

DefineMBC™: A clinical experience summary from 25 United States-based cancer centers on the clinical utility of a comprehensive liquid biopsy in metastatic breast cancer

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ABSTRACT

Breast cancer is primarily diagnosed by tissue biopsy, a gold-standard technique that allows accurate assessment of tumor histology and comprehensive molecular profiling of biomarkers that help inform treatment decisions. However, there are several challenges regarding tissue biopsies in accurately characterizing metastatic breast cancer (MBC). First, they often do not assess tumor heterogeneity and typically reflect the biology of only a few singular accessible lesions. Furthermore, invasiveness and cost hinder their use for monitoring cancer evolution over time. Noninvasive liquid biopsies have emerged as viable diagnostic tools that facilitate longitudinal cancer profiling. While useful, most of the currently available liquid biopsies are limited to assessing cell-free DNA (cfDNA). Consequently, they are unable to determine variations in important biomarkers of breast cancer, such as hormone receptor expression, which are critical for developing treatment plans. To overcome these limitations and to expand the scope of liquid biopsies, DefineMBC was developed as the first and only blood-based liquid biopsy that can assess both tumor biomarkers from circulating tumor cells (CTCs), and genetic variations from CTCs via single-cell genomics as well as from cfDNA.

The clinical utility of DefineMBC was assessed in a Clinical Experience Program across 25 different United States-based cancer centers at 34 locations with 43 recruited oncologists. Participating physicians used DefineMBC liquid biopsy to test patients with MBC; subsequently, case reviews and a survey were conducted to analyze their clinical and product experiences. Herein we provide a topline overview of the 172 DefineMBC reports generated during the program, as well as the results of the survey undertaken by 17 of the participating physicians.

Overall, CTCs were detected in 70% of DefineMBC tests. This enabled a comprehensive assessment of estrogen receptor and human epidermal growth factor receptor 2 (HER2) protein expression in these samples, resulting in 41% being deemed HER2 actionable. This contrasts with only 10% HER2-actionable samples indicated by the last available tissue biopsy. Combined with the analysis of CTC single-cell genomics and circulating tumor DNA genomic alterations, DefineMBC tests provided actionable information in 96% of the samples analyzed. Coupled with its ease of use and noninvasiveness, 82% of survey respondents agreed that DefineMBC is a valuable clinical tool in informing and improving patient care.

INTRODUCTION

Epidemiology of metastatic breast cancer (MBC)

Globally, breast cancer is the most frequently diagnosed malignancy among women.¹ In 2020, an estimated 279,100 new cases of breast cancer were diagnosed in the United States (US) with 42,690 deaths reported.² In the US, while 63% of breast cancers are confined to the breast at diagnosis (5-year survival: 99%), 6% of patients present with de novo metastatic disease (eg, stage IV) and an intact primary tumor (5-year survival: 29%).³ Furthermore, approximately 30% of women diagnosed with early-stage breast cancer will relapse and develop metastatic disease.⁴ While MBC remains incurable, advances in early diagnosis and comprehensive treatment strategies have improved long-term outcomes for patients through the tailoring of regimens to a patient's disease status, wishes, and to the specific cancer subtype.⁵

Current standard-of-care diagnostic workup of MBC

Treatment strategies for metastatic breast tumors depend on the subtype and biology of the tumor. The National Comprehensive Cancer Network's (NCCN's) guidelines recommend that patients with metastatic disease at presentation or first recurrence should be biopsied as a part of the initial workup.⁶ The current standard of care for recurrent/metastatic disease is to attempt a tissue biopsy of 1 lesion even if multiple lesions are available. However, the use of repeat tissue biopsies to guide treatment decisions for patients with progressive MBC is not currently established. Neither the NCCN⁶ nor the American Society of Clinical Oncology guidelines⁷ recommend rebiopsy of a metastatic lesion for reassessment, and it is not routinely performed in clinical practice.⁸

Importance of hormone receptor (HR) status in guiding treatment and the challenges of assessing status in patients with MBC

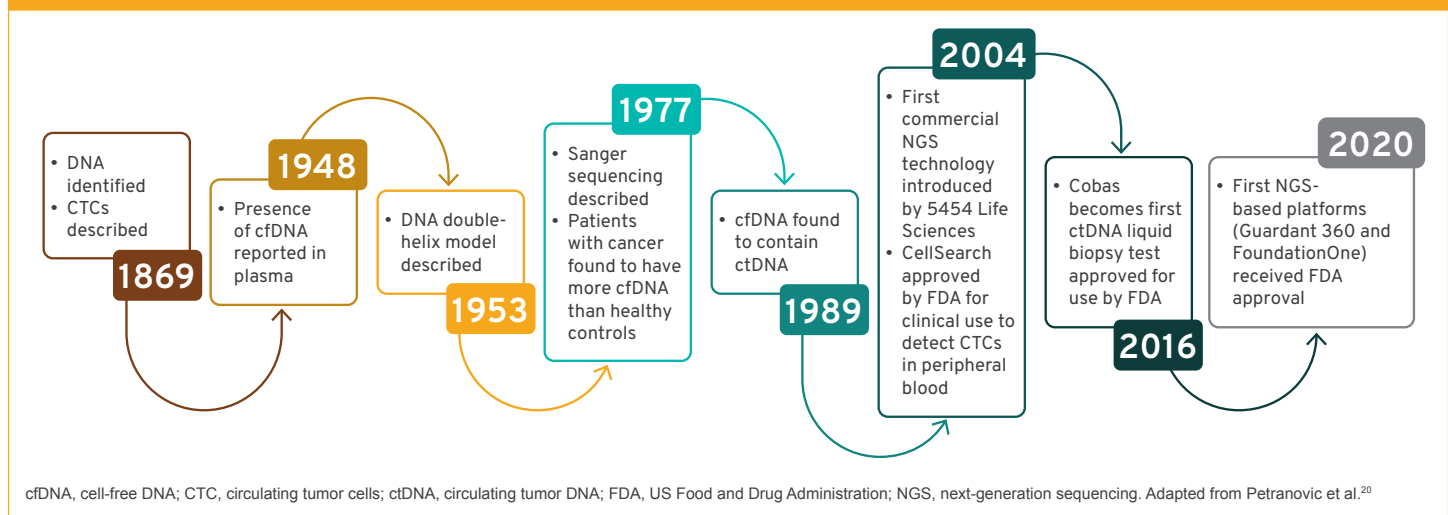
Several distinct molecular subtypes of breast cancer have been defined on the basis of tumor biomarkers. These subtypes include: (a) HR positive (estrogen receptor [ER] or progesterone receptor [PR]), human epidermal growth factor receptor 2 (HER2) negative (70% of patients); (b) HER2 positive, HR positive or negative (15%–20% of patients); and (c) triple negative (tumors lacking ER, PR, and HER2 [15% of patients]). Breast cancer subtype is routinely evaluated because of its utility in guiding treatment decisions.^{5,9}

