Circulating tumor DNA-based predictive biomarkers in breast cancer clinical trials: a narrative review

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Abstract: Breast carcinoma is the most frequent and the second leading cause of cancer mortality in women worldwide. Current treatment decisions are based on tumor profiling of the initial tissue biopsy. Cancer though evolves both spatially and temporarily in a significant percentage of patients during treatment. However, sequential biopsies from the primary tumor or its metastatic sites are not either convenient or feasible in the majority of cases. In the era of precision medicine, analysis of circulating blood-based biomarkers in the field of liquid biopsies provides an insight into the dynamic molecular profiling of the primary tumor and its metastases, in a relatively non-invasive way. The latter permits not only patient stratification but also longitudinal evaluation of treatment response, when incorporated into clinical trials. This review summarizes the results from recent and ongoing circulating tumor DNA (ctDNA)-based biomarker-driven clinical trials, with respect to ctDNA analysis’ predictive role, both in adjuvant, neo-adjuvant, and metastatic setting. Furthermore, current challenges in ctDNA analysis applications are critically discussed, including pre-analytical and analytical issues, and future perspectives in this field, through the conduct of well-designed, multicenter, randomized, large-scale, biomarker-stratified trials, with robust statistical methods. Despite in its infancy, ctDNA analysis holds great promise as a minimally invasive tool regarding tailored, personalized treatment guidance for breast cancer patients.

Keywords: Breast cancer; circulating tumor DNA (ctDNA); clinical trials; predictive biomarkers

Introduction

Breast cancer is the most prevalent cancer and the second leading cause of cancer mortality in women (1,2). Next-generation sequencing (NGS)-based diagnostics have identified around 40 genomic alterations, shedding light into the heterogeneity of this disease (3,4). Currently, only a few of these somatic alterations have been validated as therapeutic targets, whereas, there are multiple targeted therapies, effective as signalling blockade, in the adjuvant, neo-adjuvant, and metastatic settings.

In particular, trastuzumab (5-7), pertuzumab (8-10), ado-trastuzumab emtansine (11), lapatinib (12) and neratinib (13)
are human epidermal growth factor receptor 2 (HER2) inhibitors for the treatment of HER2+ disease; palbociclib (14), ribociclib (15) and abemaciclib (16) are cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors for the treatment of hormone-receptor (HR)+, HER2- disease, in combination with hormonal treatment, like aromatase inhibitors (AIs), tamoxifen or fulvestrant; everolimus (17) is a mammalian target of rapamycin (mTOR) inhibitor, also, for the treatment of HR+, HER2- disease, in combination with hormonal treatment; olaparib (18) and talazoparib (19) are poly adenosine diphosphate (ADP) ribose polymerase (PARP) inhibitors for the treatment of BRCA gene (BRCA)+ disease; while alpelisib (20) is a phosphoinositide-3-kinase (PI3K) inhibitor for the treatment of phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA)+ disease.

Despite the rapid advance in personalized medicine strategies, metastatic breast cancer remains an incurable disease, with a 5-year survival rate of approximately 25% (21,22). Breast cancer's plasticity, over time and under treatment pressure, represents the greatest challenge in its therapeutics, due to disease recurrence and drug resistance (23,24). Thus, both American and European guidelines recommend reassessment of biomarkers, like HR and HER2 status, if feasible, in the metastatic setting (25).

Unfortunately, tissue biopsies are fraught with several caveats; they are invasive, patient-unfriendly procedures, not always feasible either because of patient's condition and comorbidities or because of tumor's accessibility, and they don't permit longitudinal monitoring of tumor (26-30). Thus, the ideal approach to address the diverse molecular profile of breast tumors would be a minimally invasive method that could capture the entire genetic make-up of the tumor, in 'real-time', during the course of treatment. Currently, analysis of circulating blood biomarkers, like circulating tumor DNA (ctDNA), under the umbrella-term of 'liquid biopsies', offers an attractive approach to evaluate patient's entire tumor burden, in a non-invasive, convenient, repetitive, dynamic, and cost-effective way (27,31-36).

Several studies have evaluated the emerging role of ctDNA in monitoring treatment response or resistance and in predicting early relapse (37-48). Nevertheless, studies investigating the potential capacity of serial ctDNA monitoring for treatment guidance are still scarce, small-scale, and lack a strict clinically-centered protocol. To the best of our knowledge, there is no other review focused on the incorporation of ctDNA-based predictive biomarkers in breast cancer patients enrolled in clinical trials, therefore, we performed this review of the published literature, to assess the potential of ctDNA in optimizing disease management.

We present the following article in accordance with the Narrative Review reporting checklist (available at http://dx.doi.org/10.21037/atm-20-1175).

Methods

A review of published literature was conducted to assess the predictive value of ctDNA analysis in the setting of clinical trials in breast cancer patients. All eligible studies were identified by a search in www.clinicaltrials.gov, MEDLINE/PubMed database and Cochrane Database of Systematic Reviews (CDSR) for the period up to August 31, 2019. Clinical Trials incorporating ctDNA analysis, as source of potential predictive biomarkers, in patients with breast cancer were considered for inclusion. To create a search strategy, medical subject heading (MeSH) terms [breast, cancer, neoplasm, carcinoma, clinical trial, ctDNA, cell free DNA (cfDNA), predictive, biomarker] were used in addition with Boolean search terms (AND, OR).

