Association of 17q22 Amplicon Via Cell-Free DNA With Platinum Chemotherapy Response in Metastatic Triple-Negative Breast Cancer

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PURPOSE
To determine whether specific somatic copy-number alterations detectable in circulating tumor DNA (ctDNA) from patients with metastatic triple-negative breast cancer (mTNBC) are associated with sensitivity to platinum chemotherapy.

MATERIALS AND METHODS
In this secondary analysis of a large cohort of patients with mTNBC whose ctDNA underwent ultralow-pass whole-genome sequencing, tumor fraction and somatic copy-number alterations were derived with the ichorCNA algorithm. Seventy-two patients were identified who had received a platinum-based chemotherapy regimen in the metastatic setting. Gene-level copy-number analyses were performed with GISTIC2.0. Cytobands were associated with progression-free survival (PFS) to platinum chemotherapy using Cox proportional hazards models. The Cancer Genome Atlas and Molecular Taxonomy of Breast Cancer International Consortium data sets were interrogated for frequency of significant cytobands in primary triple-negative breast cancer (pTNBC) tumors.

RESULTS
Among 71 evaluable patients, 17q21 and 17q22 amplifications were most strongly associated with improved PFS with platinum chemotherapy. There were no significant differences in clinicopathologic features or (neo)adjuvant chemotherapy among patients with 17q22 amplification. Patients with 17q22 amplification (n = 17) had longer median PFS with platinum (7.0 vs 3.8 months; log-rank P = .015) than patients without 17q22 amplification (n = 54), an effect that remained significant in multivariable analyses (PFS hazard ratio 0.37; 95% CI, 0.16 to 0.84; P = .02). Among 39 patients who received the nonplatinum chemotherapy agent capecitabine, there was no association between 17q22 amplification and capecitabine PFS (log-rank P = .69). In The Cancer Genome Atlas and Molecular Taxonomy of Breast Cancer International Consortium, 17q22 amplification occurred in more than 20% of both pTNBC and mTNBC tumors, whereas 17q21 was more frequently amplified in mTNBC relative to pTNBC (16% vs 8.1%, P = .015).

CONCLUSION
The 17q22 amplicon, detected by ctDNA, is associated with improved PFS with platinum chemotherapy in patients with mTNBC and warrants further investigation.

INTRODUCTION
Triple-negative breast cancer (TNBC), defined by the absence of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) protein, makes up 10%-15% of all breast cancers, yet disproportionately accounts for more than one third of breast cancer–related deaths.1,3 TNBC is characterized by genomic instability, frequent loss of TP53, and widespread somatic copy-number alterations (SCNAs).2,4 Metastatic TNBCs (mTNBCs) shed high amounts of circulating tumor DNA (ctDNA) relative to other tumors, allowing for minimally invasive genomic profiling.5,7 We previously published the largest genomic characterization to date of mTNBCs (n = 164) via low-coverage (0.1×) whole-genome sequencing (WGS) of ctDNA. Using our published ichorCNA algorithm, we evaluated the fraction of cell-free DNA (ctDNA) in circulation attributable to the tumor, or tumor fraction (TFx), and SCNAs.5,7 We demonstrated that copy number is highly concordant between ctDNA and metastatic tissue biopsies, compared the copy-number landscape of mTNBC relative to primary TNBC (pTNBC) tumors, and found a significant association of TFx with metastatic survival, independent of clinicopathologic features.7 We previously investigated the association of SCNAs with...
Detection of 17q22 amplification on ultralow-pass whole-genome sequencing of circulating tumor DNA is a potential noninvasive biomarker to select for patients who will benefit from platinum chemotherapy. Further investigation and validation is warranted.

Information

Key Objective
Is there a somatic copy-number alteration detectable in circulating tumor DNA from patients with metastatic triple-negative breast cancer that is associated with response to platinum chemotherapy?

Knowledge Generated
Among 71 patients with metastatic triple-negative breast cancer, amplifications on chromosome 17 at 17q21 and 17q22 were most strongly associated with longer progression-free survival with platinum-containing chemotherapy regimens. This remained significant in a multivariable model when accounting for BRCA1/2 mutation status, percent of genome altered, line of platinum therapy, and bone-only metastases, among other covariates.

