Clinical spectrum and prognostic value of TP53 mutations in circulating tumor DNA from breast cancer patients in China

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Abstract
Background: TP53 mutations are common in breast cancer. There is currently no large-scale cohort study to investigate the TP53 landscape in breast cancer patients from China. The predictive value of TP53 mutations for the efficacy of human epidermal growth factor receptor 2 (HER2)-targeted therapy in breast cancer remains controversial. In the present study, we aimed to analyze the clinical spectrum and prognostic value of TP53 mutations in circulating tumor DNA (ctDNA) from breast cancer patients in China.

Methods: We retrospectively analyzed the clinical data and TP53 mutation features in ctDNA samples from 804 patients with metastatic breast cancer. TP53 mutations were detected by target region capture-based next-generation sequencing. The relationship between TP53 mutation status and disease-free survival (DFS) was analyzed in 444 patients with metastatic breast cancer. Moreover, the relationship between TP53 mutation status and progression-free survival (PFS) was analyzed in 55 HER2-positive patients treated with first-line trastuzumab-based therapy. Kaplan-Meier analysis was performed to estimate the survival curves of the different subgroups, and the log-rank test was used to compare the curves. A Cox regression model was used to estimate multivariable-adjusted hazard ratios and their 95% confidence intervals (CIs) associated with the DFS and PFS.

Results: Among the 804 investigated patients, 431 (53.6%) patients harbored TP53 mutations. TP53 mutations were differentially distributed among different molecular subtypes of breast cancer (P < 0.05). Patients with TP53 mutations had a shorter...
DFS than those with wild-type TP53 (hazard ratio = 1.32, 95% CI = 1.09-1.61, \( P = 0.005 \)). TP53 mutations in exons 5-8 were associated with worse outcome (hazard ratio = 1.50, 95% CI = 1.11-2.03, \( P = 0.009 \)). However, TP53 mutation status was not significantly associated with PFS in HER2-positive patients who received first-line trastuzumab-based therapy (\( P = 0.966 \)). Interestingly, in the taxane combination group, patients with TP53 mutations exhibited longer PFS than those without TP53 mutations (hazard ratio = 0.08, 95% CI = 0.02-0.30, \( P < 0.001 \)). However, in the non-taxane combination group, patients with TP53 mutations displayed shorter PFS than those with wild-type TP53 (hazard ratio = 4.84, 95% CI = 1.60-14.66, \( P = 0.005 \)).

**Conclusions:** TP53 mutations in exons 5-8 may be an independent prognostic marker for short DFS in patients with metastatic breast cancer. TP53 mutations had opposite effects on trastuzumab-treated patients treated with and without taxanes.

**Keywords**

breast cancer, TP53 mutation, circulating tumor DNA, next-generation sequencing, Chinese, prognosis, trastuzumab, taxanes

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## 1 | BACKGROUND

TP53 is a tumor suppressor gene that plays a key role in many cellular pathways and regulates essential cell activities, such as proliferation, differentiation, cell death, DNA repair, and the formation of blood vessels [1, 2]. Mutations in TP53 are common in cancers [3, 4] and present in approximately 25%-30% of all breast cancer cases [5, 6]. Several studies have investigated the TP53 mutational spectrum in various subtypes of breast cancer [5-8]. However, the TP53 genetic landscape in Chinese breast cancer patients remains unclear.

Numerous studies have indicated that TP53 is a biomarker for predicting poor prognosis in breast cancer patients [5, 9-11]. TP53 mutations located in the regions encoded TP53 DNA-binding domains have been reported to be associated with poor prognosis in patients with breast cancer [10, 12]. However, their predictive value for the efficacy of human epidermal growth factor receptor 2 (HER2)-targeted therapy in breast cancer remains controversial. Several studies most of which relied upon immunohistochemistry (IHC) to assess p53 alterations have indicated that TP53 mutations were not associated with the response to anticancer therapy [8, 13-16]. Nevertheless, IHC cannot be used to detect all gene mutations, as many mutations do not lead to p53 protein accumulation. One early study indicated that p53 status, as determined by IHC, was not associated with the response to trastuzumab-based treatment [15]. However, two clinical studies have indicated that patients with TP53 mutations exhibited good responses to trastuzumab [14, 17]. Since most of the patients in these studies received chemotherapy in addition to trastuzumab, these findings must be confirmed by further studies.

