate targeted treatment, and access to a clinical trial at the same institution can help improve rates of therapy matching. Additional reasons for not receiving matched therapy included obtaining standard-of-care treatment (but perhaps the matched therapy could be considered later in the patient's disease course), the lack of easy access to some targeted treatments, and the presence of advanced or heavily pretreated disease, similar to what has been observed in other studies (18, 20, 21).

Patients with an actionable cfDNA mutation who went on to receive matched versus nonmatched therapy had better OS, even after controlling for disease subtype, age, visceral disease, and presence of brain metastases, with the caveat that our definition of matched therapy was based primarily on the Guardant360 treatment classifications, which are supported by varying levels of evidence, as described earlier. However, a similar improvement in OS was observed after removal of standard nongenotyping biomarker selective therapies such as CDK4/6 inhibitors, DNA-damaging chemotherapy, and HER2-directed therapy (if known $HER2^+$ tumor), and this finding remained significant after controlling for disease subtype, age, visceral disease, and presence of brain metastases. Possible explanations include a true survival benefit to receiving matched therapy during a patient's MBC course, and increased treatment options for patients who were able to receive matched therapy. It is possible that for patients in whom an actionable mutation is identified, particularly if this is a driver mutation, the application of matched therapy helps address tumor biology. Further studies are needed to validate this novel finding, as a limitation of our work is the retrospective nature of our analyses and cohorts at a single institution, and also that some patients may not have received matched therapy due to advanced disease, multiple prior therapies, or hospice care. The benefit of genotype-directed therapy is likely dependent on disease subtype, genotyping platform, mutations identified, and patient demographics, which we considered in our multivariable analysis. However, the degree of matching of the actionable mutation and therapy, mutant allele frequency, presence of additional mutations, number of prior treatments, and sequencing of treatment may also play a role, and the impact of these factors needs to be determined in future studies. Ultimately, randomized prospective data are needed from initiatives such as the TAPUR (22) and NCI-MATCH (20) studies. These studies will help validate the approach of genotype-directed therapy, similar to the plasmaMATCH study, which has demonstrated the feasibility of matching patients with MBC and actionable mutations to selected targeted therapies (23). These endeavors are particularly important as more oncologists are obtaining tumor genotyping tests (24), necessitating the validation of this approach for cost-effectiveness considerations and the development of practice guidelines for oncology care. One prospective trial (SAFIR01) utilized biopsy samples in patients with metastatic breast cancer for targeted therapy trial matching, and identified genomic alterations in 46% of patients, with personalized therapy in 13%, which are similar to our findings in patients who underwent tumor tissue genotyping (25). Additional clinical questions that need to be answered in future research include whether combination therapy may be more successful than single-agent therapy in patients found to have multiple actionable mutations, and the best time frame to initiate genotype-directed therapy. In the interim, molecular tumor boards may aid in the interpretation of cfDNA and tumor tissue genotyping results, and the selection of matched therapy, taking into account the genomic data, the clinical context, and growing landscape of novel targeted therapies.

In conclusion, in this retrospective study of patients with MBC, we discerned that tissue- and plasma-based genotyping assays can identify a large number of actionable mutations, which may inform the selection of genotype-directed therapy. Plasma-based assays may identify high proportions of actionable mutations, and the selection of matched therapy may be associated with an improvement in

outcomes, although further prospective research is needed to validate this finding.

Authors' Disclosures

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Authors' Contributions

N. Vidula: Conceptualization, data curation, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **A. Niemierko:** Data curation, software, formal analysis, validation, investigation, methodology, writing–review and editing. **G. Malvarosa:** Data curation, writing–review and editing. **M. Yuen:** Data curation, writing–review and editing. **J. Lennerz:** Validation, writing–review and editing. **A.J. Iafrate:** Writing–review and editing. **S.A. Wander:** Writing–review and editing. **L. Spring:** Writing–review and editing. **D. Juric:** Writing–review and editing. **S. Isakoff:** Writing–review and editing. **J. Younger:** Writing–review and editing. **B. Moy:** Writing–review and editing. **L.W. Ellisen:** Writing–review and editing. **A. Bardia:** Conceptualization, data curation, formal analysis, validation, investigation, methodology, writing–review and editing.