Relevance
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CONTEXT

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MATERIALS AND METHODS

Cohort Identification
Patients with metastatic biopsy-proven TNBC enrolled on clinical data and biospecimen banking protocols for metastatic breast cancer were identified for analyses, as described previously. TNBC was defined as < 5% staining for the ERs and PRs, and HER2 immunohistochemistry (IHC) 0 to 1+ and/or HER2:Cep17 fluorescent in situ hybridization ratio < 2.0. Clinicopathologic data were abstracted from the medical record. PFS was determined as time from treatment initiation to progression or therapy switch. If patients received multiple lines of platinum chemotherapy, we used the PFS of the earliest platinum-containing regimen. The study was approved by the institutional review boards of the Dana-Farber Cancer Institute and Ohio State University and was conducted in accordance with the Declaration of Helsinki. All patients provided written consent.

Ethics Declarations
Patients provided consent for under approval by local human research protections programs and institutional review boards at Dana-Farber Cancer Institute and Ohio State University, and studies were conducted in accordance with the Declaration of Helsinki.

Blood Sample Collection, Processing, and Sequencing
As described previously, venous blood samples were processed to component parts within 4 hours of collection, cfDNA was extracted from plasma, and DNA quantification was performed. Three to 20 ng of cfDNA input (median 5 ng), or approximately 1,000-7,000 haploid genome equivalents, was used for ultralow-pass WGS (ULP-WGS). Constructed
sequencing libraries were pooled (2 µL of each × 96 per pool) and sequenced using 100 bp paired-end runs over 1 × lane on a HiSeq2500 (Illumina, San Diego, CA) to average genome-wide fold coverage of 0.1x. Segment copy number and TFx were derived with ichorCNA. Samples were excluded if the median absolute deviation of copy ratios (2 log2 ratio) between adjacent bins, genome-wide, was > 0.20 suggesting poor quality sequencing data. We previously demonstrated that high-confidence SCNAs are detectable in samples with TFx > 10%. So only patients with at least one ctDNA sample with TFx > 10% were considered for analyses (n = 101).

Cytoband-Level Copy-Number Analyses

GISTIC2.0 output was used for all gene-level copy-number analyses. Segmented data files derived from ichorCNA for mTNBC ctDNA for the single highest TFx and publicly available segmented data for Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) were purity- and ploidy-corrected and then input into GISTIC2.0 with amplification–deletion threshold log2 ratio > 0.3, confidence level 0.99, and Q-value threshold 0.05. Publicly available GISTIC2.0 data were used from The Cancer Genome Atlas. (TCGA) Cytobands were defined as amplification (GISTIC value 1, corresponding to three copies or GISTIC value 2, corresponding to 4+ copies) versus diploid (GISTIC value 0).

Identification of Patients in Publicly Available Data Sets

Patients with triple-negative breast cancer were identified in TCGA (n = 156) and METABRIC (n = 277) based on study-reported negative for ER and PR via IHC, and HER2-receptor copy number diploid (GISTIC2.0 value of 0) or IHC 0-1.

Statistical Analyses and Data Visualization

All statistical analyses and data visualizations were performed in R version 3.3.1. Categorical variables were compared using Fisher’s exact test and medians of continuous variables were compared using the Mann-Whitney U test. Cox proportional hazards models were calculated using the survival package and Kaplan-Meier curves with log-rank test were plotted with packHV.

RESULTS

From 101 patients with mTNBC who had ULP-WGS performed on ctDNA samples as previously published, 72 patients were identified who had received a platinum-based chemotherapy regimen in the metastatic setting (n = 40 single-agent platinum, n = 32 platinum in combination), and had a ctDNA sample collected with a TFx > 10% (adequate for high confidence SCNA calls). The specific chemotherapy regimens are listed in the Data Supplement. We evaluated the association of SCNAs with PFS on platinum-containing chemotherapy regimens in the metastatic setting. Seventy-one of 72 patients were evaluable for PFS (flowchart diagram; Fig 1). Of the 71 evaluable patients, 21 (30%) had the ctDNA collected before starting platinum chemotherapy and 50 (70%) had ctDNA collected after starting a platinum-containing regimen. Across the genome, 17q21 and 17q22 amplification were the copy-number gains most strongly associated with improved PFS with first platinum chemotherapy (Fig 2A). Although the amplicon may extend from 17q21 into 17q22, within chromosome 17, amplifications of 17q22 were most strongly associated with improved PFS with first platinum chemotherapy (Fig 2B); so, 17q22 was used for all further analyses. Patients with 17q22 amplification (n = 17) had significantly longer median PFS with first platinum (7.0 v 3.8 months; log-rank P = .015; Fig 2C) than patients without 17q22 amplification (n = 54). Table 1 shows the demographic and clinical characteristics of the patient cohorts with and without 17q22 amplification that received a platinum agent. There were no significant differences in baseline clinicopathologic features (germline BRCA1/2 status, age at primary tumor diagnosis, stage at primary diagnosis, or receipt or regimen of (neo)adjuvant chemotherapy) or metastatic clinicopathologic features (age at metastatic diagnosis, sites of metastatic disease, or number of lines of therapy before platinum chemotherapy).