In the present study, we used target region capture-based next-generation sequencing to analyze the TP53 mutation spectrum of circulating tumor DNA (ctDNA) from 804 Chinese breast cancer patients and analyzed the relationships between TP53 mutations, prognosis, and the efficacy of first-line trastuzumab-based therapy.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

TP53 mutation status was determined in the plasma of patients with metastatic breast cancer treated at National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, China) between March 2015 and October 2018. The inclusion criteria were as follows: (1) patients who were at least 18 years old; (2) patients who had a histologic/cytologic diagnosis of invasive breast cancer; (3) patients who had received radical surgery for primary tumor; (4) patients who were female; (5) patients who had complete clinicopathological data; and (6) patients who had sufficient blood samples for ctDNA analysis. The exclusion criteria were as follows: (1) patients who were male; and (2) patients who had received chemotherapy within the past month before the peripheral blood samples collection. This study was approved by the Ethics Committee of National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College...
Follow-up

2.2 | Blood sample collection

The testing for patients was ordered by the treating physician to identify clinically relevant genomic alterations that could potentially help to determine prognosis or to make treatment decisions. Peripheral blood samples for ctDNA analysis were collected in Streck tubes (Streck, Omaha, NE, USA) and centrifuged (1,600 × g for 10 min) followed by transfer to new microcentrifuge tubes, and centrifugation at 16,000 × g at 4°C for 10 min to remove remaining cell debris) within 72 hours to separate the plasma from the peripheral blood.

2.3 | DNA extraction and quantification

A QIAamp Circulating Nucleic Acid Kits (Qiagen, Hilden, Germany) was used to isolate circulating DNA from plasma samples. Genomic DNA (gDNA) was isolated from peripheral lymphocytes using the QIAamp DNA Blood Mini Kit (Qiagen) as a control. All DNA extractions were performed according to the manufacturer’s protocols.

The DNA concentration was assessed using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and the Qubit dsDNA High-sensitivity (HS) Assay Kit (Invitrogen). The size distribution of the circulating DNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and the DNA HS kit (Agilent Technologies).

2.4 | Target capture and next-generation sequencing

Library preparation, hybrid capture, and circulating DNA and gDNA sequencing were performed as previously described [19, 20]. The targeted region for sequencing covered ∼1.1 Mb, including 1,021 genes [21], and encompassed all introns and exons of \( TP53 \). DNA sequencing was performed using the HiSeq 3000 Sequencing System (Illumina, San Diego, CA, USA) with 2 × 101 bp paired-end reads.

Low-quality reads and terminal adaptor sequences were filtered out. Burrows-Wheeler Aligner (BWA, version 0.7.12-r1039) was carried out to align the clean reads to the reference human genome (hg19), and Genome Analysis Toolkit (GATK, version 3.4-46-gbc02625) was used for realignment and recalibration. Single-nucleotide variants (SNVs) were characterized by MuTect (version 1.1.4) and NChot [22]. Small insertions and deletions (indels) were identified by GATK.

2.5 | Follow-up

All patients were regularly followed-up at the out-patient clinic every 3 months within 2 years and every 6 months 2-5 years after the surgery. The efficacy, safety, and survival data of advanced breast cancer patients were followed-up every 2-3 months. Last follow-up time was October 30, 2018.

2.6 | Statistical analysis

The chi-square test and Fisher’s exact test were used to compare categorical variables. Disease-free survival (DFS) was defined as the duration from radical resection of breast cancer to the time of recurrence or death of any cause. PFS was calculated from the date of treatment initiation to the date of disease progression or death of any cause. Patients without an endpoint (progression or death) were censored at the date of the last follow-up. Kaplan-Meier survival plots were generated based on gene mutation status, and curves were compared using log-rank tests. A Cox proportional hazards model was used to analyze the association between DFS or PFS and gene mutations and clinical characteristics. All statistical tests used were two-sided, and results with \( P \) values less than 0.05 were considered significant. All statistical analyses were performed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA) or GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA).
### TABLE 1  Clinical characteristics of 804 patients with metastatic breast cancer