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References

1. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. The Journal of clinical investigation 2011;121:2750–67.
2. Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, Fuqua SA, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res 2015;21:1688–98.
3. Bertucci F, Ng CKY, Patsouris A, Droin N, Piscuoglio S, Carbuccia N, et al. Genomic characterization of metastatic breast cancers. Nature 2019;569:560–4.
4. Oza AM, Tinker AV, Oaknin A, Shapira-Frommer R, McNeish IA, Swisher EM, et al. Antitumor activity and safety of the PARP inhibitor rucaparib in patients with high-grade ovarian carcinoma and a germline or somatic BRCA1 or BRCA2 mutation: Integrated analysis of data from Study 10 and ARIEL2. Gynecol Oncol 2017;147:267–75.
5. Andre F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med 2019;380:1929–40.
6. Rodon J, Soria JC, Berger R, Miller WH, Rubin E, Kugel A, et al. Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. Nat Med 2019;25:751–8.
7. Zheng Z, Liebers M, Zhelyazkova B, Cao Y, Panditi D, Lynch KD, et al. Anchored multiplex PCR for targeted next-generation sequencing. Nat Med 2014;20: 1479–84.
8. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. Cancer Discov 2014;4:650–61.
9. Aggarwal C, Thompson JC, Black TA, Katz SI, Fan R, Yee SS, et al. Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. JAMA Oncol 2019;5: 173–80.
10. Lanman RB, Mortimer SA, Zill OA, Sebisanovic D, Lopez R, Blau S, et al. Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. PLoS One 2015; 10:e0140712.
11. Leighl NB, Page RD, Raymond VM, Daniel DB, Divers SG, Reckamp KL, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. Clin Cancer Res 2019;25:4691–700.
12. Gagan J, Van Allen EM. Next-generation sequencing to guide cancer therapy. Genome Med 2015;7:80.
13. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol 2014;32:579–86.
14. Odegaard JI, Vincent JJ, Mortimer S, Vowles JV, Ulrich BC, Banks KC, et al. Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. Clin Cancer Res 2018;24: 3539–49.
15. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the association for molecular pathology, american society of clinical oncology, and college of american pathologists. J Mol Diagn 2017;19:4–23.
16. U.S. Food and Drug Administration. FDA approves alpelisib for metastatic breast cancer; 2020. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-alpelisib-metastatic-breast-cancer.
17. Malvarosa G, Spring L, Juric D, Moy B, Bardia A. Comparison of genotyping results from tissue and circulating DNA (ctDNA) in patients with metastatic breast cancer [abstract]. In: Proceedings of the 2016 San Antonio Breast Cancer Symposium; 2016 Dec 6–10; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2017;77(4 Suppl):Abstract nr P1-05-05.
18. Goncalves A, Bachelot T, Lusque A, Arnedos M, Campone M, Bieche I, et al. High-throughput genome analysis and therapeutic decision for patients with HER2-negative metastatic breast cancer: first feasibility and molecular results of the randomized phase II study SAFIR02 BREAST (UCBG-0105/1304) [abstract]. In: Proceedings of the 2016 San Antonio Breast Cancer Symposium; 2016 Dec 6–10; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2017;77(4 Suppl): Abstract nr PD1-08.
19. Finn RS, Liu Y, Zhu Z, Martin M, Rugo HS, Dieras V, et al. Biomarker analyses of response to cyclin-dependent kinase 4/6 inhibition and endocrine therapy in women with treatment-naive metastatic breast cancer. Clin Cancer Res 2020;26: 110–21.
20. Johnson DB, Dahlman KH, Knol J, Gilbert J, Puzanov I, Means-Powell J, et al. Enabling a genetically informed approach to cancer medicine: a retrospective evaluation of the impact of comprehensive tumor profiling using a targeted next-generation sequencing panel. Oncologist 2014;19:616–22.
21. Meric-Bernstam F, Brusco L, Shaw K, Horombe C, Kopetz S, Davies MA, et al. Feasibility of large-scale genomic testing to facilitate enrollment onto genomically matched clinical trials. J Clin Oncol 2015;33:2753–62.
22. TAPUR: Testing the use of Food and Drug Administration (FDA) approved drugs that target a specific abnormality in a tumor gene in people with

advanced stage cancer (TAPUR). Available from: https://clinicaltrialsgov/ct2/show/NCT02693535.

23. Turner N, Kingston B, Kilburn L, Kernaghan S, Wardley AM, Macpherson I, et al. Results from the plasmaMATCH trial: a multiple parallel cohort, multi-centre clinical trial of circulating tumor DNA testing to direct targeted therapies in patients with advanced breast cancer (CRUK/15/010) [abstract]. In: Proceedings of the 2019 San Antonio Breast Cancer Symposium; 2019 Dec 10–14; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res 2020;80(4 Suppl):Abstract nr GS3-06.
24. Freedman AN, Klabunde CN, Wiant K, Enewold L, Gray SW, Filipski KK, et al. Use of next-generation sequencing tests to guide cancer treatment: results from a nationally representative survey of oncologists in the United States. JCO Precision Oncology 2018, November 13.
25. Andre F, Bacehlot T, Commo F, Campone M, Arnedos M, Dieras V, et al. Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicenre, prospective trial (SAFIR01/UNICANCER). Lancet Oncol 2014;15:267–74.