

## Targeted gene panel sequencing of liquid and tissue biopsies reveals actionable genomic alterations in Ghanaian metastatic breast cancer cases

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### ARTICLE INFO

#### Keywords:

Breast cancer

Ghanaian women, liquid biopsy, actionable genomic alterations, targeted treatment

### ABSTRACT

**Purpose:** Breast cancer is a major cause of cancer-related mortality among African women. The adoption of molecular genomic technologies in the management of cancer cases is limited in Africa. To provide much-needed insights on the feasibility and utility of such precision medicine paradigms in Africa, we conducted a prospective, non-interventional study involving combined tissue and plasma Next-generation sequencing (NGS)-based testing in cancer patients in Ghana.

**Methods:** We recruited 20 newly diagnosed, histologically confirmed, treatment-naïve women with metastatic breast cancer at the Cape Coast Teaching Hospital in Ghana. Tissue (NGS) and cell-free DNA (cfDNA) liquid biopsy analysis were ordered on all 20 patients.

**Results:** All 20/20 (100 %) liquid biopsy samples were acceptable for analysis, whereas only 6/20 (30 %) passed quality control for tissue NGS testing. Liquid biopsy detected 42 cfDNA mutations in 17/20 patients. Of the 17 patients, 3 (17.6 %) had mutations previously associated with African ancestry, including *BRCA1* p.K719E, *ARAF* p.S262I and *GATA3* p.G125dup. Eight potentially actionable alterations specific to breast cancer were found in 6/17 (35.3 %) liquid biopsy samples, while potentially actionable mutations non-specific to breast cancer were detected in 12/17 (70.6 %). Tissue biopsy analysis detected mutations in all 6 patients tested, with 3/6 (50 %) patients presenting potentially actionable mutations relevant to breast cancer.

**Conclusion:** Liquid biopsy detected multiple additional actionable variants in Ghanaian women with breast cancer. Plasma cfDNA analysis featured fewer variations in sample preparation which is a key consideration in resource-limited settings. Liquid biopsy presents a great opportunity to improve cancer care in Africa.

### Introduction

Breast cancer in Africans remains understudied and poorly understood despite the high case fatality rates [1] and the observation of more aggressive breast cancer phenotypes [2] [3]. Mutations in *BRCA1*,

*BRCA2*, *PALB2*, and other cancer-associated genes, have been reported [4–6] and women of African ancestry often associate with a disproportionately high number of triple-negative breast cancer cases (estrogen receptor negative (ER-), progesterone receptor negative (PR-), human epidermal growth factor receptor 2 negative (HER2-) [3] [7], an

This work was submitted as an abstract at the 2023 ASCO Annual Meeting (Abstract number e13017).

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<https://doi.org/10.1016/j.tranon.2024.102100>

Received 25 January 2024; Received in revised form 22 May 2024; Accepted 11 August 2024

Available online 16 August 2024

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aggressive molecular subtype [8]. There is, therefore, a need to better understand the biology of breast cancer in Africans.

Genomics technologies like cfDNA sequencing can provide insights for screening, diagnosing, prognosis, and treating African breast cancer [9]. The molecular genetic landscape of breast cancer among Ghanaian women has been previously described using cfDNA [10] and tissue sequencing [11], with driver copy number alterations reported. As well as profiling single nucleotide variations, cfDNA assays can also detect insertions, deletions, gene fusions, and copy number alterations in cancer-associated genes [9,12].

The benefits of pairing genetic studies, and by extension, cfDNA liquid biopsy assays with targeted treatments, have been highlighted previously [13,14]. Examples include the use of Trastuzumab, Ado-Trastuzumab Emtansine, and Pertuzumab for treating HER2+ breast cancer [15]; Olaparib, a poly ADP-ribose polymerase (PARP) inhibitor for treating patients with *BRCA* mutations [15,16]; and the profiling of *ESR1* mutations for monitoring the development of resistance to endocrine therapy or for deciding against the use of such treatments for advanced breast cancer [14].

However, there is a lack of studies applying cfDNA technology to find actionable genomic alterations for breast cancer-targeted therapy among African women. To provide much-needed insights on the feasibility and utility of such precision medicine paradigms in Africa, we partnered with Lucence Health Inc. to investigate genomic alterations in Ghanaian women with metastatic breast cancer using the LiquidHALL-MARK™ cfDNA-targeted gene panel sequencing [12]. We aimed to use this clinically validated [12], amplicon-based NGS assay to identify potentially actionable alterations for targeted therapy. For a subset of our participants, we assessed the concordance of actionable genomic alterations detected from tissue NGS and plasma cfDNA.