Eligible for inclusion were considered all randomised and non-randomised clinical trials carried out in adult patients (≥18 years old), irrespective of gender, with breast cancer, reporting results of ctDNA analysis and its correlation with treatment efficacy. Abstracts presented in conferences were also included.

Language restrictions were applied (only articles published in English were considered eligible). Animal studies, book chapters, observational study designs, commentaries, case reports, reviews, meta-analyses and studies not in cancer patients were also excluded.

The following data were extracted from each clinical trial: clinical trial name and Identification number (ID number), status, first author, year of publication, setting (primary or advanced breast cancer), line of therapy (neo-adjuvant, adjuvant, and 1st or 2nd line for metastatic setting, etc.), pathological subtype/hormonal status, allocation of study (randomized, non-randomized), intervention model (sequential-, parallel-, single group-assignment), masking, phase, treatment modalities (intervention and control arm regimens), number of patients enrolled in biomarker sub-study, primary endpoint, ctDNA sequencing technique, results.

Results

Our search strategy retrieved initially 64 clinical trials,
which were screened at title and abstract (if it was available) using the study inclusion criteria. In 43 ongoing clinical trials no data were mature, one was observational study, thus 20 clinical trials, containing data on 5,890 patients with evaluable ctDNA analyses, were finally eligible for this review. Characteristics of studies are presented in Table 1.

Preliminary results from Palbociclib and Circulating Tumor DNA for estrogen receptor-1 gene (ESR1) Mutation Detection (PADA-1) trial demonstrated that ESR1mut detection is uncommon in untreated AI-sensitive, ER+, HER2− metastatic breast cancer patients (detection rate of 2.1% at baseline) and is related to prior AI exposure in the adjuvant setting (4.9% with AI use vs. 0% without AI use, Yates Chi²: P=0.009). Remarkably, 1-month use of AI and palbociclib, the first CDK4/6 inhibitor approved as an anticancer regimen, led to undetectable ESR1mut in 13 among the 17 patients with ESR1mut detected at baseline (49).

In the PALOMA-3 study, which compared the combination of palbociclib plus fulvestrant to placebo plus fulvestrant, in patients with HR+, HER2− advanced breast cancer, progressing on prior endocrine therapy, changes in PIK3CA ctDNA dynamics upon 15 days treatment predicted response to targeted therapy in combination with fulvestrant (HR 3.94, 95% CI, 1.61–9.64, log-rank P=0.0013), while ESR1 ctDNA levels change was less predictive on progression free survival (PFS) on palbociclib plus fulvestrant (14,43). Detection of PIK3CA and estrogen receptor-2 gene (ESR2) mutations in plasma ctDNA samples, compared with their detection in archived tissue samples, has been associated with significantly improved PFS and response to abemaciclib (another selective CDK4/6 inhibitor) plus fulvestrant, in postmenopausal women with HR+, HER2− advanced breast cancer, progressing on prior endocrine therapy (62,63).

On the contrary, ctDNA sequencing from 494 patients enrolled in the randomized MONALEESA-2 trial of letrozole ± ribociclib, showed a consistent PFS benefit for the combination of endocrine therapy plus CDK4/6 inhibitor, regardless of the baseline status of ctDNA biomarkers [PIK3CA, tumor protein 53 (TP53), Zinc finger protein 703 (ZNF703)/fibroblast growth factor receptor 1 (FGFR1), ESR1] (15,51). Consistent treatment benefit was observed for fulvestrant and ribociclib, irrespective of baseline ctDNA alteration status [PIK3CA, ESR1, TP53, CDC20 homolog 1 (CDH1), FGFR1/ZNF703/Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1)] in Phase III MONALEESA-3 study (52,53).

In BELLE-2, which evaluated the combination of the panPI3 kinase inhibitor buparlisib with fulvestrant in patients with refractory to AI, HR+, HER2− advanced breast cancer, the presence of PIK3CA mutations in ctDNA corresponded to improved PFS in the buparlisib arm (7.0 vs. 3.2 months; HR =0.58; 95% CI, 0.41–0.82; 1-sided nominal P=0.001) (54,55). Clinical benefit of the addition of buparlisib to fulvestrant in HR+, HER2− advanced breast cancer patients, with prior use of mTOR inhibitors, has also been observed in the randomized Phase III BELLE-3 trial, even if this benefit was irrespective of PIK3CA status in ctDNA (56). Both, BELLE-2 and BELLE-3 highlighted the potential of PIK3CA mutational status in plasma ctDNA as predictive biomarker for benefit of buparlisib treatment, in this subset of breast cancer patients; whereas the discordance in PIK3CA status between tumor tissue and ctDNA samples (76.7% in BELLE-2 vs. 84.8% in BELLE-3) underline the need for an optimal standardized assay.