| Characteristic                          | Whole cohort [cases (%)] | TP53 mutant [cases (%)] | TP53 wild-type [cases (%)] | P value |
|-----------------------------------------|--------------------------|-------------------------|---------------------------|---------|
| Total                                   | 804                      | 431                     | 373                       |         |
| Menopausal status                       |                          |                         |                           |         |
| Premenopausal                           | 469 (58.3)               | 247 (57.3)              | 222 (59.5)                | 0.526   |
| Postmenopausal                          | 335 (41.7)               | 184 (42.7)              | 151 (40.5)                |         |
| Tumor stage at diagnosis                |                          |                         |                           |         |
| Stage 1-2                               | 495 (61.6)               | 255 (59.2)              | 240 (64.3)                | 0.132   |
| Stage 3                                 | 309 (38.4)               | 176 (40.8)              | 133 (35.7)                |         |
| Histology at diagnosis                  |                          |                         |                           |         |
| ILC                                     | 42 (5.2)                 | 23 (5.3)                | 19 (5.1)                  | 0.877   |
| IDC                                     | 762 (94.8)               | 408 (94.7)              | 354 (94.9)                |         |
| Molecular subtype                       |                          |                         |                           |         |
| HR+/HER2−                               | 397 (49.4)               | 170 (39.4)              | 227 (60.9)                | <0.001  |
| HR+/HER2+                               | 138 (17.2)               | 69 (16.0)               | 69 (18.5)                 |         |
| HR−/HER2+                               | 121 (15.0)               | 84 (19.5)               | 37 (9.9)                  |         |
| HR−/HER2−                               | 148 (18.4)               | 108 (25.1)              | 40 (10.7)                 |         |
| HR status†                              |                          |                         |                           |         |
| Positive                                | 535 (66.5)               | 239 (55.5)              | 296 (79.4)                | <0.001  |
| Negative                                | 269 (33.5)               | 192 (44.5)              | 77 (20.6)                 |         |
| HER2 status                             |                          |                         |                           |         |
| Positive                                | 259 (32.2)               | 153 (35.5)              | 106 (28.4)                | 0.032   |
| Negative                                | 545 (67.8)               | 278 (64.5)              | 267 (71.6)                |         |
| Line of chemotherapy                    |                          |                         |                           |         |
| 0                                       | 16 (2.0)                 | 5 (1.2)                 | 11 (2.9)                  | 0.051   |
| 1                                       | 194 (24.1)               | 96 (22.3)               | 98 (26.3)                 |         |
| ≥2                                      | 594 (73.9)               | 330 (76.6)              | 264 (70.8)                |         |
| Line of endocrine therapy for HR+ patients |                      |                         |                           |         |
| 0                                       | 221 (41.3)               | 111 (46.4)              | 110 (37.2)                | 0.057   |
| 1                                       | 146 (27.3)               | 64 (26.8)               | 82 (27.7)                 |         |
| ≥2                                      | 168 (31.4)               | 64 (26.8)               | 104 (35.1)                |         |
| Line of anti-HER2 therapy for HER2+ patients |                    |                         |                           |         |
| 0                                       | 116 (44.8)               | 76 (49.7)               | 40 (37.7)                 | 0.057   |
| ≥1                                      | 143 (55.2)               | 77 (50.3)               | 66 (62.3)                 |         |

### RESULTS

#### 3.1 Patient characteristics

A total of 804 female patients with metastatic breast cancer treated between March 2015 and October 2018 were retrospectively investigated. Patient characteristics are summarized in Table 1. The median age at which the patients were initially diagnosed with breast cancer was 42 years (range: 22 to 78 years) in all of the patients. The median age was 43 years (range: 22 to 78 years) in the TP53 mutant group versus 42 years (range: 26 to 78 years) in the TP53 wild-type group (P = 0.568). Fifty-five (6.8%) patients received trastuzumab plus chemotherapy as the first-line treatment after being diagnosed with metastatic breast cancer.

#### 3.2 Characterization of TP53 mutations

We performed target region capture-based next-generation sequencing to evaluate the status of TP53 mutations in the whole cohort. We found that 431 (53.6%) patients harbored at least one TP53 mutation. Among them, 65 (15.1%) patients carried two different TP53 mutations. The TP53 mutations

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Note: Positive HR status was defined as positive for estrogen receptor and/or progesterone receptor.