## Materials and methods

### Ethical approval

This study received ethical approval from the Institutional Review Board of the Cape Coast Teaching Hospital with protocol number CCTHERC/EC/2021/051.

### Study design

A prospective, non-interventional observational study was employed to evaluate the use of liquid biopsy in detecting potentially actionable genetic mutations in African women with treatment-naïve metastatic (stage IV) breast cancer. This is a pilot study. Patients were recruited at the Cape Coast Teaching Hospital, a 400-bed capacity hospital in the Central Region of Ghana. It served as the primary point of standard of care for women enrolled in the study.

### Enrolment procedure

Initial evaluation of eligibility was done by the medical practitioner attending to the patient prior to their enrolment. If patients were deemed potential candidates for the study (i.e., treatment-naïve patients with metastatic breast cancer), they were referred to the study investigators for further discussion, consent, and enrolment. With the patient's verbal permission, the eligibility criteria were accessed from the patient and their medical record. If the patient met the eligibility criteria, they were asked to sign an informed consent form and subsequently enrolled as study participants. Twenty (20) Ghanaian women diagnosed with metastatic breast cancer met the eligibility criteria and were recruited into the study.

Participant's demographics and clinical data, including tumor histopathological and molecular subtypes, stage of disease, surgical history and treatment history, were collected into UVOSYO (Yemaachi Biotech), a clinical data management system designed to securely store and report

on both research and routine clinical care data.

Participants were seen at diagnosis and not followed up during treatment by the study team. Results from all tests conducted were shared with the primary care team as per standard of care.

### Peripheral blood sample collection

Following good phlebotomy practices, 10 ml of venous blood sample was collected into three separate Cell-free DNA BCT tubes (Streck, 7002 S. 109St. La Vista, NE 68128 USA) for plasma extraction. Samples were transported at ambient temperature to the advanced molecular biology research and diagnostics laboratory of Yemaachi Biotech (Accra, Ghana) for pre-processing and storage.

### Isolation of plasma and buffy coat

Three tubes of 10 ml venous blood for each study participant were received and transferred to 15 ml falcon tubes labeled with a unique sample identification number. Using an Eppendorf 5804R centrifuge, samples were first spun at 1600 x g at 4 °C for 10 min (acceleration = 9, deceleration = 0, with no breaks). Using a 1000 µl pipette, buffy coats were collected and stored at -86 °C. 1050 µl of plasma samples were aliquoted into 1.5 ml pre-labeled microcentrifuge tubes and spun at 16,000 x g at 4 °C for 10 min to remove any precipitates. After 10 min, 1 ml of plasma supernatant was carefully aliquoted into 2 ml pre-labeled cryotubes for storage at -86 °C. All samples were processed in a class II biological safety cabinet.

### Cell-free DNA extraction

Cell-free DNA (cfDNA) extraction, library preparation, and sequencing were performed in a CLIA-certified, CAP-accredited laboratory (Lucence Health) as described previously [12]. Briefly, cfDNA was extracted from 2 – 6 ml plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen) and quantified on a Qubit® 2.0 fluorometer (Thermo Fisher), yielding a median of 40.23 ng cfDNA/ml plasma (range 7.55 – 423.33 ng/ml plasma).

### Tissue processing

Core biopsies received at the pathology laboratory were tagged with both the unique AMBER study code and routine histopathology accession number after which they were placed in cassettes labeled with the accession number for processing. Three cores were placed in each cassette and the cassette placed in 10 % neutral buffered formalin. Tissue processing was carried out with the Sakura VIP Tissue TEK processor. After processing the tissue was embedded, trimmed and sectioned at a thickness of 3 µm. Sections were placed on slides and oven-fixed for 30 min at a temperature of 73 °C. Sections were then sent back to water and stained, mounted and cover slipped for reporting. Cores with adequate, viable tumor were marked during reporting and the matching blocks identified. Additional slides were then prepared for immunohistochemistry using quoted slides. Immunohistochemistry for estrogen receptor (ER), progesterone receptor (PR), Human epidermal growth factor receptor (Her-2) and the Ki67 was done using the fully automated Benchmark GX ventanna from Roche. Relevant marked sections of tumor were sectioned from the selected blocks for downstream tissue NGS. On average 14 unstained sections per sample were submitted for downstream NGS.

