original reports abstract

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 *v* 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% *v* 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.

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ASSOCIATED CONTENT

Data Supplement

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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 (cfDNA) 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

CONTEXT

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

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.

survival, and amplification of 17q21 was among the five regions most strongly associated with improved survival in mTNBC,[7] with amplification of 17q22 and 17q23 also associated with improved survival. However, the association of specific SCNAs with response to defined treatment regimens has not previously been investigated in mTNBC to our knowledge.

There is a lack of predictive biomarkers in mTNBC, where the treatment paradigm primarily involves the use of sequential single-agent chemotherapy, until progression or unacceptable toxicity. The only widely adopted predictive biomarkers in clinical practice are programmed death-ligand 1 expression in tissue to predict benefit from treatment with nab-paclitaxel plus atezolizumab[8] and germline *BRCA1/2* mutations to predict benefit from poly (ADP-ribose) polymerase inhibitors.[9-11] A predictive biomarker of response to a particular chemotherapy regimen would allow a more refined precision oncology approach. In particular, the role of platinum chemotherapy remains a critical outstanding question in the clinical management of both early-stage and advanced TNBC. In early-stage TNBC, multiple trials of platinum-containing neoadjuvant chemotherapy regimens have shown higher pathologic complete response rates with a possible disease free survival benefit, but at the cost of higher toxicity with no evidence for overall survival benefit.[12-18] In the metastatic setting, platinum chemotherapy is active in an unselected patient population with mTNBC, but patients with germline *BRCA1/2* mutations particularly benefit.[19,20] An inclusive predictive biomarker of response to platinum chemotherapy in either the neoadjuvant or metastatic setting would be valuable to ensure that all patients who would benefit receive platinum chemotherapy.

Here, we report a secondary analysis of SCNAs in ctDNA from a cohort of patients with mTNBC to identify chromosomal cytobands associated with improved progression-free survival (PFS) with platinum-based chemotherapy to identify a novel, minimally invasive biomarker for sensitivity to platinum chemotherapy. We further characterize the frequency of the significant cytoband amplifications in breast cancer in publicly available data sets.

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.[7] 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,[7] 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 0.1×. Segment copy number and TFx were derived with ichorCNA.[5] Samples were excluded if the median absolute deviation of copy ratios ($2^{\log 2 \text{ ratio}}$) 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%,[5,7] 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[21] 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)[1] were purity- and ploidy-corrected and then input into GISTIC2.0[21] with amplification–deletion threshold $\log_2$ratio > 0.3, confidence level 0.99, and Q-value threshold 0.05. Publicly available GISTIC2.0[21] data were used from The Cancer Genome Atlas. (TCGA)[4] 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[4] (n = 156) and METABRIC[1] (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[22] and Kaplan-Meier curves with log-rank test were plotted with packHV.[23]

RESULTS

From 101 patients with mTNBC who had ULP-WGS performed on ctDNA samples as previously published,[7] 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[5,7]). 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 cfDNA collected before starting platinum chemotherapy and 50 (70%) had cfDNA 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

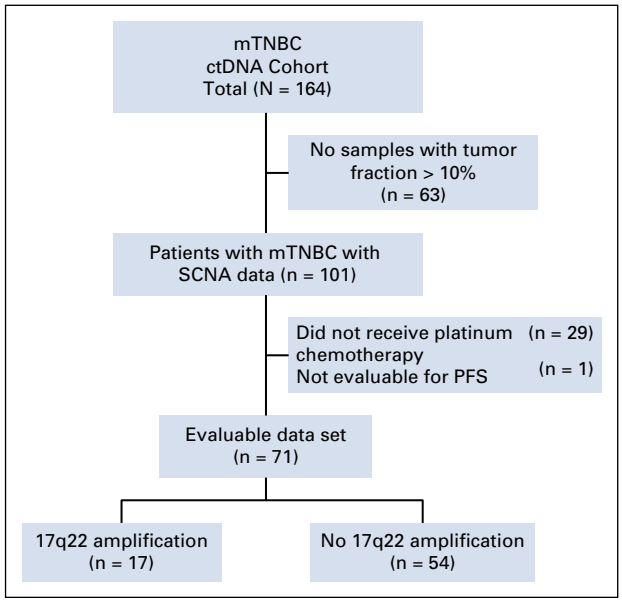

FIG 1. Flowchart diagram. ctDNA, circulating tumor DNA; mTNBC, metastatic triple-negative breast cancer; PFS, progression-free survival; SCNA, somatic copy-number alteration.

