


Prognostic Value of the *TP53* Mutation Location in Metastatic Breast Cancer as Detected by Next-Generation Sequencing

This article was published in the following Dove Press journal:
Cancer Management and Research

Han Bai¹
Jianjun Yu²
Shidong Jia²
Xiaoran Liu¹
Xu Liang¹
Huiping Li¹ 

¹Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Breast Oncology, Peking University Cancer Hospital and Institute, Beijing, 100142, People's Republic of China; ²Huidu Shanghai Medical Sciences, Shanghai, 201499, People's Republic of China

Purpose: The status of *TP53* mutations was measured in cell-free DNA from patients with metastatic breast cancer (MBC) to investigate disease characteristics and the prognostic role of different locations of the *TP53* mutation site.

Patients and Methods: Blood samples were taken from a total of 187 patients diagnosed with MBC who were treated at the Department of Breast Oncology, Peking University Cancer Hospital between January 2013 and March 2020. Next-generation sequencing was used to investigate the *TP53* mutation spectra of circulating free DNA in these blood samples.

Results: Among the 187 MBC patients, *TP53*-mutated patients had a significantly shorter median disease-free survival (DFS) and overall survival (OS) compared with *TP53* wild-type patients ($P=0.001$ and $P=0.006$, respectively). Additionally, in hormone receptor positive/HER2 negative (HR+/HER2-) and triple negative (TNBC) cohorts, *TP53*-mutated patients had a significantly shorter median DFS than *TP53* wild-type patients ($P=0.038$ and $P=0.023$, respectively). The 79 patients with *TP53* mutations carried 87 somatic *TP53* mutations, of which most (77.0%) mapped to the DNA-binding domain (DBD) of the protein encoded by *TP53* exons 5–8. In patients with *TP53* mutations, those occurring in the non-DBD had a significantly shorter median DFS and OS than *TP53* wild type ($P<0.001$ and $P=0.001$, respectively). Additionally, patients with non-missense mutations in the DBD had a significantly shorter median DFS and OS than *TP53* wild-type patients ($P=0.001$ and $P<0.001$, respectively). *TP53*-mutated patients had a significantly shorter DFS than *TP53* wild-type patients in the adjuvant endocrine therapy sensitive group ($P=0.008$), but differences in the endocrine therapy resistant group were not significant.

Conclusion: *TP53*-mutated MBC patients had a significantly worse outcome than *TP53* wild-type patients especially those in HR+/HER2- and TNBC cohorts. Of *TP53*-mutated patients, those with non-missense mutations in the DBD had worse breast cancer-related survival. *TP53* mutations were also associated with endocrine resistance.

Keywords: advanced breast cancer, *TP53* mutation, NGS, adjuvant endocrine therapy, DNA-binding domain

Correspondence: Xu Liang; Huiping Li
Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Breast Oncology, Peking University Cancer Hospital and Institute, No. 52, Fucheng Road, Haidian District, Beijing, 100142, People's Republic of China
Tel/Fax +86-10-88196739
Email liangxu15@outlook.com; huipingli2012@hotmail.com

Introduction

As a tumor suppressor and DNA binding transcription factor, *TP53* is actively involved in the regulation of the cell cycle, apoptosis, and genomic stability.^{1,2} *TP53* is one of the most frequently mutated genes in human cancers, including breast cancer,³ and numerous studies have reported it as a biomarker for predicting an aggressive and metastatic phenotype in breast cancer.^{4–7} Most of these studies

used first-generation sequencing or automated DNA extraction from formalin-fixed and paraffin-embedded tissue (FFPE). However, real-time (RT)-PCR results and first-generation sequencing could not be used to detect all *TP53* mutations to further investigate *TP53* status more accurately and reliably.

TP53 is located on chromosome 17p13.1 and contains 11 exons and 10 introns. Most *TP53* mutations map to exons 5–8, which encodes the DNA binding domain (DBD), and most are missense mutations.^{8–10} Hotspot codons 175, 213, 245, 248, 273, and 282 account for at least 2% of all mutations within the DBD.² Patients with acute myeloid leukemia carrying *TP53* mutations in the DBD had a worse prognosis than those with wild-type *TP53*.¹¹ Furthermore, another clinical trial showed that truncating mutations in the DBD had a significant independent prognostic value in breast cancer, being associated with increased recurrence compared with patients with non-modified p53 proteins.¹²