### Tissue DNA and RNA extraction

Extraction of nucleic acid from tissue samples was performed as described previously [17]. For each sample, Formalin-fixed paraffin-embedded (FFPE) sections with minimally 30 % tumor content were used for simultaneous DNA and RNA extraction using Norgen Biotek

RNA/DNA FFPE Purification Plus Kit (Cat No 54300), following the manufacturer's recommendations. The extracted nucleic acids were subsequently quantified using Qubit dsDNA HS kit (Cat No. Q32851) and Qubit RNA HS Kit (Cat No Q32855). Nucleic acid was quality controlled using Agilent Genomic DNA TapeStation and Agilent High Sensitivity RNA TapeStation on a TapeStation 4200, following the manufacturer's protocol.

#### Tissue NGS using Tissue500™ technology

Sequencing libraries were constructed using DNA and RNA extracted from Formalin-fixed Paraffin-Embedded (FFPE) tissues. DNA library constructs were used for identifying 572 genes, whereas the RNA libraries were used to identify 71 genes (Supplementary Table 1) using the Tissue500™ assay. Quality control and concentration of constructed libraries were determined and then sequenced on an Illumina NextSeq instrument with 2×150 paired-end reads. Bioinformatics data analysis was performed using an in-house bioinformatics pipeline developed by Lucence, and a proprietary sequencing error-correction methodology applied on raw sequencing data.

#### Liquid biopsy ctDNA analysis using LiquidHALLMARK™ technology

Amplicon-based targeted sequencing analysis of cfDNA was performed using LiquidHALLMARK™, a proprietary technology owned by Lucence Health Inc. Cell-free DNA extracted from plasma samples were used to construct DNA libraries for genes targeted by the LiquidHALLMARK™ assay (Supplementary Table 2). Quality control and concentration of constructed libraries were determined using a KAPA library quantification kit and sequenced on an Illumina NextSeq instrument with 2×150 paired-end reads. Using an in-house bioinformatics pipeline developed by Lucence, sequence data were analyzed for structural rearrangement (fusion), and other genetic alterations including deletions/insertions in homopolymeric regions associated with six microsatellite loci (BAT25, BAT26, NR21, NR24, NR27, MONO27). Sequences were aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Copy number changes were calculated based on adjusted read count, and its variation from normalized baseline read count was determined across control samples. Genomic findings and clinical actionability were extracted from reports generated by Lucence. All sequence alterations are described according to the Human Genome Variation Society (HGVS) nomenclature guidelines [18]. Variant allele frequency (%) and copy number fold change (FC) are reported for sequence variants and copy number alterations respectively. For this

study, copy number alterations were classified as amplification ( $FC \geq 4.0$ ), gain ( $1.5 \leq FC < 4.0$ ), loss ( $0.5 \leq FC < 1.0$ ), deletion ( $FC < 0.5$ ) using FC rounded to one decimal place. Clinical actionability of genomic findings is determined based on curated databases from publicly available data sources, including peer-reviewed publications of genomic alterations and biomarkers and associated drugs. Categorization of Clinical actionability is guided by Association for Molecular Pathology/American Society of Clinical Oncology/College of American Pathologists (AMP/ASCO/CAP) consensus recommendation for the interpretation and reporting of sequence variants in cancer [19,20]. Clinical actionability could be designated sensitive or resistant to a given therapy and corresponds to Tier 1 evidence level (FDA, NCCN guidelines, well-powered studies with expert consensus), of the AMP/ASCO/CAP consensus recommendations. Genomic alterations were visualized using the maftools package [21] in the R programming language for statistical computing (<https://www.R-project.org/>).

The complete study workflow is demonstrated in Fig. 1 below.

## Results

### Clinical and molecular features of patients

We recruited 20 Ghanaian patients with metastatic breast cancer into the study (Table 1). Triple-negative breast cancer was the most common subtype, representing 42% (8/19), of total samples tested. Luminal B (HER2-) and Luminal B (HER2+) each made up 21% (4/19) of total samples tested, while Luminal A and Non-Luminal (HER2+) made up 5% (1/19), and 11% (2/19) of total samples tested respectively. Subtype classification was not successful for one sample.