Recent studies have identified a new molecular classification, HER2-low. This category comprises tumors that express HER2 protein at a level below the threshold expression for classification as HER2 positive (immunohistochemical score 3+ and/or in situ hybridization positive); they have been traditionally classified as HER2 negative.^{10,11} HER2-low tumors constitute about 60% of traditionally HER2-negative cases.^{11,12} While current standard-of-care therapies targeting HER2 have been ineffective against HER2-low tumors,^{11,12} HER2-low tumors are responsive to novel anti-HER2 antibody-drug conjugates, such as trastuzumab deruxtecan (Enhertu®).^{10,12-14} Hence, HER2-actionable breast cancers now include both HER2-positive and HER2-low molecular subtypes.

Challenges of current diagnostic options available for oncologists

Currently, the standard diagnostic tool in breast cancer is tissue biopsies. They are routinely used to confirm diagnosis, provide information on tumor histology and known biomarkers for subtyping and treatment planning, and determine molecular profiles for predictive and prognostic signatures. While tissue biopsies have numerous benefits, there are also limitations. They can only provide tumor characteristics at a single location, thereby potentially missing intratumor heterogeneity, and they are both invasive and costly.¹⁵ Moreover, the finite amount of available tissue also restricts the number of diagnostic tests that are feasible.¹⁶ Noninvasive techniques, such as liquid biopsy, have emerged as viable alternatives.¹⁷ Blood-based liquid biopsy allow the collection of numerous circulating components, such as circulating tumor DNA (ctDNA), circulating tumor RNA, noncoding RNAs, circulating tumor cells (CTCs), and circulating leukocytes, which represent promising biomarkers to assess tumor heterogeneity and patients' response to treatment.^{18,19} Blood-based liquid biopsy also enables repetitive sampling, potentially allowing an evolving tumor landscape to be monitored in real time. Several liquid biopsy tests have received College of American Pathologists-Clinical Laboratory Improvement Amendments approval and some have been approved by the US Food and Drug Administration with companion diagnostic designation. A timeline of notable events in the evolution of liquid biopsy is presented in **Figure 1**.²⁰

Figure 1. Notable events in the evolution of liquid biopsy



While liquid biopsy tests have significantly improved the number of targeted therapies in non-small cell lung cancer owing to their identification of actionable genetic variations in ctDNA, they have had limited impact in MBC. This is because most treatment decisions in MBC rely on cellular analysis that cannot be conducted by ctDNA alone. A list of validated ctDNA genetic variations associated with non-small cell lung cancer and breast cancer is shown in **Table 1**.^{21,22} Since the majority of liquid biopsy tests only

evaluate ctDNA, they are unable to assess essential biomarkers in MBC, such as HR status, HER2 protein expression, and genomic data that are only contained within the cancer cell. These limitations must be overcome, and the scope of noninvasive liquid biopsies expanded to include assessment of a wide range of biomarkers. This will allow liquid biopsies to realize their great promise as a routine clinical tool for diagnosis, prognosis, monitoring, and therapeutic guidance for MBC.^{19,20}

Table 1. Validated ctDNA Cancer Genotypes

Tumor Type	Indications
Non-small cell lung cancer	<p><i>EGFR</i> (for common, uncommon, exon 20 insertions, T790M and other resistance mutations, eg, C797X)</p> <p><i>ALK</i> (for fusions and acquired resistance kinase domain mutations)</p> <p><i>MET</i> (for exon 14 splice site mutations, and acquired resistance mutations)</p> <p><i>KRAS</i> (for G12C and non-tier 1 other <i>KRAS</i> mutations)</p> <p><i>BRAF</i> (for V600E)</p> <p><i>RET</i> (for fusions and acquired resistance kinase domain mutations)</p> <p><i>ROS1</i> (for fusions and acquired resistance kinase domain mutations)</p> <p><i>NTRK1/2/3</i> (for fusions and acquired resistance mutations)</p> <p><i>MET</i> (for high-level copy number gain/amplification)</p> <p><i>ERBB2</i> (for exon 20 insertions and transmembrane mutations, and amplification)</p> <p><i>BRAF</i> (for non-V600E class I–III mutations)</p>
Breast cancer	<p><i>PIK3CA</i> mutations</p> <p><i>ERBB2</i> amplification</p> <p><i>BRCA1/2</i> mutations</p> <p><i>ESR1</i> mutations</p> <p><i>MSI-H</i></p> <p><i>NTRK1/2/3</i> fusions</p>

ALK, anaplastic lymphoma kinase; *BRAF*, B-Raf proto-oncogene; *BRCA1/2*, breast cancer gene 1/2; *EGFR*, epidermal growth factor receptor; *ERBB2*, erythroblastic oncogene B; *ESR1*, estrogen receptor 1; *KRAS*, Kirsten rat sarcoma virus; *MET*, MET proto-oncogene, receptor tyrosine kinase; *MSI-H*, microsatellite instability high; *NTRK*, neurotrophic tyrosine receptor kinase; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *RET*, rearranged during transfection proto-oncogene; *ROS1*, ROS proto-oncogene 1, receptor tyrosine kinase. Adapted from Pascual et al.²²

DefineMBC

To better address barriers associated with the use of liquid biopsies, Epic Sciences developed DefineMBC, the first and only blood-based diagnostic that can provide more detailed information about the molecular characteristics/profiles of patients with MBC. DefineMBC incorporates both cell-based and cell-free analysis from a single blood draw to provide comprehensive profiling of MBC, even when tissue biopsy results are not available or practical. The test’s multi-analyte method²³ (**Table 3**) has demonstrated impressive sensitivity, specificity, accuracy, and precision in

- Detection of CTCs
- Assessment of protein expression (HER2, ER)
- Single-cell genomics on individual CTCs that identifies large scale genomic transitions and single gene copy number variations (CNVs; eg, *ERBB2* amplification)

- Plasma-based cell-free (cf)DNA analysis for identification of single-nucleotide variants, indels, fusions, and CNVs from a 56-gene panel, including tier 1 recommendations for ctDNA-targeted therapy such as *ESR1*, *PI3KCA*, and *BRCA 1/2* mutations, and *ERBB2* amplification
- Calculation of microsatellite instability and tumor mutational burden

Overall, DefineMBC enables comprehensive analysis of critical biomarkers and genomic alterations comparable to tissue biopsy, with the ease and noninvasiveness of liquid biopsy.