Early studies using first-generation sequencing or automated DNA extraction from FFPE found that *TP53* mutations were associated with poor prognosis in hormone receptor-positive (HR+) breast cancer patients.^{13–15} Moreover, in an HR+ cohort, *TP53* signaling was enriched in resistant tumors (38% in the aromatase inhibitor-resistant group vs 17% in the sensitive group) such that HR+ tumors with *TP53* mutations were mostly aromatase inhibitor-resistant.⁵ No significant result was obtained from human epidermal growth factor receptor 2 positive (HER2+) or triple-negative breast cancer (TNBC) cohorts.¹⁶ Other studies found that *TP53* mutations were associated with tumor recurrence and apoptosis, which were more common in HER2-positive and TNBC cohorts.^{17,18}

While the significance of *TP53* mutations has been shown by RT-PCR and first-generation sequencing, most clinical laboratories do not use next-generation sequencing (NGS) to determine the p53 mutational status because of high costs and complex interpretation. Therefore, it is difficult to understand the clinical applications of *TP53*.¹⁹ In the present study, we collected peripheral blood samples from Chinese patients with freshly diagnosed metastatic breast cancer (MBC) and examined the whole exons and introns of *TP53* by NGS to further investigate the relationship between *TP53* mutations, prognosis, and therapy.

Materials and Methods

Patients

From January 2013 to March 2020, patients past first-line treatment and those for whom blood samples were not

available were excluded, leaving a total of 194 at the stage of first-line treatment at the Department of Breast Oncology, Peking University Cancer Hospital. Of these, 187 consented with enrollment and had complete clinic-pathological information (Figure 1).

We defined estrogen receptor (ER), progesterone receptor (PR), and HER2 status according to recommended guidelines,^{20,21} which identified three subtypes: the HER2+ cohort, HR+/HER2- cohort, and TNBC cohort.

HR+/HER2- patients who accepted adjuvant endocrine therapy were divided into two groups: endocrine-resistant patients were defined as patients relapsing during adjuvant endocrine therapy, or <12 months after its completion. Endocrine-sensitive patients were defined as patients relapsing ≥12 months after completing adjuvant endocrine therapy in the early breast cancer stage.²²

Samples

Peripheral blood samples before first-line therapy were collected in EDTA Vacutainer tubes and centrifuged at 2000 g for 10 min at 4°C. The supernatant was then removed, and each sample of 3 mL plasma was stored at –80°C.

Circulating Free DNA Extraction

Circulating free (cf)DNA was extracted using a QIAamp Circulating Nucleic Acid Kit (QIAamp, Venlo, the Netherlands) from EDTA and citrate anticoagulant plasma. The average volume of plasma used for extraction was 2.6 mL (range, 0.7–3.9 mL). The quantity and quality of the purified cfDNA were checked using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). For samples with severe genomic contamination from peripheral blood cells, size selection was performed to remove large genomic fragments with AMPure XP beads (Beckman Coulter, Brea, CA, USA). Samples with a total yield <5 ng were considered inadequate for NGS and were removed from any further sequencing methods.

Library Preparation, Capture, and Sequencing

cfDNA was end-repaired before the dA-tailing process, and then ligated with proprietary UMI adapters. The library yield was measured after PCR amplification using a Qubit and Bioanalyzer 2100. Samples yielding >700 ng proceeded to the hybridization step. Library capture was conducted using biotin-labeled DNA probes (Thermo Fisher Scientific). In