Abbreviations: HR, hormone receptor; HER2, human epidermal growth factor receptor 2; ILC, infiltrating lobular carcinoma; IDC, infiltrating ductal carcinoma.
FIGURE 1  Frequencies of TP53 mutations in different molecular subtypes of breast cancer. A. Proportion of patients with TP53 mutations according to molecular subtype. B. Comparison of TP53 mutation frequencies between our data and the MSK-IMPACT data. Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor

3.3 | Spectra of TP53 mutations in different molecular subtypes

TP53 mutations were differentially distributed among different molecular subtypes of breast cancer (P < 0.01; Figure 1A). TP53 mutation frequency was lower in the patients with luminal-type breast cancer (hormone receptor-positive [HR⁺]HER2⁺/−; 44.7% [239/535];) than in the patients with HR−HER2⁺ breast cancer (69.4% [84/121]; P < 0.001) and triple-negative breast cancer (TNBC) (HR−HER2−; 73.0% [108/148]; P < 0.001).

Patients with HR−HER2⁺ tumors showed a higher proportion of span mutations as compared with non-HR−HER2⁺ patients (17.1% [18/105] versus 8.0% [33/414]; P = 0.005), whereas those with HR⁺HER2⁺ cancer showed a higher proportion of missense mutations compared with patients with non-HR⁺HER2⁺ cancer (67.1% [51/76] versus 53.7% [238/443]; P = 0.030). We did not find any differences in mutational hotspots among the molecular subtypes (P > 0.05; Table 2).

We further compared the data in this study (from a Chinese cohort) with those from the MSK-IMPACT cohort. A total of 1746 breast cancer patients from the MSK-IMPACT cohort were included. Among them, 626 (35.9%) patients harbored TP53 mutations. The frequency of TP53 mutations in the Chinese cohort was significantly higher than that in the MSK-IMPACT cohort (P < 0.001). We further investigated the TP53 mutation frequencies among different molecular
TABLE 2  TP53 mutation characteristics in different molecular subtypes of breast cancer

| TP53 mutation characteristic | Total  | HR+HER2− | HR+HER2+ | HR+HER2− | HR−HER2− | P value |
|-----------------------------|--------|----------|----------|----------|----------|--------|
| Number of mutations         | 519    | 206      | 76       | 105      | 132      |        |
| Variant classification      |        |          |          |          |          |        |
| Missense                    | 289 (55.7) | 120 (58.3) | 51 (67.1) | 47 (44.8) | 71 (53.8) | 0.020  |
| Frame shift                 | 90 (17.3)  | 27 (13.1)  | 11 (14.5) | 21 (20.0) | 31 (23.5) | 0.072  |
| Nonsense                    | 80 (15.4)  | 37 (18.0)  | 10 (13.2) | 17 (16.2) | 16 (12.1) | 0.481  |
| Span                        | 51 (9.8)    | 16 (7.8)    | 4 (5.3)   | 18 (17.1) | 13 (9.8)  | 0.028  |
| In-frame Del/Ins            | 9 (1.7)     | 6 (2.9)     | 0 (0)     | 2 (1.9)   | 1 (0.8)   | 0.371  |
| Mutational hotspots         |        |          |          |          |          |        |
| R213*                       | 17 (3.3)    | 9 (4.4)     | 1 (1.3)   | 5 (4.8)   | 2 (1.5)   | 0.291  |
| R248Q                       | 15 (2.9)    | 7 (3.4)     | 1 (1.3)   | 1 (1.0)   | 6 (4.5)   | 0.196  |
| R273H                       | 14 (2.7)    | 4 (1.9)     | 3 (3.9)   | 4 (3.8)   | 3 (2.3)   | 0.283  |
| Y220C                       | 12 (2.3)    | 3 (1.5)     | 2 (2.6)   | 1 (1.0)   | 6 (4.5)   | 0.050  |
| R175H                       | 11 (2.1)    | 4 (1.9)     | 3 (3.9)   | 2 (1.9)   | 2 (1.5)   | 0.648  |
| Others                      | 450 (86.7)  | 179 (86.9)  | 66 (86.8) | 92 (87.6) | 113 (85.6) | <0.001 |

Abbreviations: HR, hormone receptor; HER2, human epidermal growth factor receptor 2. Del, deletion; Ins, insertion.

subtypes and compared them between the Chinese and the MSK-IMPACT cohorts. We observed significant differences between these two cohorts: in the HR+HER2− subgroup, the TP53 mutation frequency was higher in the Chinese cohort (P < 0.001); in contrast, in the TNBC subgroup, the frequency of TP53 mutation was higher in the MSK-IMPACT cohort (P < 0.001; Figure 1B).