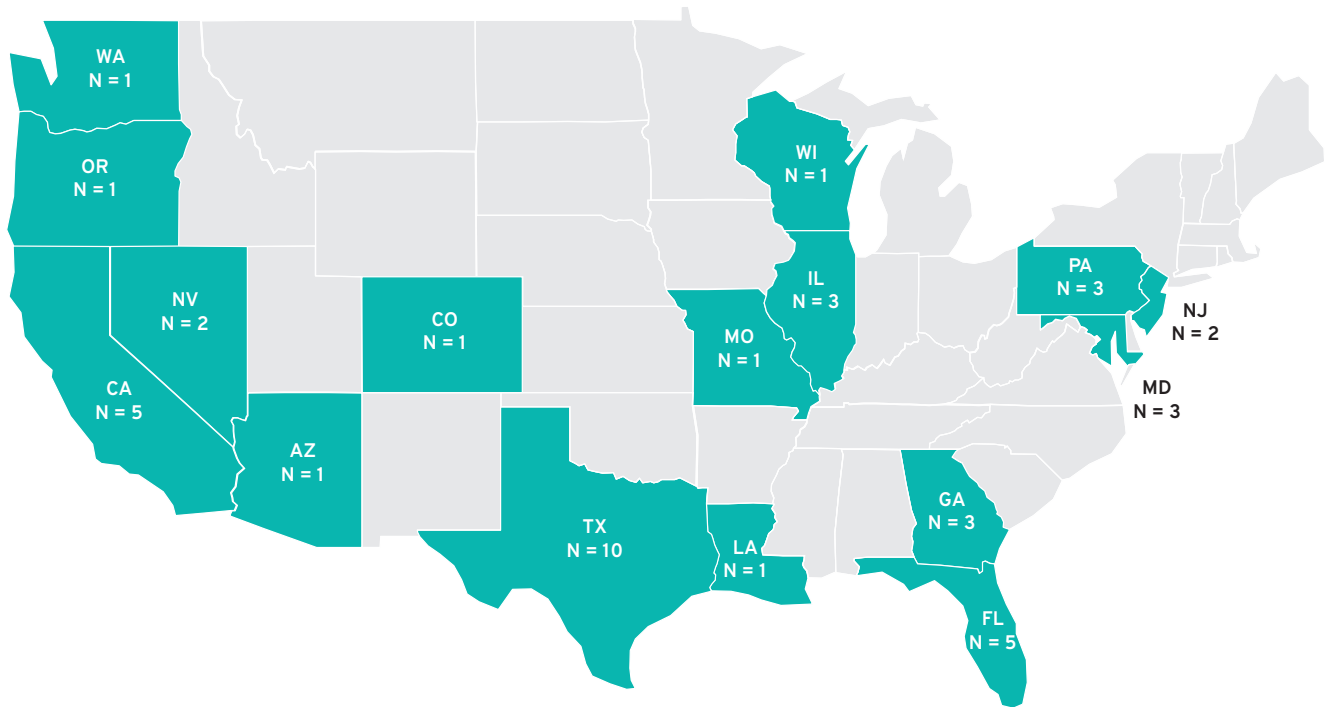
METHODS

Study Population

In order to evaluate and further define the positive impact that DefineMBC may have on patient care, Epic Sciences enrolled 25 US-based cancer centers at 34 different locations, involving a total of 43 participating oncologists, into a Clinical Experience Program (**Figure 2**).

Participants were from 16 US states, thus representing multiple geographic regions throughout the country. The main objective of the program was to enable participating physicians to evaluate the functionality of DefineMBC in patients with MBC.

Figure 2. Medical oncologists who participated in the Clinical Experience Program



Patient Population

Patient indications included: (a) prior primary breast cancer, presenting with suspected metastatic recurrence where a tissue biopsy result is unavailable, inadequate, or inconclusive; (b) patient’s tumors unresponsive to current MBC treatment and a tissue biopsy result was unavailable, inadequate, or inconclusive; or (c) the oncologist considered the patient appropriate for ctDNA-only evaluation. To ensure that the participating physicians received the most comprehensive DefineMBC clinical report, all blood draws were required to occur either prior to initiating first-line MBC treatment, or prior to initiating the next line of therapy in second-line or subsequent (second-line-plus) MBC treatment.

DefineMBC Tests

Overall, a total of 172 DefineMBC tests were performed from April 4 through July 22, 2022. Each liquid biopsy blood sample was analyzed for the presence of CTCs as well as genomic variations within the CTCs and ctDNA. CTCs were identified as white blood cells that displayed key biomarkers detected by immunofluorescence techniques. These biomarkers included cytokeratin positive, 4',6-diamidino-2-phenylindole nuclear staining positive, and cluster of differentiation (CD)45 and CD31 negative. Cells that

were identified as CTCs were further assessed for any *ERBB2* amplification, genomic alterations, chromosomal abnormalities, and large-scale transitions (chromosomal breakage resulting in 10-Mb or larger fragments and indicative of chromosomal instability),²⁴ using single-cell genomics methods. Consequently, the DefineMBC test assesses each patient’s blood sample(s) for actionable biomarkers and mutations that could be targeted for therapy.

Case reviews and Survey

Between May 19 and July 22, 2022, case reviews were performed with participating physicians to obtain feedback and to discuss the clinical utility of DefineMBC for each patient tested. After the case reviews, each physician received a 24-question survey aimed at capturing feedback on clinical and product experience with the test. Survey questions were electronically administered by a third-party and sent to identified personnel at the participating site for completion within 48 hours of receipt. For most questions, physicians were either asked to check all that applied from a list of responses or to rank options from most valuable to least valuable.

Herein, we present results from the aggregated data across the 172 DefineMBC clinical reports as well as the survey responses completed by 17 participating physicians.

