DefineMBC™

# DefineMBC is the most comprehensive liquid biopsy for patients with metastatic breast cancer

Breast cancer is primarily diagnosed by tissue biopsy, a standard of care technique that allows assessment of tumor histology, protein expression, and comprehensive molecular profiling of biomarkers which help inform treatment decisions. However, tissue biopsies face several challenges in accurately characterizing metastatic breast cancer. First, biopsy measurements are limited by tumor heterogeneity and may reflect the biology of a small portion of an accessible lesion or lesions.[1]

Tissue biopsies are also invasive, so issues of patient safety, comfort and procedure cost hinder their use for monitoring cancer evolution over time. Non-invasive liquid biopsies have emerged as viable diagnostic tools that facilitate biomarker detection and longitudinal cancer profiling.

While useful, most of the currently available liquid biopsies are limited to assessing circulating tumor DNA (ctDNA). Consequently, they are unable to determine variations in important biomarkers of breast cancer, such as protein 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 comprehensive liquid biopsy that can assess both protein biomarkers from circulating tumor cells (CTCs), and genomic variations from CTCs via single cell genomics as well as targeted ctDNA sequencing.

## Product Features

DefineMBC is a clinical test run in a CLIA-certified laboratory. DefineMBC incorporates both cell-based and cell-free analysis from a single blood draw to provide comprehensive profiling of metastatic breast cancer when tissue biopsy results are not available or practical. After analyzing all nucleated cells and ctDNA from a whole blood sample, DefineMBC provides a comprehensive clinical report that includes:

- Detection of CTCs
- Assessment of protein expression (HER2, ER) on CTCs
- Determination of ERBB2 copy number alterations (CNA) within individual CTCs
- ctDNA analysis for identification of single nucleotide variants (SNVs), indels, fusions, and CNAs from a targeted 56-gene next-generation sequencing (NGS) panel
- The calculation of microsatellite instability (MSI) and blood tumor mutational burden (bTMB)

## Intended Use

DefineMBC is designed for use in patients with breast cancer when metastatic disease is suspected or has previously been confirmed and for whom surgical tissue biopsy is not feasible, or when the results of surgical biopsy are indeterminate or inadequate, including when there is insufficient tissue sample to process.

## CTC Analysis

DefineMBC combines immunofluorescence (IF) imaging, proprietary machine learning algorithms, and individual cell retrieval to identify CTCs with high sensitivity and specificity. All nucleated cells from the white-blood-cell fraction of a blood sample are deposited on a coated glass slide and stained with fluorescent antibodies. Cells that are CD31−/CD45−, DAPI+, and CK+ and have the correct size and morphology are flagged by a propriety machine learning algorithm as prospective CTCs. Additionally, ER and HER2 protein expression on CTCs is evaluated using IF staining (Fig. 1). Final cell selection is confirmed with pathologist review.