In a single group assignment, Phase I/II trial the combination of alpelisib and nab-paclitaxel resulted in increased PFS in HER2− advanced breast cancer patients, harbouring ctDNA PIK3CA mutations (66).

A subsidiary analysis of the BOLERO-2 trial on 550 ER+ advanced breast cancer patients, demonstrated that the addition of everolimus to exemestane prolonged PFS, irrespective of cfDNA PIK3CA mutation status (HR =0.43 and 0.37 respectively) (17,69).

Furthermore, the ongoing POSEIDON trial and Neratinib HER Mutation Basket Study (SUMMIT) support the predictive value of early evaluation of ctDNA changes, before radiologic treatment response (58,59).

The translational sub-study of the ongoing I-SPY 2 trial demonstrated the significance of serial monitoring of ctDNA in predicting response to neo-adjuvant treatment (61). ctDNA analysis of the NeoALTTO trial demonstrated that the detection of PIK3CA and/or TP53 mutations, in the baseline (before neo-adjuvant therapy) plasma sample was correlated with lower rates of pathological complete response, whereas persistent ctDNA detection both at baseline and after 14 days of neo-adjuvant therapy was significantly associated with the lowest rate of pathological complete response (71,72).

In open-label WJOG6110B/ELTOP trial, whereas patients with HER2+ advanced breast cancer, were randomized to receive either lapatinib and capecitabine or trastuzumab and capecitabine, PIK3CA mutations in both tissue and ctDNA samples associated with shorter PFS, regardless of the treatment arm (57). The presence
<table>
<thead>
<tr>
<th>Clinical trial (name/ID number)</th>
<th>Status</th>
<th>Design</th>
<th>Intervention model</th>
<th>Setting</th>
<th>Population characteristics</th>
<th>Intervention vs. Control arm</th>
<th>Enrollment (biomarker analysis)</th>
<th>Patients (%) with detectable ctDNA</th>
<th>Endpoints</th>
<th>ctDNA sequencing technique</th>
<th>Concordance of tissue and plasma samples</th>
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<th>References</th>
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<tbody>
<tr>
<td>PADA-1/ NCT03079011</td>
<td>Active, not recruiting</td>
<td>Open label, randomized, phase III</td>
<td>Sequential assignment</td>
<td>1st line (metastatic setting)</td>
<td>ER+, HER2−, postmenopausal female, ECOG PS: 0–2</td>
<td>Palbociclib + AI vs. palbociclib + fulvestrant</td>
<td>803</td>
<td>17/803 (2.1%)</td>
<td>Safety, efficacy</td>
<td>ddPCR-based assay</td>
<td>76.47% of patients had undetectable ESR1m after 1 month of palbociclib + AI therapy</td>
<td>(49)</td>
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<tr>
<td>SOLAR-1/ NCT02437318</td>
<td>Active, not recruiting</td>
<td>Triple blind, randomized (1:1), phase II</td>
<td>Parallel assignment</td>
<td>2nd line (metastatic setting)</td>
<td>PIK3CAM, HR+, HER2−, male or postmenopausal female, 1 prior line of endocrine therapy, ECOG PS: 0–1</td>
<td>Alpelisib + fulvestrant vs. placebo + fulvestrant</td>
<td>549</td>
<td>186/549 (33.87%)</td>
<td>PFS</td>
<td>Assay developed by Ogen</td>
<td>94.7% PFS of 3.7 months for tissue PIK3CAM and of 10.9 months for ctDNA PIK3CAM. Treatment benefit, with the combination of alpelisib and fulvestrant, in PFS for patients with ctDNA PIK3CAM, irrespective of prior treatment for advanced breast cancer and/or prior CDK4/6 inhibitors use</td>
<td>(20,50)</td>
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<td>MONALEESA-2/ NCT01958021</td>
<td>Active, not recruiting</td>
<td>Double blind, randomized (1:1), phase III</td>
<td>Parallel assignment</td>
<td>1st line (metastatic setting)</td>