RESULTS

Summary of 172 clinical reports delivered during the Clinical Experience Program

The Clinical Experience Program generated a total of 172 DefineMBC liquid biopsy clinical reports for patients with MBC; the characteristics of patients tested are summarized in **Table 2**. A summary of the results from these reports is shown in **Table 3**. CTCs were detected in 120 (70%) of the blood samples analyzed. In a subset of samples where CTCs were not detected, it was determined to be because the patient did not have MBC, the lesion identified radiographically was not due to progressive disease, or the patient was on active and effective treatment at the time the DefineMBC sample was collected. Of the 120 samples where CTCs were detected, 93 (78%) had ER-expressing cells, with 11 (9%) harboring an actionable estrogen receptor 1 (*ESR1*) mutation identified through ctDNA analysis. In addition, ctDNA analysis identified level 1A actionable alterations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) in 27 (21%) samples, and in breast cancer gene 1 (*BRCA1*) or *BRCA2* in 4 (3%) samples. Overall, combining the results from both CTC and ctDNA analyses, actionable information was identified in 165 (96%) samples (**Table 3**).

Furthermore, while 10 (8%) samples were classified as HER2 positive, 40 (33%) were consistent with the classification of HER2-low (ie, HER2-expressing CTCs detected but *ERBB2* amplification not detected on single-cell genomic and/or ctDNA analysis). This translates to a total of 41% of

the samples with CTCs detected being identified as HER2 actionable (ie, HER2 positive and HER2-low combined; **Table 3**). In contrast, 90% of these cases were previously classified as HER2 negative (ie, not HER2 actionable) on the basis of tissue biopsy results. Thus, DefineMBC provided updated tumor characteristics compared with the most recent tissue biopsy for several patients tested.

Survey results from 17 oncologists who participated in the Clinical Experience Program

A total of 17 physicians participating in the Clinical Experience Program responded to the survey regarding their perception of clinical benefit and product experience with DefineMBC. Respondents were all medical oncologists specializing in breast cancer care.

Table 2. Characteristics of patients evaluated during the Clinical Experience Program (N = 172)

Subtype	No.	Percentage
HR positive, HER2 negative	112	71.8%
HER2 positive	9	5.8%
TNBC	12	7.7%
Not recorded	23	14.7%

HER2, human epidermal growth factor receptor 2; HR, hormone receptor; TNBC, triple-negative breast cancer.

Table 3. Summary of DefineMBC clinical reports from the Clinical Experience Program (N = 172)

Category	Result Type	No.	Percentage of Total
DefineMBC clinical reports delivered	Clinical reports delivered	172	NA
CTC and ctDNA detection	CTCs detected (IF + LST)	120	70%
ER analysis	ER expression	93	78%
	<i>ESR1</i> mut	11	9%
HER2 analysis	HER2-low	40	33%
	HER2 positive	10	8%
ctDNA analysis	<i>PIK3CA</i> mut	27	21%
	<i>BRCA1</i> or 2 mut	4	3%
DefineMBC actionability	CTCs and/or ctDNA detected	165	96%

BRCA1, breast cancer gene 1; *BRCA2*, breast cancer gene 2; CTC, circulating tumor cell; ctDNA, circulating tumor DNA; ER, estrogen receptor; *ESR1*, estrogen receptor 1; HER2, human epidermal growth factor receptor 2; IF, immunofluorescence; LST, large-scale transition; mut, mutation; NA, not applicable; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

Per test indication, all respondents (100%) utilized DefineMBC in patients with documented progression of metastatic disease (as confirmed by scans) during the Clinical Experience Program. Other clinical settings in which DefineMBC was used were in newly diagnosed recurrent MBC (65%); suspected progression of metastatic disease on the basis of clinical symptoms, but not confirmed by scans (41%); and newly diagnosed de novo MBC (35%; data not shown). Most respondents (88%) used DefineMBC prior to second- or third-line treatment for metastatic disease (**Figure 3**).

DefineMBC was viewed by 82% of respondents as a valuable clinical tool in guiding their care plan for patients with MBC for whom a tissue biopsy would not otherwise be performed or was not possible (**Figure 4**). When the DefineMBC results were consistent with the most recent tissue biopsy, 71% of respondents concurred that it significantly boosted confidence in their established care plan (**Figure 5**). Respondents also felt that the DefineMBC report could provide new or updated information compared with the known clinical history of a patient, which could facilitate identification of potential future line(s) of therapy that were not previously considered (76%), or could help obtain up-to-date information about a patient's cancer that otherwise would not have been available (59%; **Figure 6**).

Respondents understood that multiple factors could contribute to differences between DefineMBC and tissue biopsy results. For example, in cases where the CTC analysis for ER and/or HER2 from DefineMBC were not consistent with results from the most recent tissue biopsy, 53% of the respondents indicated that DefineMBC results may reflect a change in ER or HER2 status that has taken place since the time of the most recent tissue biopsy (**Figure 7**). Several respondents (53%) also stated that the differences between DefineMBC results and tissue biopsy results could reflect tumor heterogeneity. Interestingly, 24% of respondents opined that the discrepancy between the 2 results could derive from DefineMBC being able to identify a patient as potentially having HER2-low MBC that had not been identified by tissue testing (**Figure 7**). Some respondents (24%) also stated that DefineMBC results were able to identify a genomic alteration that was not assessed by the most recent tissue biopsy sample.

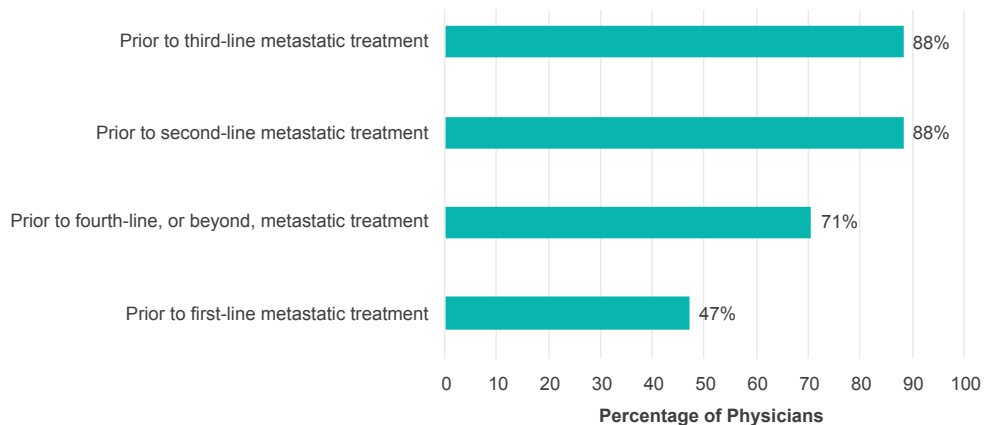
Most respondents (88%) rated DefineMBC as having more clinical value because it provides cell-based CTC and cfDNA analysis compared with a liquid biopsy test that only analyzes cfDNA using next-generation sequencing (eg, cfDNA comprehensive genomic profiling panel). Only 12% stated that both tests have the same level of clinical value (**Figure 8**). Indeed, respondents found that genomic alterations identified from CTCs via single-cell genomics were a highly valuable component of DefineMBC (66%) along with those identified from ctDNA analysis (65%; **Figure 9**).