In a multivariable analysis, 17q22 remained significantly associated with platinum chemotherapy PFS adjusting for BRCA mutation status, primary receptor status, stage at diagnosis, age at diagnosis, year the sample was collected, sample collection cohort, and percent of genome altered (PFS hazard ratio 0.37; 95% CI, 0.16 to 0.84; P = .02; Table 2). Notably, we included percent of genome altered as a covariate to ensure that the effect seen with 17q22 and platinum PFS was not secondary to general genome

FIG 1. Flowchart diagram. ctDNA, circulating tumor DNA; mTNBC, metastatic triple-negative breast cancer; PFS, progression-free survival; SCNA, somatic copy-number alteration.
instability. Furthermore, amplification of 17q22 remained significantly associated with longer PFS with platinum chemotherapy in additional sensitivity analyses of the base multivariable model with bone-only metastatic status (17q22 amplification \( P = .03 \)), and base multivariable model with both bone-only status AND platinum as first line versus later (17q22 amplification \( P = .03 \); Data Supplement). To evaluate whether this was a nonspecific effect of
chemotherapy, we also evaluated PFS with capecitabine, an oral fluorouracil prodrug commonly used in the management of mTNBC. Among the 39 patients who had received capecitabine, there was no association between 17q22 amplification and median PFS from capecitabine (log-rank \( P = .69 \); Fig 2D). In our discovery data set, for predicting a PFS of at least 3 months, presence of 17q22 amplification has a sensitivity 0.349, specificity 0.929, positive-predictive value 0.883, and negative-predictive value 0.481. For predicting a clinically meaningful PFS of at least 6 months,

| TABLE 1. Characteristics of Patients Who Received Platinum Chemotherapy With and Without 17q22 Amplicon |
|-------------------------------------------------|-----------------------------------------------|
| Characteristic                                   | Patients With 17q22 Amplification Who Received Platinum (n = 17) | Patients Without 17q22 Amplification Who Received Platinum (n = 54) | \( P^* \) |
| Female, No. (%)                                  | 17 (100)                                      | 54 (100)                                      | .09 |
| BRCA status, No. (%)                             |                                               |                                               |     |
| \( \text{BRCA1-mutated} \)                       | 2 (12)                                        | 7 (13)                                        |     |
| \( \text{BRCA2-mutated} \)                       | 1 (6)                                         | 1 (2)                                         |     |
| \( \text{BRCA1/2 wild-type} \)                   | 14 (82)                                       | 41 (76)                                       |     |
| Not available                                    | 0                                             | 5 (9)                                         |     |
| Age at primary tumor diagnosis, median (range), years | 45 (32-63)                                  | 46 (29-74)                                   | .92 |
| Primary tumor receptor status, No. (%)           |                                               |                                               | .92 |
| \( \text{HR+/HER2–} \)                          | 3 (18)                                        | 5 (9)                                         |     |
| \( \text{HER2+} \)                               | 2 (12)                                        | 0                                             |     |
| TNBC                                             | 12 (71)                                       | 46 (85)                                       |     |
| Indeterminate                                    | 0                                             | 3 (6)                                         |     |
| Stage at primary diagnosis, No. (%)              |                                               |                                               | .47 |
| I                                                | 2 (12)                                        | 10 (19)                                       |     |
| II                                               | 10 (59)                                       | 23 (43)                                       |     |
| III                                              | 2 (12)                                        | 15 (28)                                       |     |
| IV                                               | 3 (18)                                        | 5 (9)                                         |     |
| Not available                                    | 0                                             | 1 (2)                                         |     |
| Neoadjuvant or adjuvant chemotherapy, No. (%)    |                                               |                                               | .10 |
| Anthracycline ± taxane                           | 13 (76)                                       | 44 (81)                                       |     |
| Chemotherapy (no anthracycline)                  | 0 (0)                                         | 5 (9)                                         |     |
| No chemo or de novo or unknown                   | 4 (24)                                        | 5 (9)                                         |     |
| Age at metastatic diagnosis, median (range), years | 47 (35-66)                                  | 50 (30-77)                                   | .91 |
| Sites of metastatic disease, No. (%)             |                                               |                                               |     |
| Breast or chest wall                             | 5 (29)                                        | 15 (28)                                       | 1   |
| Bone                                             | 10 (59)                                       | 19 (35)                                       | .10 |
| CNS                                              | 5 (29)                                        | 18 (33)                                       | 1   |
| Liver                                            | 5 (29)                                        | 25 (46)                                       | .27 |
| Lung                                             | 7 (41)                                        | 32 (59)                                       | .27 |
| Lymph nodes                                      | 6 (35)                                        | 27 (50)                                       | .40 |
| No. of lines of metastatic therapy before first platinum, No. (%) | 10 (59)                                      | 26 (48)                                       |     |
| 3+                                               | 3 (18)                                        | 10 (19)                                       |     |
| Duration on platinum chemo, median (range), months | 6.7 (1.0-37.1)                              | 3.2 (0.4-28.4)                               | .008 |