### Actionable genomic alterations detected in plasma cfDNA from metastatic breast cancer cases

We performed high-coverage targeted next-generation gene panel sequencing on plasma cfDNA samples from these 20 Ghanaian metastatic breast cancer cases using the LiquidHALLMARK™ test. Mean read depth per sample ranged from 7003.96x to 12,100.58x. We found genomic alterations including single nucleotide variants (SNVs), insertions and deletions (indels), and copy number alterations (CNAs) in 17 (85%) of the samples tested (Fig. 2). *TP53*, *PIK3CA*, and *BRCA2* are among the top mutated genes while *MYC*, *ERBB2*, and *CCND1* were involved in the majority of copy number alterations in our samples. Most samples were microsatellite instability stable (MSI-stable) with only 1 sample found to have low microsatellite instability (MSI-L).

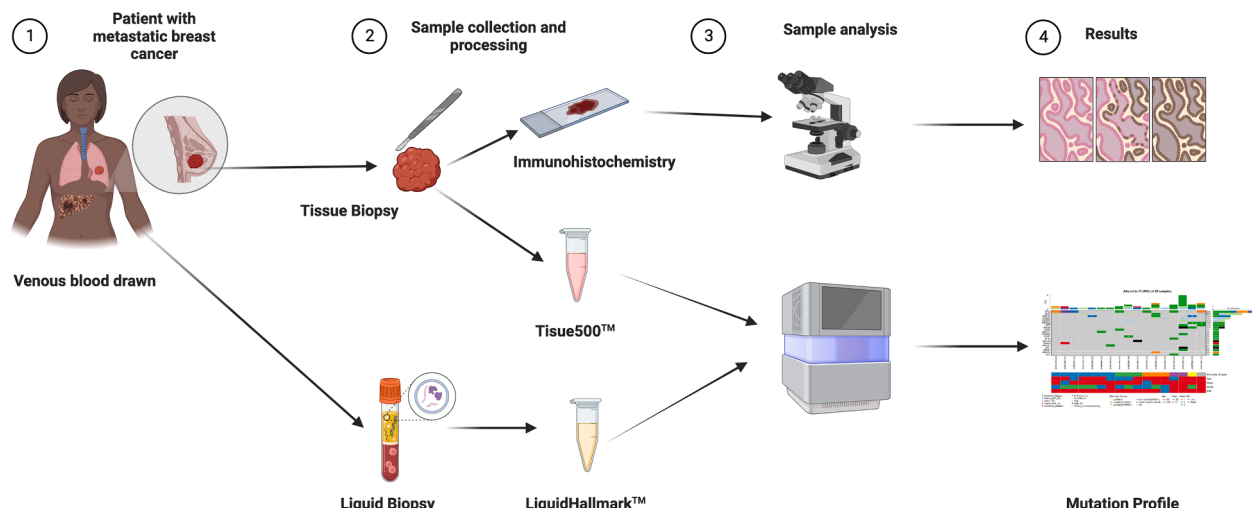


Fig. 1. Study workflow and methodology. Created with BioRender.com.

**Table 1**  
Clinical and molecular characteristics of AMBER patients tested with LiquidHALLMARK™.

| Sample      | Age at diagnosis | Stage | Grade | HR & HER2 status | Molecular subtype        |
|-------------|------------------|-------|-------|------------------|--------------------------|
| AMBER01-001 | 37               | IV    | 3     | HER2 - ER- PR -  | Triple negative (Ductal) |
| AMBER01-002 | 46               | IV    | 2     | HER2 - ER+ PR+   | Luminal A                |
| AMBER01-003 | 98               | IV    | 2     | HER2 - ER- PR -  | Triple negative (Ductal) |
| AMBER01-004 | 39               | IV    | 1     | HER2+ ER- PR-    | Non-luminal (HER2+)      |
| AMBER01-005 | 43               | IV    | 1     | NA               | NA                       |
| AMBER01-006 | 52               | IV    | 1     | HER2+ ER- PR-    | Non-luminal (HER2+)      |
| AMBER01-007 | 45               | IV    | 2     | HER2 - ER- PR -  | Triple negative (Ductal) |
| AMBER01-008 | 42               | IV    | 3     | HER2 - ER- PR -  | Triple negative (Ductal) |
| AMBER01-009 | 55               | IV    | 2     | HER2- ER+ PR+    | Luminal B (HER2-)        |
| AMBER01-010 | 46               | IV    | 2     | HER2 - ER- PR -  | Triple negative (Ductal) |
| AMBER01-021 | 47               | IV    | 2     | HER2 - ER- PR -  | Triple negative (Ductal) |
| AMBER01-022 | 38               | IV    | 3     | HER2+ ER+ PR+    | Luminal B (HER2+)        |
| AMBER01-023 | 58               | IV    | 1     | HER2 - ER- PR -  | Triple negative (Ductal) |
| AMBER01-024 | 47               | IV    | 2     | HER2 - ER- PR -  | Triple negative (Ductal) |
| AMBER01-025 | 36               | IV    | 2     | HER2+ ER+ PR-    | Luminal B (HER2+)        |
| AMBER01-031 | 40               | IV    | 3     | HER2- ER+ PR+    | Luminal B (HER2-)        |
| AMBER01-032 | 43               | IV    | 3     | HER2+ ER+ PR+    | Luminal B (HER2+)        |
| AMBER01-033 | 51               | IV    | 3     | HER2- ER+ PR+    | Luminal B (HER2-)        |
| AMBER01-034 | 41               | IV    | 3     | HER2+ ER+ PR-    | Luminal B (HER2+)        |
| AMBER01-035 | 50               | IV    | 3     | HER2- ER+ PR+    | Luminal B (HER2-)        |