Additionally, respondents reported that DefineMBC was able to identify patients as potentially having HER2-low breast cancer (59%), identify HER2 status changes vs the last known biopsy status (53%), and identify a genomic alteration (eg, *ESR1* or *PIK3CA* mutation) that had not been previously identified/detected (47%; **Figure 10**). Indeed, 76% of the respondents agreed that they were more likely to use this test to reassess ER and HER2 status prior to second-line-plus treatment selection, due to the less invasive nature of a single blood draw in DefineMBC compared with tissue biopsy (**Figure 11**).

The survey also captured the ease of use of DefineMBC, with 77% of the respondents finding value in the ability to access all the relevant biomarkers for MBC in a single DefineMBC report, as opposed to multiple tissue biopsy reports (**Figure 12**). Importantly, 88% of respondents believed that their patients had a better experience with the DefineMBC test compared with an invasive tissue biopsy. Only 12% noted that their patients had the same experience with both tests (**Figure 13**).

The survey also assessed the respondents' perceived clinical utility of DefineMBC by identifying the potential future uses of the assay following the conclusion of the Clinical Experience Program. Most respondents (76%) planned to use DefineMBC for patients with newly diagnosed MBC when a tissue biopsy is not feasible, 59% would use the test for patients with bone-only metastatic disease, and 59% would also use the test for patients prior to starting second-line-plus treatment for MBC when a tissue biopsy is not feasible or desired. Additional anticipated clinical settings are listed in **Figure 14**.

Figure 3. Survey question^a: When in the treatment journey of patients with metastatic breast cancer did you utilize DefineMBC during the Clinical Experience Program? (N = 17)



^aRespondents could choose more than one answer.

Figure 4. Survey question: Overall, how would you rate the clinical value of DefineMBC in helping to guide your care planning for patients with metastatic breast cancer for whom a tissue biopsy would not be done or is not possible? (N = 17)

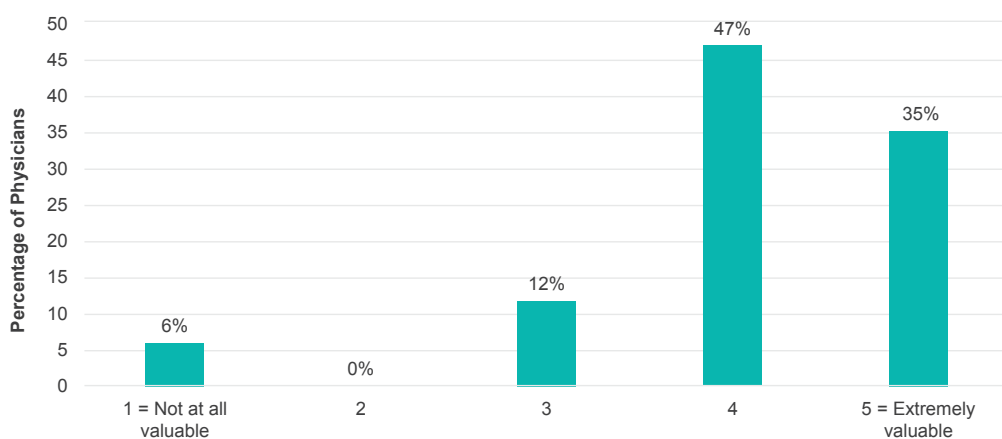


Figure 5. Survey question: For results that were consistent with the most recent tissue biopsy, how did the DefineMBC results impact your confidence in the established care plan? (N = 17)