<td>HR+, HER2−, postmenopausal female, ECOG PS: 0–1</td>
<td>Ribociclib + letrozole vs. placebo + letrozole</td>
<td>494</td>
<td>427/494 (86%)</td>
<td>PFS</td>
<td>NGS</td>
<td>ctDNA genomic alterations: PIK3CA (33%), TP53 (12%), ZNF703/FGFR1/WHSC1L1 (11%), ESR1 (4%), and in genes involved in RTK signaling (12%). Treatment benefit, with the combination of ribociclib and letrozole, irrespective of ctDNA genetic alterations at baseline</td>
<td>(15,51)</td>
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<tr>
<td>MONALEESA-3/ NCT02422615</td>
<td>Active, not recruiting</td>
<td>Double blind, randomized (1:1), phase III</td>
<td>Parallel assignment</td>
<td>≤2nd line (metastatic setting)</td>
<td>HR+, HER2−, postmenopausal female, ≤1 prior line of endocrine therapy ECOG PS: 0–1</td>
<td>Ribociclib + fulvestrant vs. placebo + fulvestrant</td>
<td>600</td>
<td>124/600 (20.66%) for PIK3CAM</td>
<td>PFS</td>
<td>NGS</td>
<td>ctDNA genomic alterations: PIK3CA (35%), ESR1 (14%), TP53 (19%), CDH1 (12%), FGFR1/ZNF703/WHSC1L1 (11%), CCC genes (16%), genes involved in RTK signaling (20%) and genes involved in the MAPK pathway (10%). Treatment benefit, with the combination of ribociclib and fulvestrant, irrespective of ctDNA genetic alterations; shorter PFS was correlated with altered genetic status</td>
<td>(52,53)</td>
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<td>BELLE-2/ NCT01610284</td>
<td>Completed</td>
<td>Double blind, randomized (1:1), phase III</td>
<td>Parallel assignment</td>
<td>2nd line (metastatic setting)</td>
<td>HR+, HER2−, postmenopausal female, AI-refractory disease</td>
<td>Buparlisib + fulvestrant vs. placebo + fulvestrant</td>
<td>587</td>
<td>200/587 (34%)</td>
<td>PFS</td>
<td>Sanger sequencing</td>
<td>64 of 307 (21%) patients with PIK3CAM tumour tissue had PIK3CAM ctDNA, indicating evolution between initial diagnosis and the present time, ctDNA PIK3CAM corresponded to improved median PFS in the buparlisib arm (7.0 vs. 3.2 months; HR =0.58; 95% CI: 0.41–0.82; 1-sided nominal P =0.001)</td>
<td>(54,55)</td>
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<td>BELLE-3/ NCT01633060</td>
<td>Terminated</td>
<td>Double blind, randomized (2:1), phase III</td>
<td>Parallel assignment</td>
<td>≥2nd line (metastatic setting)</td>
<td>HR+, HER2−, postmenopausal female, prior treatment with AI, progression to the combination of mTORi and endocrine therapy, ECOG PS: 0–2</td>
<td>Buparlisib + fulvestrant vs. placebo + fulvestrant</td>
<td>348</td>
<td>135/348 (39%)</td>
<td>PFS</td>
<td>Inostics BEAMing assay</td>
<td>Treatment benefit, with the combination of buparlisib and fulvestrant, irrespective of ctDNA PIK3CA mutational status (PFS of 4.2 vs. 1.6 months; HR =0.66; 95% CI 0.28–0.73; P=0.00031 for PIK3CAM and 3.9 vs. 2.7 months; HR =0.73; 95% CI: 0.53–1.00; P=0.026 for PIK3CAM)</td>
<td>(56)</td>
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Table 1 (continued)
Early ctDNA HER2 dynamics were predictive on response to neoadjuvant treatment (HR =0.60; 95% CI, 0.16–0.93; P=0.54), whereas for ctDNA PIK3CA PFS was 4.1 and 6.1 months for the lapatinib arm and for the trastuzumab arm, respectively (HR =0.38; 95% CI, 0.11–3.13; P=0.54). Early ctDNA HER2 PFS was 8.2 and 4.9 months for the lapatinib arm and for the trastuzumab arm, respectively (HR <0.38; 95% CI, 0.16–0.93; P<0.035), whereas for ctDNA PIK3CA PFS was 4.1 and 6.1 months for the lapatinib arm and for the trastuzumab arm, respectively. Especially, for ctDNA HER2 PFS was 8.2 and 4.9 months for the lapatinib arm and for the trastuzumab arm, respectively (HR <0.38; 95% CI, 0.16–0.93; P<0.035).