Molecular subtypes are based on histology and immunohistochemistry expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and proliferation marker Ki67. HR - Hormone receptor.

Copy number alterations were consistent with molecular subtypes defined by immunohistochemistry with *ERBB2* amplification observed in two HER2-positive samples. For a subset of cases with matching tumor tissue ( $n=6$ ), we found 13 alterations. Of that number, 92% (12/13) of plasma cfDNA genomic alterations were found in the matching tumor tissue sample (Table 2). Of note, 3 participants had mutations previously associated with African ancestry [22,23], including *BRCA1* p. K719E, *ARAF* p.S262I and *GATA3* p.G125dup. Tumor tissue and plasma cfDNA samples were sequenced to similar read depth. Genomic alterations with potentially strong clinical actionability (as described above) were found in plasma cfDNA from six (30%) samples (Table 3).

Discussion

Our study reveals potentially actionable genomic alterations in 30 % (6/20) of Ghanaian metastatic breast cancer cases. This study illustrates the feasibility of detecting actionable genomic alterations from liquid biopsy genomic profiling and follows prior work by others showing that circulating tumor DNA is readily detectable for copy number profiling from liquid biopsy samples from Ghanaian breast cancer cases [10]. We found alterations with strong clinical actionability based on US FDA approval and NCCN guidelines that could form the basis for choosing

targeted treatments for patients with metastatic breast cancer who may not respond to conventional treatment. These alterations comprise mutations in *PIK3CA*, *BRCA2*, and *ESR1*, as well as copy number alterations in *ERBB2*. Corresponding targeted therapies for these alterations include PARP inhibitors (Olaparib or Talazoparib) for *BRCA2* mutation, PI3K inhibitor in combination with ER antagonist (Alpelisib + Fulvestrant) for *PIK3CA* mutation, ER antagonist (Elaeestrant) for *ESR1* mutation, and anti-HER2 monoclonal antibodies or antibody-drug conjugates for *ERBB2* copy number amplification (Table 2; Supplementary Table 3).