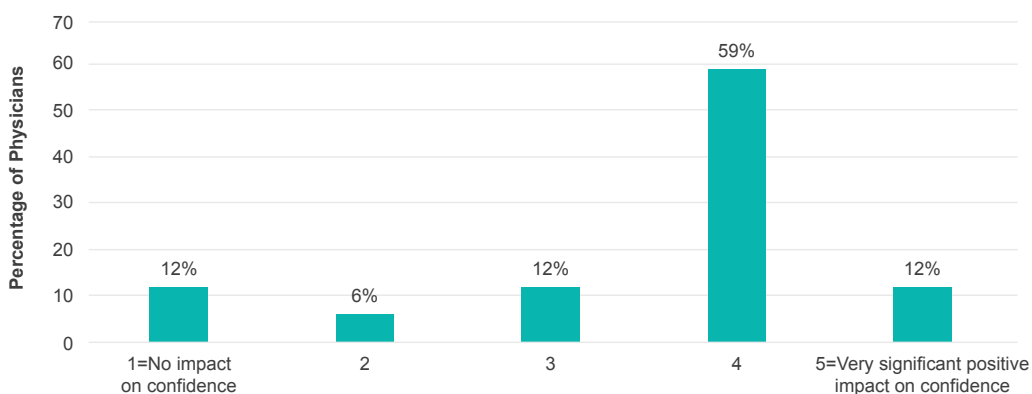
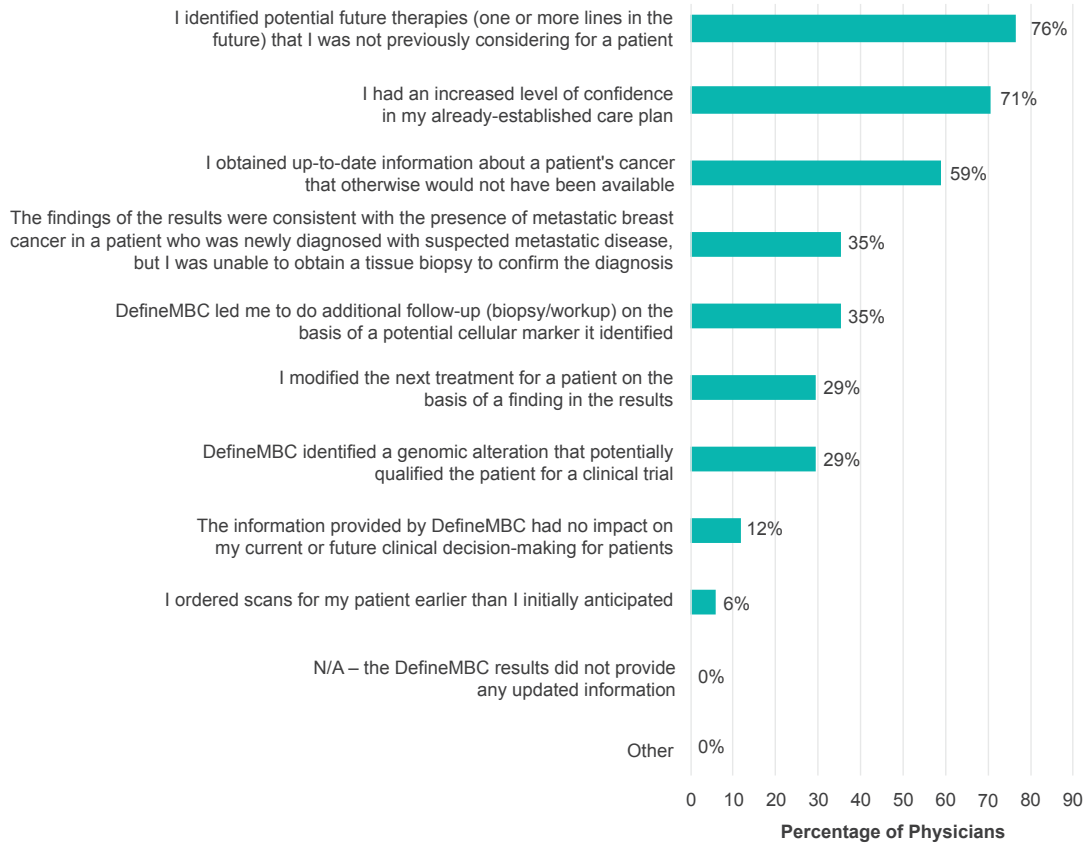
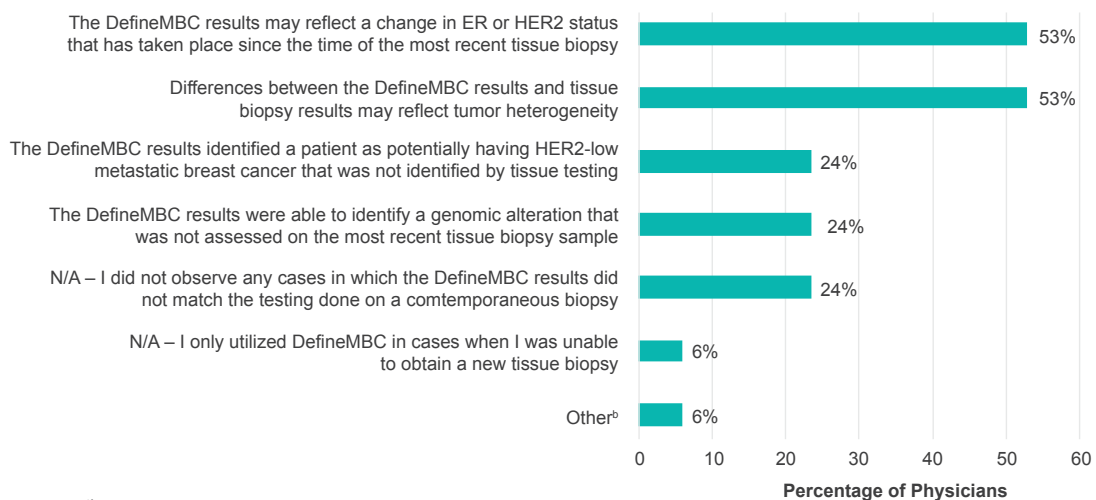


Figure 6. Survey question^a: In cases where the DefineMBC report provided updated information compared with the known clinical history for a patient, what impact did the results have on your care planning for patients you tested? (N = 17)



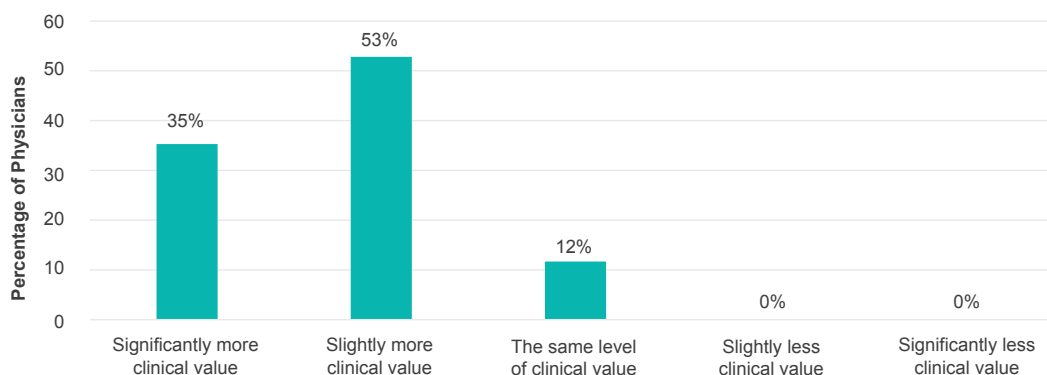
^aRespondents could choose more than one answer.
N/A, not applicable.