Both PIK3CA mutant copies and wild-type allele and ESR1 mutant copies and wild-type allele were significantly lower in the Paclitaxel treatment group (Wilcoxon signed-rank test, P<0.0001). Early ctDNA PIK3CA dynamics (after 2 weeks of therapy) were predictive on response to paclitaxel and fulvestrant.

PIK3CAm in both tissue and plasma samples correlated with shorter PFS, irrespective of the treatment arm. Especially, for ctDNA PIK3CAwt PFS was 8.2 and 4.9 months for the lapatinib arm and for the trastuzumab arm, respectively (HR <0.38; 95% CI, 0.16–0.93; P<0.035), whereas for ctDNA PIK3CA PFS was 4.1 and 6.1 months for the lapatinib arm and for the trastuzumab arm, respectively (HR <0.38; 95% CI, 0.16–0.93; P<0.035).

Table 1 continued.

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<th>Patients (%) with detectable ctDNA</th>
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<td>PALOMA-3/ NCT01942135</td>
<td>Active, not recruiting</td>
<td>Double blind, randomized (2:1), phase III</td>
<td>Parallel assignment</td>
<td>2nd line (metastatic setting)</td>
<td>HR+, HER2+, female of any menopausal status, progression to prior adjuvant or metastatic endocrine therapy, ECOG PS: 0-1</td>
<td>Paclitaxel+ + fulvestrant vs. placebo + fulvestrant</td>
<td>455</td>
<td>100/455 (22%) for PIK3CAm and 114/445 (25.6%) for ESR1m</td>
<td>PFS</td>
<td>ddPCR-based assay</td>
<td>Both PIK3CA mutant copies and wild-type allele and ESR1 mutant copies and wild-type allele were significantly lower in the Paclitaxel+ treatment group (Wilcoxon signed-rank test, P&lt;0.0001). Early ctDNA PIK3CA dynamics (after 2 weeks of therapy) were predictive on response to paclitaxel and fulvestrant. (14,43)</td>
<td></td>
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<tr>
<td>WJOG6110B/ ELTOP/ UMIN000005219</td>
<td>Completed</td>
<td>Open label, randomized (1:1), phase II</td>
<td>Parallel assignment</td>
<td>≥1st line (metastatic setting)</td>
<td>HER2+, female, prior use of taxanes, progression on trastuzumab-containing regimens, ECOG PS: 0-2</td>
<td>Lapatinib + capcitabine vs. trastuzumab + capcitabine</td>
<td>35</td>
<td>8/35 (23%)</td>
<td>PFS</td>
<td>ddPCR-based assay</td>
<td>85% PIK3CAm in both tissue and plasma samples correlated with shorter PFS, irrespective of the treatment arm. (57)</td>
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<tr>
<td>POSEIDON/ NCT02285179</td>
<td>Recruiting</td>
<td>Double blind, randomized (1:1), phase III (3:3 design)</td>
<td>Parallel assignment</td>
<td>≥2nd line (metastatic setting)</td>
<td>HR+, HER2+, female of any menopausal status, prior endocrine therapy, ≤5 chemotherapy lines in the metastatic setting</td>
<td>Taselisib + tamoxifen vs. placebo + tamoxifen</td>
<td>22</td>
<td></td>
<td>PFS</td>
<td>ddPCR/Tagged ampilon deep-sequencing</td>
<td>ctDNA PIK3CA dynamics were predictive on response to taselisib and tamoxifen, before radiologic treatment response. (58)</td>
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<td>SUMMIT/ NCT03433274</td>
<td>Recruiting</td>
<td>Open label, non-randomized, phase IV</td>
<td>Single group assignment</td>
<td>BASKET trial: colon, lung, breast, bladder cancer, fibromellar carcinoma, any line of therapy</td>
<td></td>
<td>Neratinib</td>
<td>14</td>
<td>11/14 (78.5%) Clinical benefit rate</td>
<td>70-gene digital sequencing assay</td>
<td>93.5% Early ctDNA HER2 dynamics were predictive on response to neratinib; ctDNA HER2mut frequency decreased in 9 of 11 paired samples, at week 4, followed by an increase upon radiographical disease progression at week 8 (59)</td>
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<tr>
<td>BEECH/ NCT01625286</td>
<td>Active, not recruiting</td>
<td>Double blind, randomized (1:1), phase III (adaptive design)</td>
<td>Parallel assignment</td>
<td>1st line (metastatic setting)</td>
<td>ER+, HER2-, WHO PS: 0-1</td>
<td>CAPIvastebert+ + pacitaxel vs. placebo + pacitaxel</td>
<td>148</td>
<td></td>
<td>Dose-liming toxicity events, PFS</td>
<td>ddPCR-based assay for ctDNA quantification, Roche cobas PIK3CA assay for PIK3CAwt identification</td>
<td>Early ctDNA dynamics were predictive on PFS for irinotecan/patritumab ± pertuzumab/ganetespib/2206 ± trastuzumab/T-DM1 arm and for the trastuzumab arm, respectively (HR =0.60; 95% CI, 0.11–3.13; P=0.54). (60)</td>
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<td>I-SPY 2/ NCT01042379</td>
<td>Recruiting</td>
<td>Open label, randomized, phase II (adaptive design)</td>
<td>Parallel assignment</td>
<td>Locally advanced breast cancer (stage II, III), neoadjuvant setting</td>
<td>Any tumor ER/PR/HER2 status, female, no prior cytotoxic regimens, ECOG PS: 0-1</td>
<td>AMG 386 + trastuzumab/ AMG 479 + metformin/MK-2206 + trastuzumab/TDM1 + pertuzumab/ganetespib/ABT-888/nabkrab/PLX3397/pembrolizumab/falazaparib + irinotecan/patritumab + trastuzumab/SN-3814A/durvalumab + olaparib/SD-101 + pembrolizumab/tucatinib vs. standard therapy/pertuzumab + trastuzumab</td>
<td>84</td>
<td></td>
<td>pCR after the use of experimental agents</td>
<td>Mutational profiles derived from pretreatment tumor biopsy and germline DNA whole exome sequencing were used to design personalized assays</td>
<td>Early ctDNA dynamics were predictive on response to neoadjuvant treatment. (61)</td>
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Table 1 (continued)