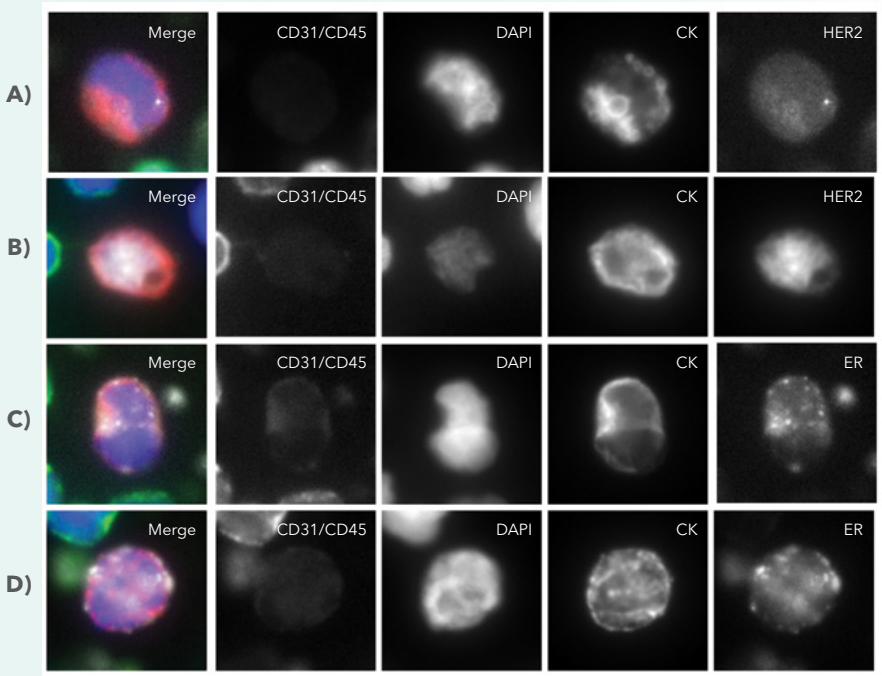


Figure 1: Representative IF images of CTCs isolated from patient samples. A) HER2-negative CTC; B) HER2-positive CTC; C) ER-negative CTC; D) ER-positive CTC

## Single-Cell Genomic Analysis

Individual CTCs are isolated, and the genomic material is amplified and sequenced using low-pass whole-genome sequencing (WGS). Single-cell sequencing data are used to evaluate ERBB2 amplification and can also add insight on genomic instability by detecting large-scale transitions (Fig. 2).

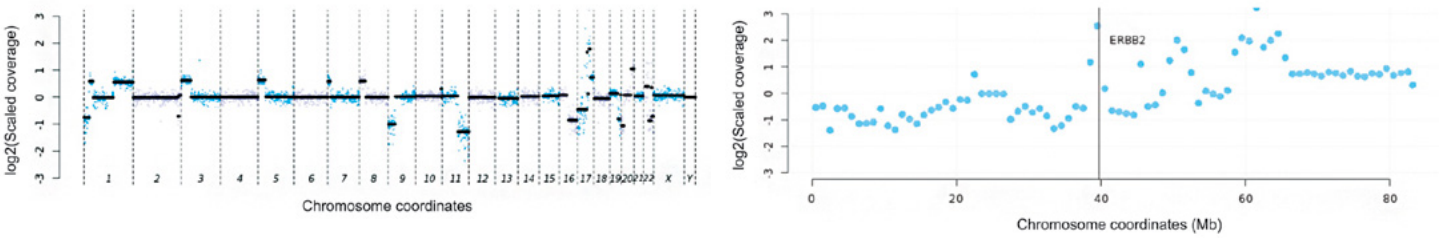


Figure 2: Representative single-cell genomic analysis results from a patient with HER2-positive breast cancer. A) Genomic instability results. B) Close-up of CNA on chromosome 17 showing ERBB2 copy number gain.

## ctDNA Genomic Alterations

Targeted next-generation sequencing (NGS) is used for analysis of SNVs, CNAs, indels, and fusions in 56 clinically relevant genes, and MSI and bTMB to assess immunotherapy biomarker status. Genomic alterations are categorized according to criteria recommended by a joint working group of the Association for Molecular Pathology, American College of Medical Genetics and Genomics, American Society of Clinical Oncology, and College of American Pathologists.2 bTMB is the number of nonsynonymous mutations within the coding region of a tumor genome and is reported as a score of mutations per megabase (muts/Mb). MSI is determined by analyzing 76 homopolymer marker sites. The percentage of unstable MSI sites to total assessed MSI sites is reported as a sample-level microsatellite score and reported as stable or unstable.

## Technical Specifications and Performance Characteristics[3]

Performance of cell-based and cell-free assays are shown below.

### Overview of CTC Analytical Validation Data

Table 1: Summary of CTC-based assay performance*

| Limit of detection (LOD) | 1 CTC in 6 million White Blood Cells (WBCs) | |
|---|---|---|
| Sensitivity of the assay | HER2: 94% | ER: 91% |
| Specificity of the assay | HER2: 97% | ER: 100% |
| Accuracy | HER2: 95% | ER: 94% |
| Overall Precision | HER2: 4.5% CV | ER: 7.4% CV |

*Protein expression results are determined using mean fluorescence intensity (MFI).