Figure 7. Survey question^a: In cases where the CTC analysis for ER and/or HER2 from DefineMBC were not consistent with testing done on the most recent tissue biopsy, why do you believe there was a discrepancy between the 2 results? (N = 17)



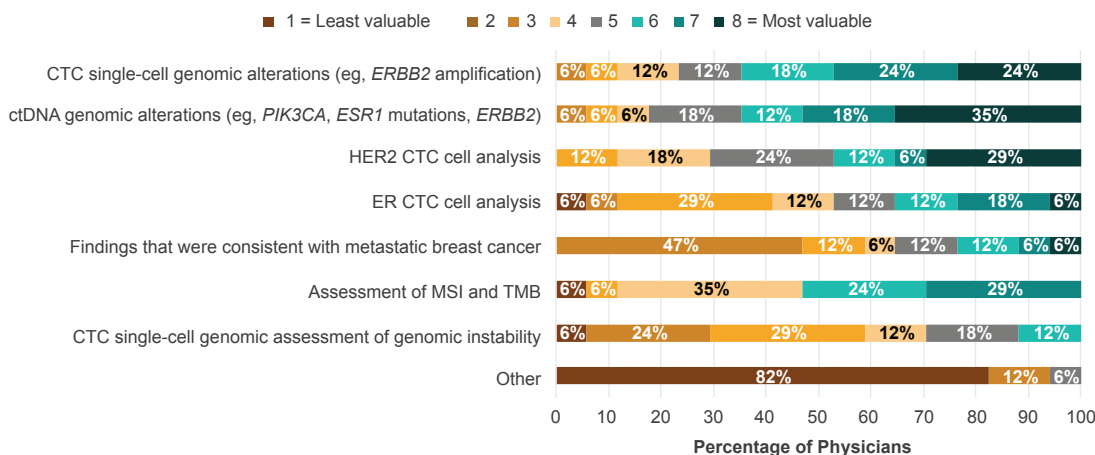
^aRespondents could choose more than one answer.
^bER was positive on CTC, but subsequent tissue was ER 0 and patient did not respond to ER directed therapy. I suspect that ER expression was very low by DefineMBC.
CTC, circulating tumor cell; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; N/A, not applicable.

Figure 8. Survey question: How would you rate the clinical value of DefineMBC in providing both CTC and cfDNA compared with a liquid biopsy test that only includes cfDNA analysis using NGS (a cfDNA comprehensive genomic profiling panel)? (N = 17)



cfDNA, cell-free DNA; CTC, circulating tumor cell; NGS, next-generation sequencing.

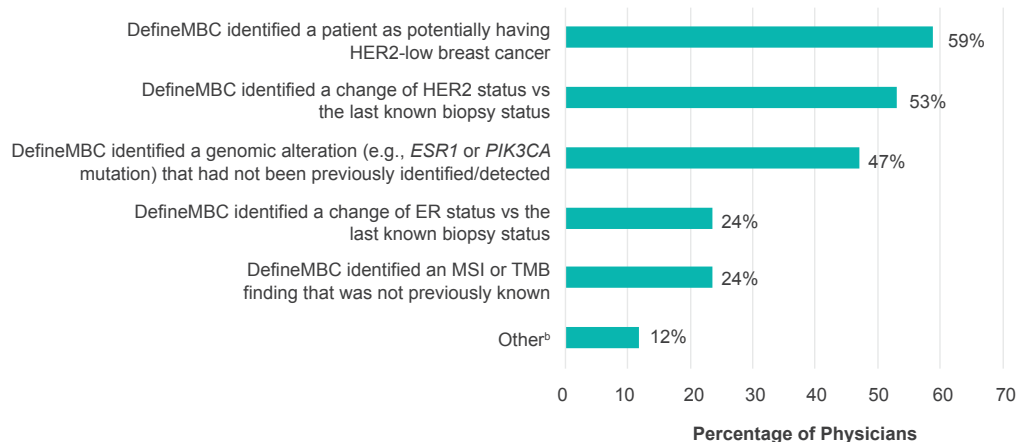
Figure 9. Survey question^a: Which component of the DefineMBC test did you find most valuable in establishing a care plan for patients with metastatic breast cancer? (N = 17)



^aRespondents rank options in descending order.

CTC, circulating tumor cell; ctDNA, circulating tumor DNA; ER, estrogen receptor; *ERBB2*, erythroblastic oncogene B; *ESR1*, estrogen receptor 1; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TMB, tumor mutational burden.

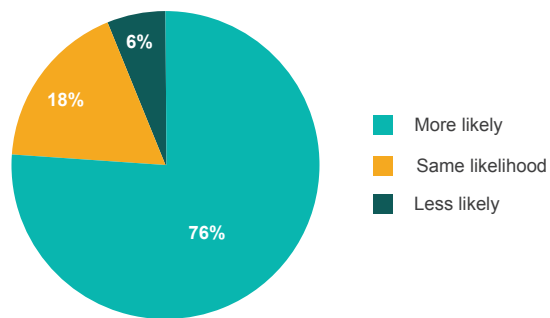
Figure 10. Survey question^a: What clinical features were identified by the DefineMBC report that impacted your clinical decision making? (N = 17)



^aRespondents could choose more than one answer.

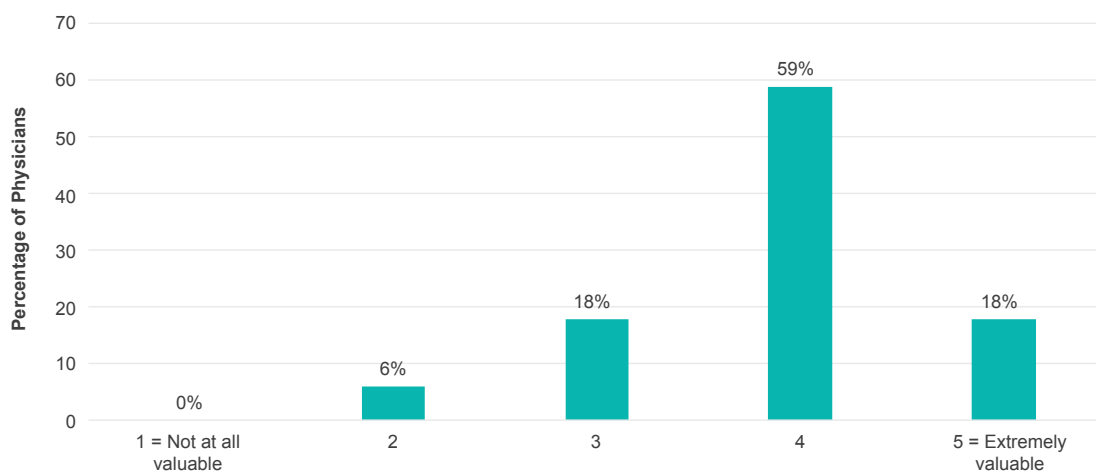
^bDefineMBC identified *BRCA1* mutation that did not change *BRCA1*, breast cancer gene 1; ER, estrogen receptor; *ESR1*, estrogen receptor 1; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TMB, tumor mutational burden.