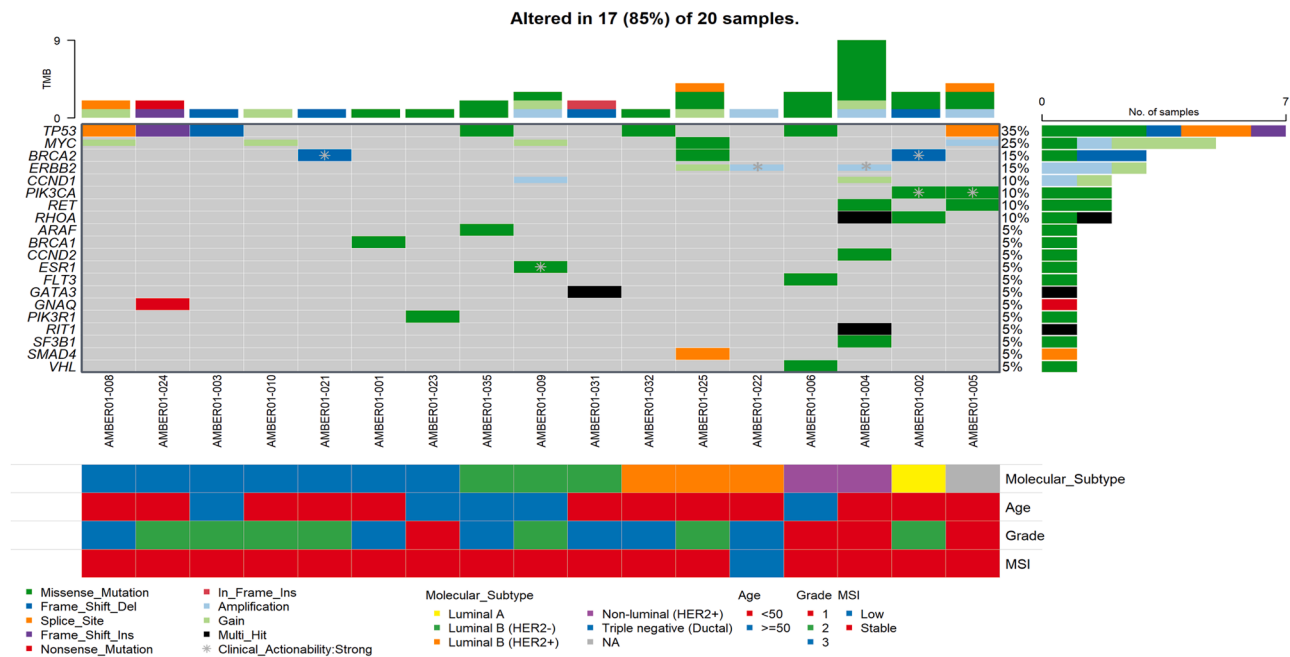
Genomic alterations with strong clinical actionability were found in plasma cfDNA from six (30%) samples (Table 3). These alterations are known to confer sensitivity or resistance to specific targeted therapies. For two samples with *BRCA2* mutations, the poly (ADP-ribose) polymerase (PARP) inhibitors Olaparib [24] and Talazoparib [25] [26], are available targeted therapies. Olaparib and Talazoparib are approved for the treatment of HER2-negative metastatic breast cancer with deleterious or suspected deleterious germline mutation in *BRCA1* or *BRCA2* [27],[28]. Both *BRCA2*-mutated cases in our study are HER2-negative although AMBER01-002 is hormone receptor-positive (ER and PR) while AMBER01-021 is Triple Negative. However, the *BRCA2* mutation p.C1948Wfs\*11 in AMBER01-002 is not germline given its low VAF (0.78%). On the other hand, the *BRCA2* p.T630Qfs\*12 mutation in AMBER01-021 is likely of germline origin as it occurs at 54.45% VAF in plasma cfDNA and is also found at 70.20% VAF in tumor tissue suggesting loss of heterozygosity in the tumor. We will need further testing of whole blood DNA to determine the germline status for these variants.

Two cases (AMBER01-002, AMBER01-005) with *PIK3CA* mutations, including one case which also has a *BRCA2* mutation, could potentially be eligible for treatment with phosphatidylinositol 3-kinase (PI3K) inhibitor Alpelisib in combination with estrogen receptor antagonist fulvestrant (Fulvestrant) [29,30]. This combination is approved for HER2-negative, hormone receptor-positive, *PIK3CA*-mutated advanced or metastatic breast cancer following progression on or after an endocrine-based treatment regimen [31,32]. While AMBER01-002 is hormone receptor-positive, the hormone receptor status of AMBER01-005 is unknown because molecular subtype classification was not successful due to insufficient tissue sample for immunohistochemistry.

Two HER2-positive cases with *ERBB2* amplification could potentially be treated with anti-HER2 monoclonal antibodies Trastuzumab or Margetuximab, or antibody-drug conjugates Trastuzumab Deruxtecan, Trastuzumab Emtansine as monotherapy or combination are common treatment options [33-36]. Additionally, HER2 inhibitors (neratinib, lapatinib, and pyrotinib) are also treatment options [37-39].

For an *ESR1*-mutated, HER2-negative and ER-positive case (AMBER01-009), the estrogen receptor antagonist Elaeestrant is a potential treatment option [40]. However, *ESR1* mutations are associated with resistance to anti-estrogen drugs letrozole (Femara) and Exemestane [41,42].