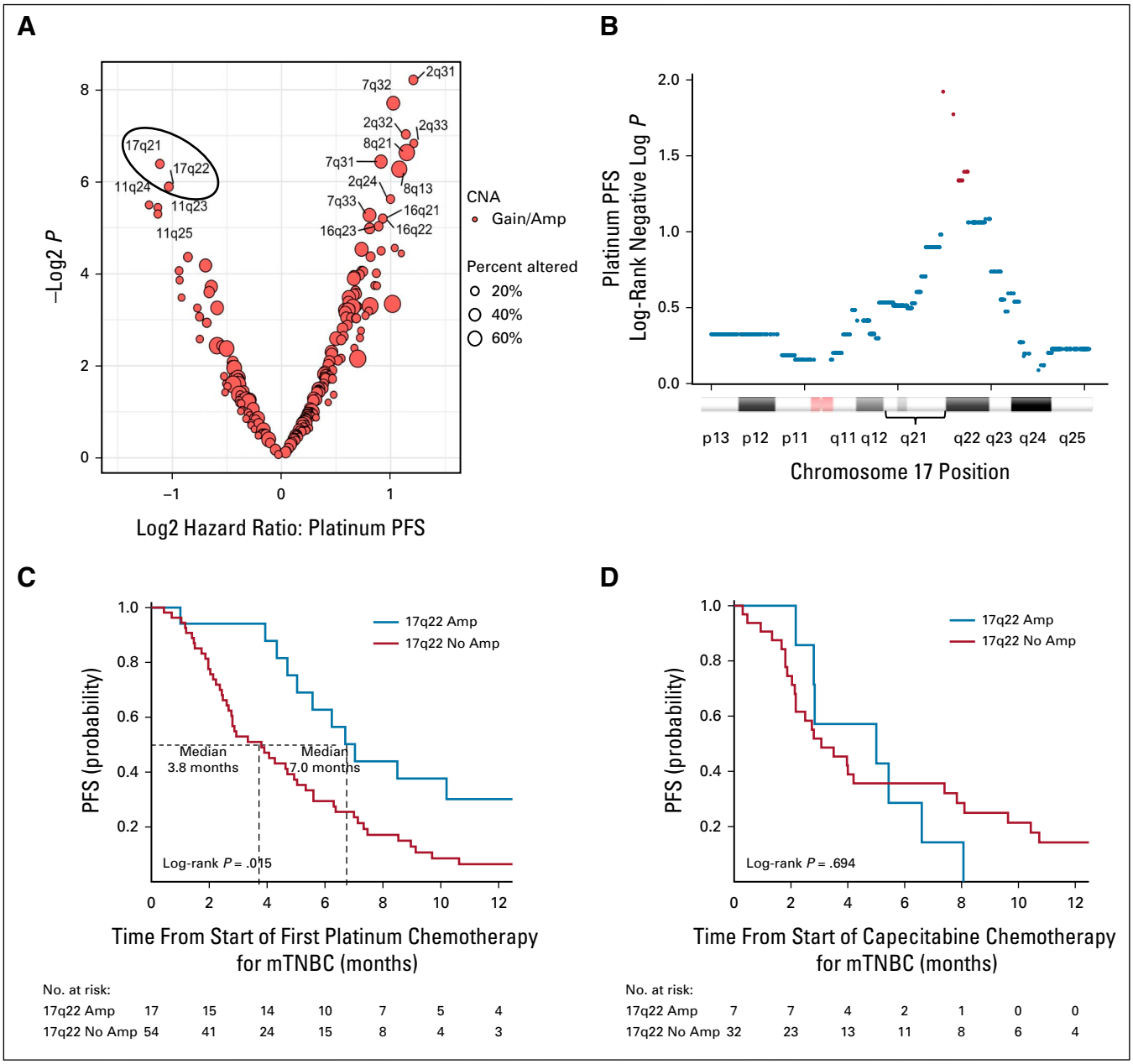

FIG 2. Association of 17q amplicon in circulating tumor DNA with platinum chemotherapy benefit in mTNBC. (A) Volcano plot of hazard ratio significance versus $\log_2$ hazard ratio for PFS of first platinum chemotherapy in the metastatic setting by chromosomal cytoband from highest tumor fraction blood draw. Only cytobands with gain in at least 10% of patients with mTNBC or primary TNBC are plotted, as determined by gene-level GISTIC gain/Amp. Size of point indicates the frequency altered among patients with mTNBC. Black circle indicates 17q21 and 17q22. (B) Association of Amp along chromosome 17 with PFS of platinum chemotherapy in patients with mTNBC (n = 101), visualized as log-rank negative $\log_{10}$ *P* value (*y*-axis). Each point represents an individual gene based on GISTIC output. *x*-axis indicates chromosome 17 loci, with the pink region indicating the centromere; chromosome graphic from National Center for Biotechnology Information Genome Decoration Page. (C and D) Kaplan-Meier curve of PFS of (C) platinum chemotherapy and (D) capecitabine for patients with mTNBC stratified by presence or absence of 17q22 Amp. Amp, amplification; CNA, copy-number alteration; mTNBC, metastatic triple-negative breast cancer; PFS, progression-free survival.