Figure 11. Survey question: Given the less invasive nature of DefineMBC (single blood draw) compared with tissue biopsy, are you more or less likely to use this test in place of tissue biopsy to reassess ER and HER2 status in the second-line-plus settings? (N = 17)



ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.

Figure 12. Survey question: How would you rate the value to you and your staff of DefineMBC providing all relevant biomarkers for metastatic breast cancer (ER, HER2, *ERBB2* amplification, analysis of genomic alterations, MSI, TMB) in a single report compared with assessing information from several different reports with testing done on tissue samples? (N = 17)



ER, estrogen receptor; *ERBB2*, erythroblastic oncogene B; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; TMB, tumor mutational burden.

Figure 13. Survey question: How do you believe your patients with metastatic breast cancer would rate the experience of having the DefineMBC test, compared with an invasive tissue biopsy? (N = 17)

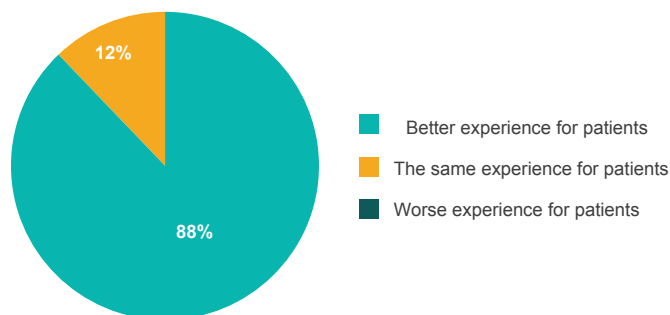
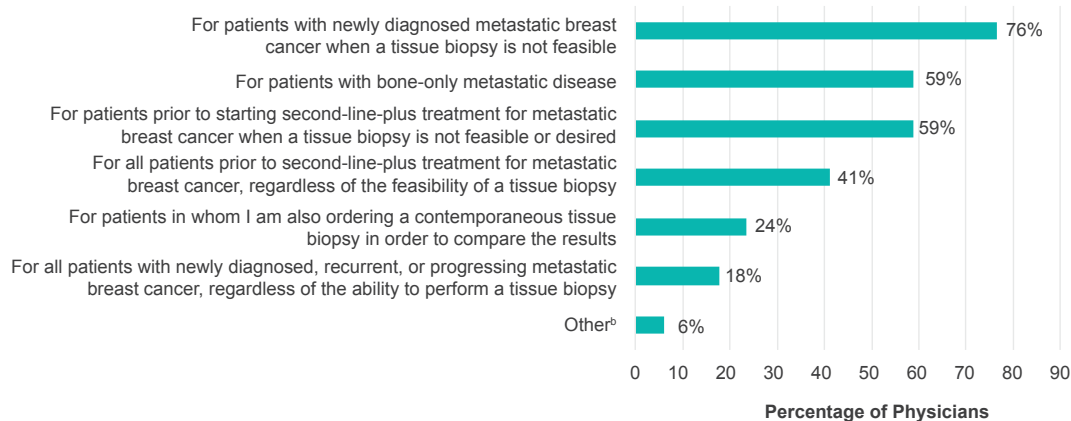


Figure 14. Survey question^a: Now that the Clinical Experience Program has concluded, in which of the following clinical settings do you anticipate utilizing DefineMBC in the future? (N = 17)



^aRespondents could choose more than one answer.

^bProbably will not use

CONCLUSIONS

While tissue biopsies remain the standard-of-care diagnostic tool for MBC, they are limited by tumor accessibility and the amount of tissue that can be obtained from a single biopsy. Consequently, they may miss any spatial intratumor heterogeneity, potentially introduce sample bias, or provide inadequate or incomplete information.¹⁵ Moreover, their invasive nature also restricts the number of biopsies that can be undertaken, which in turn makes it difficult to monitor the evolution of a tumor over time. Longitudinal determination of tumor receptor status is important, due to the frequently reported changes in initial biomarker status between primary and recurrent breast tumors, as well as during disease progression.^{25,26} This likely reflects changes in disease biology, differential elimination of clonal subsets, tumor heterogeneity, tumor progression across multiple lines of therapy, or imperfect accuracy and reproducibility of assays.²⁶ Indeed, changes in HR and HER2 status during disease evolution have been documented in approximately 10% to 30% of cases,²⁷ illustrating the importance of re-evaluating tumor biology as MBC progresses and underscoring the need for tools that enable longitudinal monitoring.

DefineMBC is a liquid biopsy that incorporates cell-based and cell-free analysis and enables comprehensive profiling of MBC. The Clinical Experience Program survey provided real-world insights on the use of the DefineMBC liquid biopsy diagnostic in patients with MBC. Overall, the survey confirmed that the DefineMBC assay can be utilized across multiple clinical settings and at various time points for patients with MBC. DefineMBC is easier to undertake than a

tissue biopsy, given the noninvasiveness of the single-blood-draw technique. Moreover, DefineMBC is a comprehensive liquid biopsy that can analyze CTCs for essential biomarkers in MBC such as HR and HER2 protein expression. It can also determine genomic variations in CTCs via single-cell genomics, in addition to assessing ctDNA for genetic mutations. Thus, DefineMBC can outperform other currently available liquid biopsies that can only evaluate ctDNA.

Although there are numerous factors that may hinder tissue biopsy in patients with MBC, it remains the gold-standard diagnostic tool according to survey respondents. While DefineMBC results can differ from the most recent tissue biopsy, the survey results highlight that awareness exists among physicians that these discordant results could be reflective of changes in tumor biology, tumor heterogeneity, and identification of new molecular subtypes and genomic alterations. Such information could be used to better inform decisions on the optimal treatment strategies. Nevertheless, further clinical research studies are necessary to better understand how to interpret discordant results between DefineMBC and tissue biopsy. In addition, further education among oncologists is important to highlight that DefineMBC can be a powerful diagnostic tool in providing comprehensive information regarding tumor heterogeneity among different metastatic sites, unlike tissue biopsies. Further research and education will also help diminish any skepticism regarding the predictive value and diagnostic utility of CTCs that are uniquely analyzed by DefineMBC, unlike most other liquid biopsies.

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