3.4 Association of TP53 mutation status and the DFS of breast cancer patients

To analyze the association between TP53 mutation and DFS, we retrospectively calculated the DFS of the eligible patients. A total of 444 patients recorded DFS, including 243 (54.7%) patients with TP53 mutations. We found that patients with TP53 mutations had a shorter DFS than those with wild-type TP53. The median DFS for patients with TP53 mutations was 28.5 months (95% confidence interval [CI] = 23.6-33.3 months) versus 40.6 months (95% CI = 33.5-47.7 months) for patients without TP53 mutations (hazard ratio = 1.32; 95% CI = 1.09-1.61; P = 0.005; Figure 2).

The results of the univariate Cox regression analysis with clinical characteristics and TP53 mutation status are shown in Table 3. HER2 status, HR status, tumor stage at diagnosis, histology at diagnosis, TP53 mutation status, and exons 5-8 mutation status were related to DFS of patients with metastatic breast cancer (all P < 0.05). A multivariate Cox regression analysis was conducted to investigate the association between these characteristics and DFS. TP53 mutation status was not significantly associated with DFS (hazard ratio = 1.18, 95% CI = 0.97-1.45, P = 0.101). However, the DFS of patients with TP53 mutations in exons 5-8 was significantly shorter than the DFS of patients with wild-type TP53 (hazard ratio = 1.50, 95% CI = 1.11-2.03, P = 0.009, Table 3).

3.5 Association of TP53 mutation status and the response to first-line trastuzumab-based therapy in breast cancer patients

A total of 55 metastatic breast cancer patients who received trastuzumab-based treatment as first-line therapy were selected to evaluate the relationship between TP53 mutation status and the response to trastuzumab. As shown in Supplementary Table S2, there were no significant differences in clinical characteristics between TP53 mutant and wild-type groups (all P > 0.05). Additionally, TP53 mutation status
TABLE 3 Univariate and multivariate Cox regression analyses for DFS in patients with breast cancer

| Variable                                      | Univariate Cox regression analysis | Multivariate Cox regression analysis |
|------------------------------------------------|------------------------------------|-------------------------------------|
| | Hazard ratio (95% CI) | P value | Hazard ratio (95% CI) | P value |
| HER2 status (positive vs. negative)            | 1.42 (1.15-1.77) | 0.001 | 1.28 (1.02-1.60) | 0.031 |
| HR status (positive vs. negative)              | 0.09 (0.56-0.85) | 0.001 | 0.76 (0.62-0.95) | 0.015 |
| Tumor stage at diagnosis (stages 1-2 vs. stage 3) | 0.71 (0.59-0.86) | 0.001 | 0.47 (0.24-0.91) | 0.005 |
| Histology at diagnosis (ILC vs. IDC)           | 0.46 (0.24-0.88) | 0.020 | 0.76 (0.62-0.92) | 0.026 |
| TP53 mutation status (mutation vs. wild-type)  | 1.32 (1.09-1.61) | 0.005 | 1.18 (0.97-1.45) | 0.101 |
| TP53 exons 5-8 mutation status (mutation vs. wild-type) | 1.61 (1.20-2.17) | 0.002 | 1.50 (1.11-2.03) | 0.009 |

1 Positive HR status was defined as positive for estrogen receptor and/or progesterone receptor.
Abbreviations: DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; ILC, infiltrating lobular carcinoma; IDC, infiltrating ductal carcinoma.

was not significantly associated with PFS. The median PFS for patients with TP53 mutation was 9.1 months versus 5.4 months for those without TP53 mutation (hazard ratio = 1.01; 95% CI = 0.59-1.75; P = 0.966; Figure 3A).