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

*Fisher’s exact test used to compare categorical variables; Mann-Whitney \( U \) test used to compare medians of continuous variables.
presence of 17q22 amplification has a sensitivity 0.400, specificity 0.848, positive-predictive value 0.588, and negative-predictive value 0.722. Comprehensive biomarker performance metrics with 95% CIs and receiver operating characteristic curves are shown in the Data Supplement.

In this mTNBC cohort, chromosome 17q22 amplifications were common, present in 21.8% (22/101) of patients. To more broadly assess the frequency of 17q22 amplification, we investigated the frequency of chromosome 17 amplifications in additional cohorts. Specifically, we compared pTNBC tissue SCNAs in the publicly available data sets METABRIC1 and TCGA4 (total n = 433) with all mTNBCs in our cohort (n = 101). Detailed analysis of chromosome 17 gains demonstrated that 17q22 amplification occurs in more than 20% of both pTNBC (Fig 3A) and mTNBC (Fig 3B). Among chromosome 17 cytobands, only 17q21 demonstrated significantly more frequent amplification in mTNBC relative to pTNBC (16% v 8.1%, genome-wide false discovery rate-corrected Fischer exact P = .015; Fig 3C). To evaluate for association of homologous recombination deficiency (HRD) with 17q22 amplification, we compared the Van’t Veer BRCA1 gene expression signature24 between the groups with and without 17q22 amplification in METABRIC and found no association (t-test, P = .59; Data Supplement).

**DISCUSSION**

Using shallow WGS of ctDNA in a large cohort of patients with mTNBC, we found that amplification of 17q22 is independently associated with improved platinum chemotherapy PFS. Analysis of the TCGA and METABRIC data sets shows that 17q21-22 amplifications are common, occurring in both pTNBCs and mTNBCs. Altogether, this study suggests that identification of 17q21-q22 amplification in ctDNA has the potential to be a blood-based biomarker of platinum sensitivity in mTNBC, and possibly also early-stage breast cancer.

To date, potential biomarkers of platinum sensitivity in breast cancer have involved germline genetic testing for BRCA1/2 mutations or tissue-based DNA/RNA markers of HRD. In the metastatic setting, TNBC response to single-agent platinum chemotherapy has been associated with germline BRCA1/2 mutations and scores of genomic instability including HRD-loss of heterozygosity and HRD large-scale state transitions, but was not associated with p63/p73 expression, p53 mutations, PIK3CA mutations, PAM50 gene expression subtype, BRCA1 methylation, low BRCA1 messenger RNA levels, or a Myriad HRD assay.19,20 In the neoadjuvant setting, TNBC response to platinum-containing chemotherapy regimens has been associated with HRD score (based on HRD loss of heterozygosity,
telomeric allelic imbalance, and HRD large-scale state transitions), but was not associated with tumor-infiltrating lymphocytes.15,25-28 More recently, the HRDetect29 algorithm, which incorporates single-nucleotide variant mutational signatures and SCNA features to predict BRCA1/2 deficiency, has been applied in breast cancer.30,31 It has shown promise in assessing HRD in a population-based trial (SCAN-B: NCT02306096)31 and a phase II window clinical trial of a poly (ADP-ribose) polymerase inhibitor for patients with pTNBC, the RIO trial (EudraCT 2014-003319-12).30 However, there are conflicting reports in the neoadjuvant setting whether or not patients with germline BRCA1/2 mutations benefit from adding platinum to neoadjuvant chemotherapy.13,32-35 A better biomarker of platinum chemotherapy benefit in breast cancer is needed.