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<th>Patients (%) with detectable ctDNA</th>
<th>Patients (%) with detectable ctDNA or NGS</th>
<th>Endpoints</th>
<th>ctDNA sequencing technique</th>
<th>Concordance of tissue and plasma samples</th>
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<td>MONARCH 2/ NCT02107703</td>
<td>Recruiting</td>
<td>Double blind, randomized (2:1), phase III</td>
<td>Parallel assignment</td>
<td>2nd line (metastatic setting)</td>
<td>HER2−, HER2−, postmenopausal female, ECOG PS: 0–1</td>
<td>Abemaciclib + fulvestrant vs. placebo + fulvestrant</td>
<td>334</td>
<td>PFS</td>
<td>96/238 (40.3%) for PIK3CAm and 190/295 (64.4%) for ESR1m</td>
<td>ddPCR-based assay</td>
<td>ctDNA mutational status associates with improved PFS and response to abemaciclib and fulvestrant arm. For ctDNA PIK3CAm PFS was 15 and 5.7 months for the abemaciclib arm and for the control arm, respectively (HR =0.46; 95% CI, 0.27–0.78), whereas for ctDNA ESR1m PFS was 2.9 and 10.3 months for the abemaciclib arm and for the control arm, respectively (HR =0.49; 95% CI, 0.33–0.73)</td>
<td>(62,63)</td>
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<td>LOTUS/ NCT02162719</td>
<td>Active, not recruiting</td>
<td>Double blind, randomized (1:1), phase II</td>
<td>Parallel assignment</td>
<td>1st line (metastatic setting)</td>
<td>TNBC, female of any menopausal status, ECOG PS: 0–1</td>
<td>Ipilimumab + nivolumab vs. placebo + nivolumab</td>
<td>88</td>
<td>PFS</td>
<td>96/238 (40.3%) for PIK3CAm and 190/295 (64.4%) for ESR1m</td>
<td>FoundationACT assay (plasma samples) and FoundationOne genomic profiling (tumor tissue samples)</td>
<td>ctDNA dynamics were predictive on PFS and objective response irrespective of treatment arm</td>
<td>(64,65)</td>
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<td>NCT02378047</td>
<td>Active, not recruiting</td>
<td>Open label, non-randomized, phase III</td>
<td>Single group assignment</td>
<td>≥2nd line (metastatic setting)</td>
<td>HER2−, female, prior chemotherapy for metastatic disease, ECOG PS ≤2</td>
<td>Apelisib + nab-paclitaxel</td>
<td>42</td>
<td>17/42 (40%)</td>
<td>Recommended phase II dose, objective response rate, PFS</td>
<td>NGS</td>
<td>PFS of 13 months for ctDNA PIK3CAm and 7 months for ctDNA PIK3CAwt (HR =0.39; P =0.03)</td>
<td>(66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INSPIRE/ NCT02644369</td>
<td>Active, not recruiting</td>
<td>Open label, non-randomized, phase II</td>
<td>Single group assignment</td>
<td>2nd line (metastatic setting)</td>
<td>BASKET trial: squamous cell Ca of the head and neck, TNBC, high-grade serous ovarian cancer, melanoma, mixed advanced solid tumors, any line of therapy</td>
<td>Pembrolizumab</td>
<td>20 (TNBC)</td>
<td>Changes in genomic and immune biomarkers that will be measured in blood and tumor pre-treatment, on treatment and at progression</td>
<td>Single cell suspensions were pooled for exome/RNA sequencing, flow cytometry for immunophenotyping</td>
<td>Early ctDNA dynamics were predictive on PFS, OS and overall clinical RR</td>
<td>(67,68)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOLERO-2/ NCT0083655</td>
<td>Completed</td>
<td>Double blind, randomized (2:1), phase III</td>
<td>Parallel assignment</td>
<td>2nd line (metastatic setting)</td>
<td>ER+, postmenopausal female, disease refractory to NSAI, recurrence or progression on or after the last systemic therapy</td>
<td>Everolimus + exemestane vs. placebo + exemestane</td>
<td>550</td>
<td>PFS</td>
<td>238/550 (43.3%)</td>
<td>ddPCR-based assay</td>
<td>70.4%</td>
<td>Treatment benefit, with the combination of everolimus and exemestane, irrespective of ctDNA PIK3CA status (HR =0.43 for PIK3CAwt tumors and 0.57 for PIK3CAm tumors)</td>
<td>(17,69)</td>
<td></td>
</tr>
<tr>
<td>BLTN-ic/ NCT0336112</td>
<td>Completed</td>
<td>Open label, non-randomized, phase I</td>
<td>Single group assignment</td>
<td>2nd line (metastatic setting)</td>
<td>HER2+, female, invas breast cancer &gt;2cm diameter, ECOG PS: 0–1</td>
<td>Pyrotinib + capcetabine</td>
<td>28</td>
<td>MTD</td>
<td>Median PFS of 15.8 months for ≥2ctDNA gene mutations, irrespective of treatment and at serial plasma samples was predictive of low rates of pathological response</td>
<td>Median PFS of 15.8 months for ≥2ctDNA gene mutations, irrespective of treatment and at serial plasma samples was predictive of low rates of pathological response</td>
<td>(70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neo ALLTO/ NCT00553358</td>
<td>Active, not recruiting</td>
<td>Open label, randomized (2:2:2), phase III</td>
<td>Parallel assignment</td>
<td>Primary invasive breast cancer, neoadjuvant treatment</td>
<td>HER2−, female, invasive breast cancer &gt;2cm diameter, ECOG PS: 0–1</td>
<td>Lapatinib + palbociclib + trastuzumab vs. palbociclib + trastuzumab</td>
<td>124</td>
<td>Number of participants with pCR at the time of surgery</td>
<td>NGS</td>
<td>ctDNA PIK3CAm and/or TP53m detection at baseline and at serial plasma samples was predictive of low rates of pathological response</td>
<td>(71,72)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MONALEESA-7/ NCT02278120</td>
<td>Active, not recruiting</td>
<td>Double blind, randomized (1:1:1), phase III</td>
<td>Parallel assignment</td>
<td>1st line (metastatic setting)</td>
<td>ER+ and/or PR+, HER2−, premenopausal or postmenopausal female, ECOG PS: ≤1</td>
<td>Ribociclib + tamoxifen/letrozole/ anastrazole + goserelin vs. placebo + tamoxifen/letrozole/ anastrazole + goserelin</td>
<td>565</td>
<td>489/565 (86.54%)</td>
<td>PFS</td>
<td>NGS</td>
<td>Treatment benefit, with the combination of ribociclib and NSAI or tamoxifen and goserelin, irrespective of ctDNA mutational status at baseline</td>
<td>(73,74)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AI, aromatase inhibitor; AKT, protein kinase B; BEAMing, beads, emulsion, amplification, magnetic; CCC, cell cycle-related; CDH1, CDH20 homolog 1; CDK4/6, cyclin-dependent kinase 4 and 6; CI, confidence interval; ctDNA, circulating tumor DNA; ddPCR, droplet digital polymerase chain reaction; dPCR, digital polymerase chain reaction; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; ESR1, estrogen receptor-1 gene; FGFR1, fibroblast growth factor receptor 1; HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; HR, hazard ratio; HR, hormone receptor; m, mutant; MAPK, RAS-mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NGS, next generation sequencing; NSA1, non-steroidal aromatase inhibitor; OS, overall survival; pCR, pathologic complete response; PFS, progression free survival; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; PR, progesterone receptors; RR, response rate; RTK, receptor tyrosine kinase; TNBC, triple negative breast cancer; TP53, tumor protein 53; WHSC1L1, Wolf-Hirschhorn syndrome candidate 1-like 1; wt, wild type; ZNF703, zinc finger protein 703.
of concomitant genetic alterations of HER2, PI3K/protein kinase B (AKT)/mTOR pathway and TP53 in ctDNA analysis was significantly correlated with worse PFS, compared to ≤1 genetic alteration, in the open-label, Phase I BLTN-Ic trial, of the combination of pyrotaxel plus capcitabine in HER2+ advanced breast cancer patients (70).