instability. Furthermore, amplification of 17q22 remained significantly associated with longer PFS with platinum chemotherapy in additional sensitivity analyses of the base multivariable model with platinum chemotherapy as first-line therapy versus later (17q22 amplification $P = .04$), 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

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[a]
Female, No. (%)	17 (100)	54 (100)	
BRCA status, No. (%)			.09
BRCA1-mutated	2 (12)	7 (13)	
BRCA2-mutated	1 (6)	1 (2)	
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
HR+/HER2–	3 (18)	5 (9)	
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. (%)			.71
0	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.
[a]Fisher's exact test used to compare categorical variables; Mann-Whitney *U* test used to compare medians of continuous variables.

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 2. Multivariable Analysis of 17q22 Amplification With Platinum Chemotherapy Progression-Free Survival in Metastatic TNBC

Variables	Hazard Ratio	95% CI	*P*
17q22 status			
No amplification	Ref	Ref	Ref
Amplification	**0.37**	**0.16 to 0.84**	**.02**
BRCA status			
BRCA status unknown	Ref	Ref	Ref
BRCA wild-type	0.74	0.26 to 2.09	.57
BRCA mutant	0.81	0.19 to 3.39	.78
Primary receptor status			
Indeterminate	Ref	Ref	Ref
HR-positive/HER2-negative	2.43	0.48 to 12.4	.29
HER2	1.63	0.15 to 17.5	.69
TNBC	1.27	0.30 to 5.32	.75
Primary stage at diagnosis			
Stage I	Ref	Ref	Ref
Stage II	1.74	0.74 to 4.11	.21
Stage III	2.04	0.80 to 5.18	.14
Stage IV	1.75	0.48 to 6.41	.40
Age at primary diagnosis (per decade over 40), years	0.99	0.74 to 1.34	.95
Year sample collected	0.88	0.71 to 1.10	.25
Sample collection cohort	0.97	0.82 to 1.15	.73
Percent genome altered	3.78	0.67 to 21.1	.13

Bold text indicates significant association in multivariable model.
Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor; TNBC, triple-negative breast cancer.

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 METABRIC[1] and TCGA[4] (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 signature[24] 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,