Advantages of a ctDNA-based biomarker include being minimally invasive, feasible even if tissue is not available, and available for collection at the time of treatment decisions. Many studies have looked at whole-exome sequencing and targeted panel ctDNA assays as biomarkers of response to...
therapy, but few studies have explored the use of more affordable low-coverage WGS to analyze copy-number alterations (CNAs) as a predictive biomarker. Low-coverage WGS of cfDNA has been used to characterize CNAs in many cancers including TNBC, and the burden of CNAs may be prognostic. In some cancers, CNA burden may be predictive of response to checkpoint inhibitors. But, to our knowledge, no previous study has associated a focal CNA detected by ctDNA ULP-WGS with response to a particular therapy for any cancer.

Amplification of 17q21-q22 has been previously described in breast cancer. Notably, HER2 is located on adjacent 17q12 and BRCA1 is located on 17q21. In this study, the association of 17q22 with platinum PFS appears to peak at KIF2B (Fig 1B), kinesin family member 2B, a microtubule-dependent motor required for spindle assembly and chromosome movement. Of the many genes in 17q21-q22, several others may conceivably be related to the platinum sensitivity seen in our study, and deserve further investigation. Overexpression of TRIM37, a centrosomal ubiquitin ligase, delays centrosome maturation and separation in late G2 and M phase, promoting mitotic errors and genomic instability. The G2/M checkpoint prevents progression of cells with damaged DNA from entering mitosis, and alterations in proteins associated with this cell-cycle checkpoint may make cancer cells more sensitive to DNA-damaging therapies. High expression of NR1D1 prevents DNA damage repair, inhibiting both nonhomologous end joining and homologous recombination, which results in chemosensitivity. DLX4 encodes BP1, a transcription factor, which binds to and suppresses BRCA1. Overexpression of KIF2B, TRIM37, NR1D1, or DLX4 may contribute to TNBC sensitivity to DNA-damaging platinum chemotherapy.

Given the well-established association of HRD with platinum sensitivity, exploration of the association between 17q21-22 amplification and HRD is warranted. Based on the lack of association with 17q22 with BRCA1/2 mutations in our ctDNA data set and the lack of association of a BRCA1 gene expression signature with 17q22 amplification in the METABRIC data set, 17q22 amplification does not appear to be related to BRCA1/2 mutation status. However, the association of other measures of HRD should be explored in the future, but unfortunately deeper whole-genome or whole-exome sequencing are needed to calculate such metrics.

Furthermore, 17q22 amplification presented here is a specific, but not optimally sensitive, potential biomarker of platinum response in mTNBC. Although specificity is a favorable characteristic in a predictive biomarker, future studies should consider combination of 17q22 amplification with other minimally invasive biomarkers of platinum response. Recently, Sipos et al published biomarker analyses of the TNT study demonstrating that intermediate chromosomal instability measurements were most associated with platinum benefit. However, the association of individual amplicons such as 17q22 was not evaluated in that study and will be important to validate the findings from the current report in prospective studies, such as TNT.

Limitations of our study include the retrospective design and a modest sample size; however, a modern cohort of patients with mTNBC were included, who had received current standard of care treatment. Confirmation of our findings in a validation cohort of patients with mTNBC is needed. We investigated potential validation cohorts such as the TNT trial and TBCRC009, two completed clinical trials of platinum chemotherapy for mTNBC; copy number is not available for either but we anticipate may be in the future. Although we suspect based on previous work from our group that SCNAs are an early event in the natural history of a cancer and remain stable over time, it would be valuable in future work to show with serial ctDNA samples from each individual that 17q21-22 amplifications do not change with platinum chemotherapy or other treatments. Finally, a similar analysis of ULP-WGS of ctDNA from patients with ER+/HER2– or HER2+ metastatic breast cancer or advanced ovarian cancer may also identify a potential biomarker for platinum chemotherapy benefit.