Dynamic ctDNA analysis of plasma samples from Phase I/II trial BEECH, whereas patients with ER+ metastatic breast cancer randomized to either paclitaxel plus AKT inhibitor capivasertib or paclitaxel plus placebo, predicted long-term outcome (PFS of 11.1 months in patients with suppressed ctDNA at 21 days vs. 6.4 months in patients with high levels of ctDNA, HR =0.20; 95% CI, 0.083–0.50; P<0.0001), thus serving as a surrogate for PFS (60).

The double-blind, Phase II LOTUS trial, comparing the combination of ipatasertib plus paclitaxel with paclitaxel monotherapy in triple negative advanced breast cancer patients, demonstrated the predictive value of dynamic evaluation of ctDNA in evaluating both objective response and PFS, consistently in both arms (64,65).

As part of the Phase II, INSPIRE basket trial, a secondary analysis of ctDNA at baseline and before the initiation of 3rd cycle of the single-agent immune checkpoint inhibitor pembrolizumab in 10 triple negative metastatic breast cancer patients, strongly correlated with PFS, overall survival (OS) and overall clinical response rate (ORR) (67,68).

In the 1st comprehensive genomic analysis of ctDNA of premenopausal patients with ER+ and/or progesterone receptors (PR)+, HER2- advanced breast cancer, the combination of the CDK 4/6 inhibitor ribociclib and non-steroidal aromatase inhibitor (NSAI) or tamoxifen and goserelin resulted in PFS benefit, irrespective of the baseline genetic landscape status (73,74).

Based on the results of SOLAR-1, Food and Drug Administration (FDA) approved, on May 24, 2019, the use of PIK3CA selective inhibitor alpelisib in combination with fulvestrant for the treatment of men and postmenopausal women, with HR+, HER2-, PIK3CA-mutated advanced breast cancer, following disease progression on or after an endocrine-based regimen. In particular, the combination of alpelisib and fulvestrant resulted in significant prolongation of PFS (HR 0.55; 95% CI, 0.39–0.79; n=186) in patients with ctDNA PIK3CA mutant status. Concurrently, FDA also approved the companion diagnostic test PIK3CA Rotor-Gene Q real-time polymerase chain reaction (RGQ PCR) kit to detect the PIK3CA mutation in a tissue and/or a liquid biopsy. Thus, the assessment of PIK3CA mutations in ctDNA became the first liquid biopsy to be used in the clinical setting for breast cancer patients (20,50).