To further explore the relationship between TP53 mutation status and taxanes (including paclitaxel and docetaxel) treatment response in the breast cancer patients who received trastuzumab-based treatment as first-line therapy, we divided these patients into the taxane and non-taxane combination groups (Supplementary Table S3). Interestingly, we found that in the taxane combination group, patients with TP53 mutations had longer median PFS than those without TP53 mutations (12.1 months vs. 2.7 months; hazard ratio = 0.08, 95% CI = 0.02-0.30, P < 0.001; Figure 3B). However, in the non-taxane combination group, the opposite pattern was observed. Patients with TP53 mutations had shorter median PFS than those with wild-type TP53 (4.1 months vs. 8.1 months, (hazard ratio = 4.84, 95% CI = 1.60-14.66, P = 0.005; Figure 3C).

4 | DISCUSSION

In the present study, we performed a TP53 mutation analysis within a large cohort of 804 Chinese patients with metastatic breast cancer and further compared our data to those from the MSK-IMPACT cohort. Our results indicated that TP53 mutation was a prognostic marker for poor outcomes in breast cancer. Additionally, it may affect the response of breast cancer patients to first-line therapy based on trastuzumab.

In the present study, the frequency of TP53 mutation was 53.6%, which was higher than that in the MSK-IMPACT cohort (35.9%) [18]. Additionally, our data indicated that the TP53 mutation frequency was higher in TNBC and HER2+/HR− breast cancer than in luminal-type breast cancer. We also found that the frequency of TP53 mutation in HER2+/HR+ breast cancer was higher in the MSK-IMPACT cohort than in our cohort, while the opposite was true for the TNBC subgroup. Further study is needed to investigate these differences between cohorts. A striking feature of the patient cohort in the present study was that the median age at diagnosis was very young (42 years). This corresponds to increased proportions of HER2+ (32.2%) and TNBC (18.4%) patients in the study population. In addition, all patients in the study population had metastatic disease at the time of consent and blood drawn for the study. Moreover, most patients had received at least 2 lines of chemotherapy. It is our understanding that metastatic breast cancer are also associated with a higher frequency of TP53 mutations than cancers at earlier stages. Both of these features of the patient population may have contributed to the higher percentage of TP53 mutations found in this cohort when compared with that in the MSK-IMPACT study. Another possibility is that this discrepancy reflects differences between the ethnicities of the patients.

Previous studies have shown that the TP53 sequence encoding the DNA-binding domain was the most frequently mutated region in the TP53 gene in breast cancer [6, 8]. Our study also found that most mutations occurred in the DNA-binding domain and that p.R213*, p.R248Q, p.R273H, p.Y220C, and p.R175H were common mutations of TP53. The majority (55.7%) of the mutations were missense mutations. Previous studies have shown that TP53 mutation was a prognostic marker for poor breast cancer outcomes. Several studies have investigated the impact of different types of TP53 somatic mutations on breast cancer, with conflicting results [5, 10, 12, 23, 24]. Some studies [10, 12] have shown that only missense mutations in the DNA-binding domain affected the outcome of breast cancer patients, but this was not confirmed by other studies [10, 12, 24, 25]. However, most previous studies have failed to investigate whether the prognostic impact of TP53 mutation is independent of other clinicopathologic factors [10, 12, 24]. In the present study, univariate Cox regression analysis indicated that TP53 mutation was related to short DFS. However, in the multivariate analysis, TP53 mutation status was not significantly associated with DFS. Further analysis showed that TP53 mutation in exons 5-8 was significantly associated with shorter DFS than wild-type TP53.
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FIGURE 3  Impact of TP53 mutation on the response to trastuzumab-based first-line therapy. A. PFS of patients underwent trastuzumab-based first-line treatment based on the presence of TP53 mutation. B. PFS of patients in the taxane combination group based on the presence of TP53 mutation. C. PFS of patients in the non-taxane combination group based on the presence of TP53 mutation. Abbreviations: PFS, progression-free survival; CI, confidence interval

TP53 mutations located in exons 5-8 may predict shorter DFS of breast cancer patients than wild-type TP53. Most of the TP53 mutations found in breast cancer were missense substitutions located in the DNA binding domain (exons 5-8). The difference in prognosis of TP53 DNA-binding domain mutations and mutations in other domains is still under debate. This difference is hypothesized to be related to the degree to which the mutants function as dominant negative mutants [24]. However, this hypothesis needs to be confirmed by further studies.