In summary, we demonstrate an independent association of the 17q22 amplicon, detected via minimally invasive, shallow WGS of ctDNA, with improved platinum chemotherapy PFS in a large cohort with mTNBC. The role of the 17q22 amplicon as a biomarker of platinum sensitivity in breast cancer warrants further validation and prospective investigation.

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REFERENCES

1. Curtis C, Shah SP, Chin SF, et al: The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 486:346-352, 2012
2. Shah SP, Roth A, Goya R, et al: The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 486:395-399, 2012
3. Bauer KR, Brown M, Cress RD, et al: Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: A population-based study from the California cancer Registry. Cancer 109:1721-1728, 2007
4. The Cancer Genome Atlas Network: Comprehensive molecular portraits of human breast tumours. Nature 490:61-70, 2012
5. Adalsteinsson VA, Ha G, Freeman SS, et al: Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. Nat Commun 8:1324, 2017
6. Garcia-Murillas I, Chopra N, Comino-Méndez I, et al: Assessment of molecular relapse detection in early-stage breast cancer. JAMA Oncol 5:1473-1478, 2019

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7. Stover DG, Parsons HA, Ha G, et al: Association of cell-free DNA tumor fraction and somatic copy number alterations with survival in metastatic triple-negative breast cancer. J Clin Oncol 36:543-553, 2018
8. Schmid P, Adams S, Rugo HS, et al: Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 379:2108-2121, 2018
9. Litton JK, Rugo HS, Ett J, et al: Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med 379:753-763, 2018
10. Robson ME, Tung N, Conte P, et al: OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician’s choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Ann Oncol 30:558-566, 2019
11. Litton JK, Scoggins ME, Hess KR, et al: Neoadjuvant talazoparib for patients with operable breast cancer with a germline BRCA pathogenic variant. J Clin Oncol 38:398-404, 2020
12. Saleh R, Nadler MB, Desnoyers A, et al: Platinum-based chemotherapy in early-stage triple negative breast cancer: A meta-analysis. Cancer Res 80, 2020 (abstr P4-14-09)
13. Poggio F, Bruzzone M, Ceppi M, et al: Platinum-based neoadjuvant chemotherapy in triple-negative breast cancer: A systematic review and meta-analysis. Ann Oncol 29:1497-1508, 2018
14. von Minckwitz G, Schneeweiss A, Loibl S, et al: Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): A randomised phase 2 trial. Lancet Oncol 15:747-756, 2014
15. Loibl S, Weber KE, Timms KM, et al: Survival analysis of carboplatin added to an anthracycline/taxane-based neoadjuvant chemotherapy and HRD score as predictor of response-final results from GeparSixto. Ann Oncol 29:2341-2347, 2018
16. Sikow WM, Berry DA, Perou CM, et al: Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol 33:13-21, 2015
17. Loibl S, O’Shaughnessy J, Untch M, et al: Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BriThNess): A randomised, phase 3 trial. Lancet Oncol 19:497-509, 2018
18. Alba E, Chacón JJ, Lluch A, et al: A randomised phase II trial of platinum salts in basal-like breast cancer patients in the neoadjuvant setting. Results from the GEICAM/2006-03, multicenter study. Breast Cancer Res Treat 136:487-493, 2012
19. Tutt A, Tovey H, Cheang MCU, et al: Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: The TNT trial. Nat Med 24:628-637, 2018
20. Isakoff SJ, Mayer EL, He L, et al: TBCRC009: A multicenter phase II clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. J Clin Oncol 33:1902-1909, 2015
21. Mermel CH, Schumacher SE, Hill B, et al: GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. Genome Biol 12:R41, 2011
22. Therneau T: A Package for Survival Analysis in R (ed 3.2-7). 2020. https://CRAN.R-project.org/package=survival
23. Varet H: Package packHV
24. van ’t Veer LJ, Dai H, van de Vijver MJ, et al: Gene expression profiling predicts clinical outcome of breast cancer. Nature 415:530-536, 2002