While detecting actionable genomic alterations is an important first step to identifying targeted therapies, the choice of targeted therapy for a given patient is also based on variant allele fraction or clonality of alteration, prior treatment(s) response, molecular subtype or features of tumor, indications and contraindications of targeted therapy, among others. Variant allele frequency of actionable alteration can affect targeted treatment outcome. In NSCLC patients treated with tyrosine kinase inhibitors, patients with high variant allele frequency *EGFR* mutations were found to have improved progression-free survival compared to patients with low variant allele frequency *EGFR* mutations [43]. Ongoing treatment and treatment outcomes for cases in our study are unknown. Therefore, it is possible that not all cases harboring actionable alterations will ultimately receive targeted therapy even if they were available in Ghana. Conventional treatment for metastatic breast cancer in Ghana involves surgery, chemotherapy (adjuvant or neoadjuvant) and radiation with limited use of targeted therapies [44].



**Fig. 2.** Genomic alterations in plasma cfDNA samples from LiquidHALLMARK™ assay. Alterations with strong clinical actionability in breast cancer are indicated with an asterisk (\*). Molecular subtype, age group, and grade, and microsatellite instability (MSI) status are annotated for each sample in the bottom panel. NA - Not available.

**Table 2**  
Genomic alterations shared between plasma cfDNA and tumor tissue or unique to either sample.

| Sample      | Gene  | Alteration             | Clinical Actionability | Plasma cfDNA% VAF or copy number fold change | Tumor tissue% VAF or copy number fold change |
|-------------|-------|------------------------|------------------------|----------------------------------------------|----------------------------------------------|
| Shared      |       |                        |                        |                                              |                                              |
| AMBER01-009 | ESR1  | p.Y537S                | Strong                 | 14.16                                        | 4.00                                         |
| AMBER01-009 | CCND1 | Amplification/<br>Gain | Uncertain              | 4.55                                         | 3.39                                         |
| AMBER01-009 | MYC   | Gain                   | Uncertain              | 3.06                                         | 2.40                                         |
| AMBER01-021 | BRCA2 | p.T630Qfs*12           | Strong                 | 54.45                                        | 70.20                                        |
| AMBER01-022 | ERBB2 | Amplification          | Strong                 | 7.42                                         | 7.94                                         |
| AMBER01-024 | TP53  | p.C124Wfs*25           | Uncertain              | 21.04                                        | 34.20                                        |
| AMBER01-024 | GNAQ  | p.Q176*                | Uncertain              | 12.77                                        | 27.80                                        |
| AMBER01-025 | MYC   | p.E42D                 | Uncertain              | 52.99                                        | 47.20                                        |
| AMBER01-025 | SMAD4 | c.788-1G>C             | Uncertain              | 3.17                                         | 13.80                                        |
| AMBER01-025 | ERBB2 | Gain/<br>Amplification | Uncertain/Strong       | 3.31                                         | 9.88                                         |
| AMBER01-035 | TP53  | p.R273C                | Uncertain              | 13.01                                        | 48.40                                        |
| AMBER01-035 | ARAF  | p.S262I                | Uncertain              | 51.57                                        | 50.70                                        |
| Unique      |       |                        |                        |                                              |                                              |
| AMBER01-025 | BRCA2 | p.Q1379E               | Uncertain              | 0.86                                         | –                                            |

Where alteration is different between plasma cfDNA and tumor tissue samples, Alteration column is represented as Classification in plasma cfDNA/Classification in tumor tissue. For example, Amplification/Gain refers to copy number amplification in plasma cfDNA but copy number gain in tumor tissue sample for a specific gene.

Apart from Trastuzumab, none of the other targeted therapies is available in Ghana. In 2020 Ghana added Trastuzumab to the National Health Insurance Scheme (NHIS) medicines list, making it affordable for patients [45]. However, it can only be prescribed for one year following diagnosis; irrespective of metastatic disease status. The current administration of Trastuzumab in Ghana is based on HER2-positive status from IHC. While the addition of Trastuzumab to Ghana's NHIS coverage is a positive step, further steps are needed to ensure access to targeted therapies for patients who may benefit from them. There are currently no standardized national treatment guidelines for breast cancer treatment in Ghana, however, some clinicians adopt the National Comprehensive Cancer Network (NCCN) harmonized treatment guidelines for sub-Saharan Africa [46,47].