Discussion

Research into understanding breast cancer’s complexity, both at cellular and molecular level, and development of targeted therapies underline the urgent need of conducting novel biomarker-driven clinical trials, with the ultimate goal of optimizing disease management. The traditional process of drug research and development, where investigational drugs were evaluated for safety and optimal dosing scheme in Phase I, for early signs of efficacy in Phase II, and for confirmation of efficacy, effectiveness and safety in Phase III, gradually fades out. Over the last decade, novel clinical trial designs have found their way into clinical research, in order not only to streamline but also to expedite drug development (75).

Master Protocol (MAPs) use a single, biomarker-driven, trial design and protocol to concurrently evaluate multiple drugs and/or diseases, and include (I) basket trials, which enrol patients based on the presence of a specific biomarker (e.g., mutation), regardless of histology, to identify efficacy of a biomarker-specific, thus targeted, therapy, and (II) umbrella and (III) adaptive platform trials, where patients who share the same cancer histology are allocated to different arms, based on their biomarker status (e.g., mutation), in order to evaluate new investigational agents matches to biomarker-derived cohorts (75). The main difference between umbrella and platform trials is that the last incorporate more adaptions, during the trial, based on efficacy results of interim analyses, by permitting in a flexible way the addition or exclusion of new treatment modalities (75).

Establishing biomarker-stratified clinical-trial design frameworks in the context of spatial and temporal heterogeneity is challenging because the traditional use of archival tissue samples may not be reflective of the dynamic genomic status of the tumor, especially in the metastatic setting (26,30). Such hurdle could potentially be overcome through the incorporation of ctDNA analyses, for the longitudinal evaluation of predictive biomarkers. Overall, results emerged from the clinical trials presented in this review highlight the importance of dynamic ctDNA monitoring in the era of precision medicine; measurement of ctDNA provides representative data of spatiotemporal tracking of mutational landscape of both primary tumour and metastases, thus serving as a sensitive biomarker.
for both monitoring tumor progression and evaluating treatment response (37,76).

cDNA dynamics could serve as a predictive biomarker independent of the histology. Indeed, the I-SPY 2 trial reported that early cDNA dynamics could predict response to neo-adjuvant treatment (61), whereas in the basket trial SUMMIT a decrease in cDNA HER2 mutation variant allele frequency during treatment with the pan-HER inhibitor neratinib was followed by an increase upon radiographically proven progression (59).

Moreover, in the SUMMIT trial a number of genetic aberrations were also identified co-occurring with HER2 mutations, which highlighted the acquisition of secondary resistance to targeted therapy due to clonal evolution (59). Also, in the PALOMA-3 trial, which enrolled patients with ER+, HER2- advanced, previously progressed on endocrine treatment, breast cancer, early cDNA dynamics of truncal mutations in PIK3CA predicted sensitivity to the CDK4/6 inhibitor palbociclib; on the contrary, serial cDNA monitoring of the, commonly sub clonal, ESR1 mutations failed to predict clinical outcome (43). Taken together, these results address the importance of assessing tumor’s genetic heterogeneity and clonal evolution in real time, with minimally invasive ways, like cDNA analyses, in order not only to predict response, but also to rapidly identify acquired resistance to targeted therapies in breast cancer.

Regarding breast cancer detection, at present mammography remains the gold standard screening method, whereas gene expression profiling tests are used to stratify patients regarding recurrence (77). The potential of cDNA analysis both as a consistent detection biomarker and as an accurate predictor of breast cancer recurrence risk needs to be further investigated, given the data scarcity and the lack of standardized analytical methods.

Nowadays, digital PCR (dPCR)- and next generation sequencing (NGS)-based methods are most frequently used to detect cDNA in a background of wildtype DNA. Despite the wide variety in the number of available technologies for cDNA analysis, only 2 companion diagnostic kits are FDA-approved: cobas EGFR Mutations Test v2 for detection of epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC), and therascreen® PIK3CA RGQ PCR Kit for detection of PIK3CA mutations in advanced or metastatic breast cancer (78).

Standardization challenges for integration of cDNA analysis into routine clinical practice include: (I) biological variability (thus tumor heterogeneity), (II) pre-analytical variability (e.g., specialized collecting tubes to prevent leukocyte lysis, optimal time period between blood-draw and sample processing, centrifugation conditions, quantification methods), and (III) analytical variability (an ideal technology should be accurate, highly sensitive and specific, robust, and cost-effective) (76). To accelerate the development and establishment of liquid biopsies in clinical practice, consortium of researchers from academia, industry, regulatory agencies and public, both in United States (BloodPAC) (79), and Europe (Cancer-ID) (80) have been developed.

Conclusions

In conclusion, it can be said that the majority of published results from both recent and ongoing biomarker-driven clinical trials in breast cancer patients seem to concur that cDNA profiling may significantly correlate with response to targeted therapies, thus indicating its potential as a non-invasive predictive biomarker, both in adjuvant, neo-adjuvant, and metastatic setting.

The incorporation of cDNA analysis into sophisticated, biomarker-driven clinical trials, with adequate statistical power and sufficient sample sizes, remains the most reliable way to demonstrate not only the analytical and clinical validity, but also the clinical utility of cDNA as liquid biopsy, in tailoring decision-making in breast cancer patients.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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