25. Telli ML, Timms KM, Reid J, et al: Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. Clin Cancer Res 22:3764-3773, 2016
26. Telli M, McMillan A, Ford J, et al: Homologous recombination deficiency (HRD) as a predictive biomarker of response to neoadjuvant platinum-based therapy in patients with triple-negative breast cancer (TNBC): A pooled analysis. Cancer Res 76, 2016 (abstr P3-07-12)
27. Kaklamani VG, Jerus JS, Hughes E, et al: Phase II neoadjuvant clinical trial of carboplatin and eribulin in women with triple negative early-stage breast cancer (NCT01372579). Breast Cancer Res Treat 151:629-638, 2015
28. de Boo L, Cimino-Mathews A, Lubeck Y, et al: Tumour-infiltrating lymphocytes (TILs) and BRCA-like status in stage III breast cancer patients randomised to adjuvant intensified platinum-based chemotherapy versus conventional chemotherapy. Eur J Cancer 127:240-250, 2020
29. Davies H, Golczid D, Morganela S, et al: HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nat Med 23:517-525, 2017
30. Chopra N, Tovey H, Pearson A, et al: Homologous recombination DNA repair deficiency and PARP inhibition activity in primary triple negative breast cancer. Nat Commun 11:6662, 2020
31. Staff J, Golczid D, Bosch A, et al: Whole-genome sequencing of triple-negative breast cancers in a population-based clinical study. Nat Med 25:1526-1533, 2019
32. Byrski T, Gronwald J, Huzarski T, et al: Neoadjuvant therapy with cisplatin in BRCA1-positive breast cancer patients. Hered Cancer Clin Pract 9:A4, 2011
33. Byrski T, Huzarski T, Dent R, et al: Pathologic complete response to neoadjuvant cisplatin in BRCA1-positive breast cancer patients. Breast Cancer Res Treat 147:401-405, 2014
34. Minckwitz GV, Timms K, Untch M, et al: Prediction of pathological complete response (pCR) by homologous recombination deficiency (HRD) after carboplatin-containing neoadjuvant chemotherapy in patients with TNBC: Results from GeparSixto. J Clin Oncol 33, 2015 (abstr P3-07-12)
35. Hahnen E, Lederer B, Hauke J, et al: Germline mutation status, pathological complete response, and disease-free survival in triple-negative breast cancer: Secondary analysis of the GeparSixto clinical trial. JAMA Oncol 3:1378-1385, 2017
36. Brutman SV, Yang SY, Iaffolla MAJ, et al: Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nat Cancer 1:873-881, 2020
37. Pessoa LS, Heringer M, Ferrer VP: ctDNA as a cancer biomarker: A broad overview. Crit Rev Oncol Hematol 155:103109, 2020
38. Chen X, Chang C-W, Spoerke JM, et al: Low-pass whole-genome sequencing of circulating cell-free DNA demonstrates dynamic changes in genomic copy number in a squamous lung cancer clinical cancer cohort. Clin Cancer Res 25:2254-2263, 2019
39. Wei T, Zhang J, Li J, et al: Genome-wide profiling of circulating tumor DNA depicts landscape of copy number alterations in pancreatic cancer with liver metastasis. Mol Oncol 14:1966-1977, 2020
40. Paracchini L, Beltrame L, Grassi T, et al: Genome-wide copy number alterations in circulating tumor DNA as a novel biomarker in high grade serous ovarian cancer patients. Clin Cancer Res 27:2549-2559, 2021
41. Van Roy N, Van Der Linden M, Menten B, et al: Shallow whole genome sequencing on circulating cell-free DNA allows reliable noninvasive copy-number profiling in neuroblastoma patients. Clin Cancer Res 23:6305-6314, 2017
42. Oh CR, Kong S-Y, Im H-S, et al: Genome-wide copy number alteration and VEGFA amplification of circulating cell-free DNA as a biomarker in advanced hepatocellular carcinoma patients treated with Sorafenib. BMC Cancer 19:292, 2019
43. Nakabayashi M, Kawasaki A, Yasuhara R, et al: Massively parallel sequencing of cell-free DNA in plasma for detecting gynaecological tumour-associated copy number alteration. Sci Rep 8:11205, 2018
44. Hirsch D, Kemmerling R, Davis S, et al: Chromothripsis and focal copy number alterations determine poor outcome in malignant melanoma. Cancer Res 73:1454-1460, 2013
45. Zhang L, Feizi N, Chi C, et al: Association analysis of somatic copy number alteration burden with breast cancer survival. Front Genet 9:421, 2018