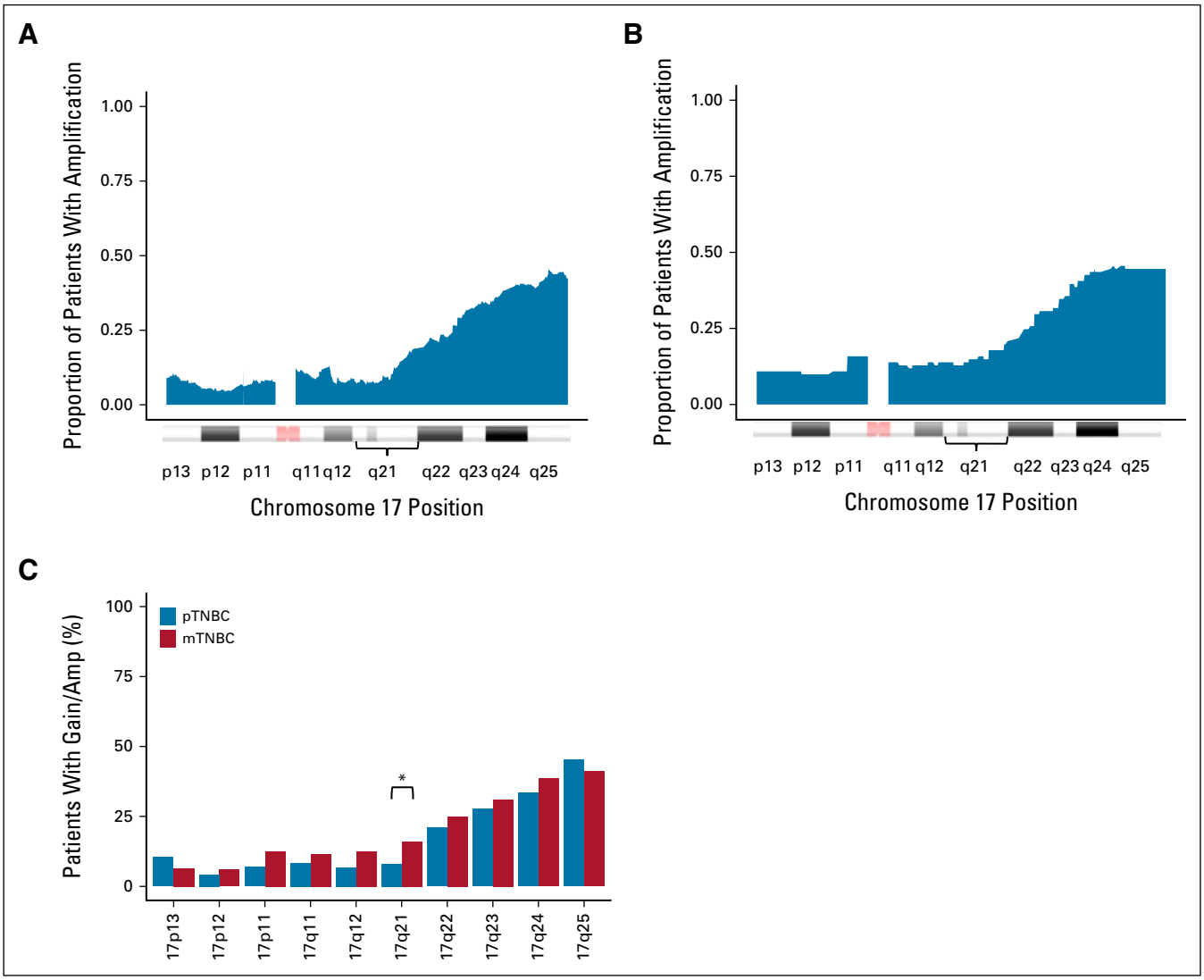

FIG 3. Frequency of 17q amplification in triple-negative breast cancer. (A and B) Proportion of patients with pTNBC in TCGA and METABRIC (total n = 433; A) and patients with mTNBC (total n = 101; B) with amplification on chromosome 17, based on gene-level copy number via GISTIC2.0. *x*-axis indicates chromosome 17 loci, with the pink region indicating the centromere; chromosome graphic from National Center for Biotechnology Information Genome Decoration Page. (C) Proportion of patients with pTNBC in TCGA and METABRIC (total n = 433; blue bars) and patients with mTNBC (total n = 101; red bars) with gain/amplification on chromosome 17 by cytoband, based on gene-level copy number via GISTIC2.0. *Fisher exact FDR $P = .015$. FDR, false discovery rate; METABRIC, Molecular Taxonomy of Breast Cancer International Consortium; mTNBC, metastatic triple-negative breast cancer; pTNBC, primary triple negative breast cancer; TGCA, The Cancer Genome Atlas.

telomeric allelic imbalance, and HRD large-scale state transitions), but was not associated with tumor-infiltrating lymphocytes.[15,25-28] More recently, the HRDetect[29] 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. An ULP-WGS ctDNA biomarker may capture a patient population who would differentially benefit from platinum chemotherapy, even in the absence of biopsy-accessible tumor tissue or a germline *BRCA1/2* mutation. Obtaining ctDNA and analyzing ULP-WGS is more feasible and affordable than bulk tissue DNA sequencing. 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.[36,37] Low-coverage WGS of cfDNA has been used to characterize CNAs in many cancers including TNBC, and the burden of CNAs may be prognostic.[7,38-45] In some cancers, CNA burden may be predictive of response to checkpoint inhibitors.[46,47] 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.[48-55] 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.[56] 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.[57,58] 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* is associated with improved early-stage TNBC PFS and overall survival treated with adjuvant chemotherapy.[59] *NR1D1* prevents DNA damage repair, inhibiting both nonhomologous end joining and homologous recombination, which results in chemosensitivity.[60,61] *DLX4* encodes BP1, a transcription factor, which binds to and suppresses *BRCA1*.[62,63] 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.[64-67]

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[68] 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,[19,20,68] 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.[69] 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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DATA SHARING STATEMENT

All sequencing data supporting the conclusions of this paper are available through dbGAP (dbGaP Accession Number: phs001417.v1.p1). The METABRIC data set is available at https://www.synapse.org/#!Synapse:syn1688369/wiki/27311. The TCGA-BRCA data set is available at https://portal.gdc.cancer.gov/projects/TCGA-BRCA.

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