Data on the association between the presence of TP53 mutations and clinical treatment response are conflicting. Although a number of prior studies of breast cancer have suggested that the presence of TP53 mutations was associated with a lack of response to a variety of chemotherapy agents, including anthracyclines and the combination of cyclophosphamide, methotrexate, and fluorouracil [8, 26, 27], some other studies have found that TP53 mutation was associated with a better response to docetaxel and trastuzumab [13, 14, 28-30]. The association between TP53 mutation and trastuzumab efficacy is also controversial. One early study indicated that p53 status, as determined by IHC, is not associated with the response to trastuzumab-based treatment [15]. Nevertheless, two clinical studies have indicated that patients with TP53 mutations may exhibit a better response to trastuzumab [14, 17]. However, trastuzumab is used in combination with chemotherapy in most cases during clinical practice. Therefore, we investigated the efficacy of trastuzumab in metastatic breast cancer. To reduce bias from other factors, we chose patients who received trastuzumab as first-line therapy. We did not find any association between TP53 mutation and the efficacy of trastuzumab-based first-line therapy. We further analyzed the impact of TP53 mutation in different chemotherapy subgroups. Interestingly, we found that in the taxane combination group (including paclitaxel and docetaxel), the presence of TP53 mutation was a sensitive biomarker of efficacy. In contrast, in the non-taxane combination group, patients with TP53 mutations exhibited a poor response. Different types of chemotherapeutics have different mechanisms of action, which may explain why patients with TP53 mutations showed a better response to taxanes. A preclinical study indicated that in vitro, TP53 mutant tumor cell lines are more sensitive to mitotic poisons, such as taxanes, than are wild-type TP53 cell lines, presumably because mutant cells lack the p53-dependent capacity to induce G1 and G2 phase arrest and cannot defend themselves from taxane-induced mitotic blockade and apoptosis [29].

A phase III clinical trial assessed the impact of TP53 status on taxane- and non-taxane-based chemotherapy regimens [31]. However, this study showed no effect of TP53 mutation on the response to taxane- and non-taxane combination treatments. This may be because this study enrolled patients with all subtypes of breast cancer, and the impact of TP53 mutation may be distinct for different subtypes of breast cancer. This study also enrolled locally advanced breast cancer patients who received neoadjuvant chemotherapy, and the dosage and intensity of chemotherapy are of crucial importance in inducing a response and a survival benefit in TP53-mutated tumors [8]. In our study, we investigated the impact of TP53 mutation specifically in HER2+ patients
who received first-line trastuzumab-based therapy. Another difference between the previous study and our study is that we used next-generation sequencing to detect TP53 mutations in ctDNA, which may limit the impacts of temporal and spatial heterogeneity among tumors.

Some limitations of this study must be acknowledged. First, DFS was retrospectively calculated, which may influence the survival analysis, as TP53 mutation status may change after distant metastasis. Second, the retrospective design for the analysis of trastuzumab-based first-line therapy did not provide sufficient power to arrive at statistically sound conclusions. The predictive value of TP53 mutation among different chemotherapy agents needs to be verified in prospective studies.

5 CONCLUSIONS

In conclusion, our data indicate that TP53 somatic mutations are common in Chinese patients with breast cancer and are more frequently observed in HER2+/HR− patients and those with TNBC than in those with other subtypes of breast cancer. TP53 mutation in exons 5–8 may be an independent prognostic marker for short DFS. TP53 mutation has opposite effects in patients treated with trastuzumab-based chemotherapy in combination with and without taxanes. Large-scale prospective studies are needed to verify our findings.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was reviewed and approved by the Institutional Review Board of National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (16-038/1117). Each participant signed the informed consent before participating to this study.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data used to support the findings of this study are available from the corresponding authors upon request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

Z.Y. designed and performed experiments, analyzed data, drafted and provided critical revision of the manuscript. F.M. contributed substantially to the conception of the study, acquired the funding, designed and performed experiments, drafted and provided critical revision of the manuscript. G.R. analyzed data and co-wrote the manuscript. Y.G. and C.L. designed and performed experiments and drafted the manuscript. B.X. contributed substantially to the conception of the study, supervised the research, acquired the funding, and drafted and provided critical revision of the manuscript. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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