46. Lu Z, Chen H, Li S, et al: Tumor copy-number alterations predict response to immune-checkpoint-blockade in gastrointestinal cancer. J Immunother Cancer 8:e000374, 2020
47. Jensen TJ, Goodman AM, Kato S, et al: Genome-wide sequencing of cell-free DNA identifies copy-number alterations that can be used for monitoring response to immunotherapy in cancer patients. Mol Cancer Ther 18:448-458, 2019
48. Bärlund M, Tirkkonen M, Forozan F, et al: Increased copy number at 17q22-q24 by CGH in breast cancer is due to high-level amplification of two separate regions. Genes Chromosomes Cancer 20:372-376, 1997
49. Wu G, Sinclair C, Hinson S, et al: Structural analysis of the 17q22-23 amplicon identifies several independent targets of amplification in breast cancer cell lines and tumors. Cancer Res 61:4951-4955, 2001
50. Jones C: 17q aberrations in breast cancer. Breast Cancer Res 1:66626, 1999
51. Orsetti B, Courjal F, Curry M, et al: 17q21-q25 aberrations in breast cancer: Combined allelotype and CGH analysis reveals 5 regions of allelic imbalance among which two correspond to DNA amplification. Oncogene 18:6262-6270, 1999
52. Bilal E, Vassallo K, Toppare J, et al: Amplified loci on chromosomes 8 and 17 predict early relapse in ER-positive breast cancers. PLoS One 7:e38575, 2012
53. Orsetti B, Nugoli M, Cervera N, et al: Genomic and expression profiling of chromosome 17 in breast cancer reveals complex patterns of alterations and novel candidate genes. Cancer Res 64:6453-6460, 2004
54. Ooi A, Inokuchi M, Horike S, et al: Amplicons in breast cancers analyzed by multiplex ligation-dependent probe amplification and fluorescence in situ hybridization. Hum Pathol 85:33-43, 2019
55. Jacot W, Fiche M, Zaman K, et al: The HER2 amplicon in breast cancer: Topoisomerase IIa and beyond. Biochim Biophys Acta 1836:146-157, 2013
56. Manning AL, Ganem NJ, Bakhoum SF, et al: The kinesin-13 proteins Kif2a, Kif2b, and Kif2c/MCAK have distinct roles during mitosis in human cells. Mol Biol Cell 18:2970-2979, 2007
57. Yeow ZY, Lambus BG, Marlow R, et al: Targeting TRIM37-driven centrosome dysfunction in 17q23-amplified breast cancer. Nature 585:447-452, 2020
58. Meitinger F, Ohta M, Lee K-Y, et al: TRIM37 controls cancer-specific vulnerability to PLK4 inhibition. Nature 585:440-446, 2020
59. Na H, Han J, Ka NL, et al: High expression of NR1D1 is associated with good prognosis in triple-negative breast cancer patients treated with chemotherapy. Breast Cancer Res 21:127, 2019
60. Ka NL, Na TY, Na H, et al: NR1D1 recruitment to sites of DNA damage inhibits repair and is associated with chemosensitivity of breast cancer. Cancer Res 77:2463-2463, 2017
61. Ka NL, Na TY, Lee MO: NR1D1 enhances oxidative DNA damage by inhibiting PARP1 activity. Mol Cell Endocrinol 454:87-92, 2017
62. Kluk BJ, Fu Y, Formilo TA, et al: BP1, an isoform of DLX4 homeoprotein, negatively regulates BRCA1 in sporadic breast cancer. Int J Biol Sci 6:513-524, 2010
63. Lou Y, Fallah Y, Yamane K, et al: BP1, a potential biomarker for breast cancer prognosis. Biomark Med 12:535-545, 2018
64. Polak PA-O, Kim J, Braunstein LZ, et al: A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. Nat Genet 49:1476-1486, 2017
65. Davies H, Glodzik D, Morganella S, et al: HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nat Med 23:517-525, 2017
66. Szutajska Z, Dlouhy M, Krzyzanek M, et al: Migrating the SNP array-based homologous recombination deficiency measures to next generation sequencing data of breast cancer. NPJ Breast Cancer 4:16, 2018
67. Raine KM, Van Loo P, Wedge DC, et al: ascatNgs: Identifying somatically acquired copy-number alterations from whole-genome sequencing data. Curr Protoc Bioinformatics 56:15.9.1-15.9.17, 2016
68. Sipos O, Tovey H, Quist J, et al: Assessment of structural chromosomal instability phenotypes as biomarkers of carboplatin response in triple negative breast cancer: The TNT trial. Ann Oncol 32:58-65, 2021
69. Weber ZT, Collier KA, Tallman D, et al: Modeling clonal structure over narrow time frames via circulating tumor DNA in metastatic breast cancer. Genome Med 13:89, 2021