### Overview of Single-Cell Genomics Analytical Validation Data

Table 2: Summary of Single-Cell Genomic Analysis performance

| | CTC ERBB2 amplification |
|---|---|
| LOD | 2-fold amplification |
| Sensitivity of the assay | 85-100%* |
| Specificity of the assay | 94% |

*Tested using MDA-MB-453 (low positive [~2-fold]) and SK-BR-3 (high positive [~10-fold]) cell lines

### Overview of ctDNA Genomic Alteration Analytical and Clinical Validation Data

Table 3: Summary of Analytical Validation Data for detection of genomic alterations in ctDNA*

| | |
|---|---|
| Liquid biopsy sample requirement† | 8 mL peripheral whole blood |
| DNA input required | 20 ng of cell-free DNA (cfDNA) |
| Panel size | 56 genes |
| Mean depth coverage | 800 M reads for sample at 35,000X coverage, with median exon coverage of 1300X and 80% of exons above 100X |

| Variant Class | Analytical Sensitivity | Analytical Specificity | Reproducibility |
|---|---|---|---|
| SNV | 99.48% | 99.99% | 99.99% |
| Indel | 98.26% | 99.99% | 99.99% |
| CNA | 100% | 99.92% | 99.93% |
| Fusions | 100% | 100% | 100% |

*Based on 95% probability of detection
†Blood must be collected in Streck tubes and received by Epic Sciences within 48 hours for CTC analysis and within 7 days for ctDNA analysis

Table 4: Summary of ctDNA Genomic Alteration Clinical Validation Data

| | Sensitivity (Positive Percent Agreement- PPA (95% CI) | Specify (Negative Percent Agreement-NPA) (95% CI) |
|---|---|---|
| SNV | 97.06% | 100% |
| Indel | 100% | 100% |
| CNA | 100% | 100% |
| Fusions | 100% | 96.61% |

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## DefineMBC ctDNA Panel Content

DefineMBC interrogates 56 genes, including 54 genes with complete exonic (coding) coverage and 2 genes with only select non-coding coverage (indicated with *). All genes are analyzed for SNVs and indels. Genes analyzed for CNAs are in bold and those analyzed for fusions are highlighted in grey.

| | | | | | | |
|---|---|---|---|---|---|---|
| *AKT1* | ***BRCA2*** | ***CHEK1*** | *FAT1* | ***KRAS*** | *NTRK2* | *RAD54L* |
| ***ALK*** | *BRIP1* | ***CHEK2*** | ***FGFR1*** | *MAP2K1** | *NTRK3* | *RB1* |
| ***AR*** | ***CCND1*** | ***EGFR*** | ***FGFR2*** | ***MDM2*** | *PALB2* | ***RET*** |
| *ARID1A* | ***CCNE1*** | ***ERBB2*** | ***FGFR3*** | ***MET*** | ***PDGFRA*** | *ROS1* |
| ***ATM*** | *CD274* | ***ERBB3*** | *FOXA1* | ***MYC*** | ***PIK3CA*** | *SPOP* |
| *BARD1* | *CDK12* | ***ESR1*** | *HOXB13* | ***MYCN*** | ***PTEN*** | *STK11* |
| ***BRAF*** | ***CDK4*** | *ETV6* | *IGF1R* | *NF1** | *RAD51B* | *TMPRSS3* |
| ***BRCA1*** | ***CDK6*** | *EZH2* | ***KIT*** | *NTRK1* | *RAD51D* | *TP53* |

References:


1. Tay TKY and Tan PH. Arch Pathol Lab Med; 2021145(6):678-686
2. Li MM, et al. J Mol Diagn. 2022;19(1):4-23
3. Data on file. Epic Sciences.



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