A limitation of our study is the absence of *PALB2* in the LHM panel. Rare, germline pathogenic variants in *PALB2* present substantial risk for breast cancer development in Ghanaian women [4]. Additionally,

pathogenic *PALB2* mutations have potential clinical actionability based on reports of sensitivity to PARP inhibitors Talazoparib or Olaparib from clinical trial [25] and case study [48] respectively. Subsequent studies will use custom gene panels including additional relevant breast cancer genes such as *PALB2*, *CHEK2*, and *RAD51* paralogs. Another limitation was the inability to sequence all matching tissue with blood samples. Most of the FFPE samples did not pass quality control. Some of the reasons cited include heavily fragmented DNA/RNA and poor DNA yield. These could have resulted from formalin overfixation and temperature variations. Subsequent studies will incorporate carefully optimized tissue handling protocols to avoid over- or under-fixation. The fact that liquid biopsy proved to be more reliable (all samples passed quality control) provides some rationale for the preferential use of cfDNA NGS over tissue NGS in resource-limited settings. Last, the sample size for this study was too small to draw relevant conclusions.

**Table 3**  
Samples harboring alterations with strong clinical actionability in breast cancer.

| Sample      | Gene   | Alteration (% VAF or copy number fold change) | Targeted Therapies                                                                                                                                                                                                       |
|-------------|--------|-----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AMBER01-002 | PIK3CA | p.E542K (2.11 %)                              | Alpelisib + fulvestrant (Fulvestrant) (Fulvestrant)                                                                                                                                                                      |
| AMBER01-002 | BRCA2  | p.C1948Wfs*11 (0.78 %)                        | Olaparib, or Talazoparib                                                                                                                                                                                                 |
| AMBER01-005 | PIK3CA | p.H1047R (33.16 %)                            | Alpelisib + fulvestrant (Fulvestrant) (Fulvestrant)                                                                                                                                                                      |
| AMBER01-009 | ESR1   | p.Y537S (14.16 %)                             | Sensitive – Elacestrant; Resistant – letrozole (Femara), Exemestane                                                                                                                                                      |
| AMBER01-021 | BRCA2  | p.T630Qfs*12 (54.45 %)                        | Olaparib, or Talazoparib                                                                                                                                                                                                 |
| AMBER01-004 | ERBB2  | Amplification (9.31)                          | Trastuzumab Deruxtecan, Margetuximab, Trastuzumab Emtansine, Neratinib, Lapatinib, Trastuzumab + Tucatinib, Trastuzumab, Pertuzumab + Trastuzumab, Lapatinib + letrozole (Femara), Lapatinib + Trastuzumab, or Pyrotinib |
| AMBER01-022 | ERBB2  | Amplification (7.42)                          | Trastuzumab Deruxtecan, Margetuximab, Trastuzumab Emtansine, Neratinib, Lapatinib, Trastuzumab + Tucatinib, Trastuzumab, Pertuzumab + Trastuzumab, Lapatinib + letrozole (Femara), Lapatinib + Trastuzumab, or Pyrotinib |

Plus sign (+) indicates drug combination.

**Conclusion**

We used liquid biopsy genomic profiling to detect clinically actionable genomic alterations and identify potential targeted therapies for prospective metastatic breast cancer cases in Ghanaian women. Our work provides initial justification for future larger studies that would form the basis for broad application of genomic and molecular profiling to facilitate integration of targeted therapies into breast cancer management, attract clinical trials for evaluating emerging targeted therapies, and overall advancement of genomic and molecular characterization of cancers for precision oncology discovery and implementation in Ghana and other African countries.

**Funding**

This work was funded by Yemaachi Biotech and Lucence Health Inc.

**CRediT authorship contribution statement**

**Emmanuella Amoako:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Setor Amuzu:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Emmanuel Owusu Ofori:** Writing – original draft, Project administration, Methodology, Investigation. **Harry Sefoga Akligoh:** Writing – original draft, Methodology, Investigation, Data curation. **Randy Tackie:** Methodology, Investigation, Data curation. **Barikisu Anna Ibrahim:** Methodology, Investigation. **Emmanuel Kofi Quaye:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Patrick Kafui Akakpo:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Luke Adagrah Aniakwo:** Methodology, Investigation. **Bashiro Jimah:** . **Kofi Ulzen-Appiah:** Methodology, Investigation. **David Hutchful:** Writing – original draft, Software,

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**Declaration of competing interest**

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Min-Han Tan is a director, shareholder and Chief executive officer of Lucence. Yaw Bediako is a director, shareholder and Chief executive officer of Yemaachi Biotech.

**Acknowledgments**

We would like to acknowledge Shrestha Bhatia, Lauren Delgado, Immanuel Imbeah, Dr. Kwablah Grant, and Patience Koggoh for their contributions to this paper.

**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.tranon.2024.102100](https://doi.org/10.1016/j.tranon.2024.102100).

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