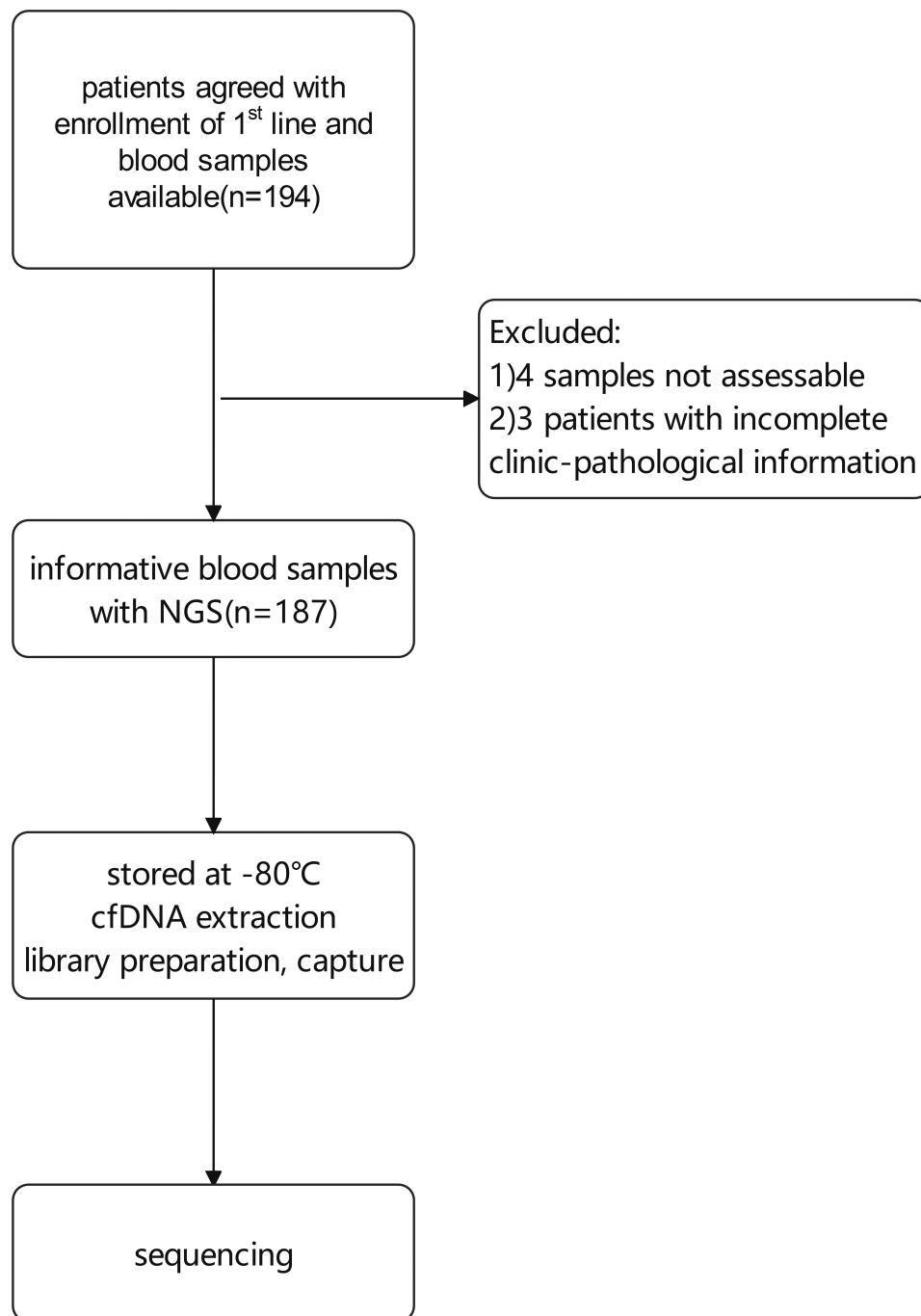


Figure 1 Flowchart of patient inclusion.

brief, the library was hybridized using PredicineCARE panel (Huidu Shanghai Medical Sciences, Inc.) overnight and captured on Dynabeads M-270 Streptavidin (Thermo Fisher Scientific).^{23,24} Unbound fragments were washed away, and the enriched fragments were amplified via PCR. For library preparation, the purified product was checked using Bioanalyzer 2100 and loaded into the HiSeq X Ten system

(Illumina, San Diego, CA, USA) for NGS with paired-end 150 bp sequencing kits.

Analyses of NGS Data Generated from cfDNA

Consensus binary alignment map (BAM) files were derived by merging paired-end reads that originated from

the same molecules (based on mapping location and unique molecular identifiers) as single-strand fragments. Single-strand fragments from the same double-strand DNA molecules were merged to be double-stranded for suppressing sequencing and PCR errors during this process. NGS quality-checking was performed by examining the percentage of targeted regions with >1500x unique consensus coverage. Samples with <80% regions having >1500x unique coverage were deemed to be QC failed and excluded. Candidate variants, consisting of point mutations, small insertions and deletions, were identified using Huidu proprietary bioinformatics pipeline. Candidate variants with low base quality, mapping scores, and other quality metrics were filtered. Candidate variants in repeat regions were also excluded.

A variant identified in cfDNA was considered to be a candidate somatic mutation-based if all of the following pre-defined criteria were present. These criteria were 1) the presence of at least 4 distinct paired reads in the mutation in the plasma; 2) the number of distinct paired reads containing a particular mutation in the plasma is at least 0.1% of the total distinct read pairs (if the nucleotide change and amino acid change are identical to an alteration observed in ≥ 20 cancer cases reported in the COSMIC database or previously reported as a cancer hotspot [<http://www.cancerhotspots.org>]) or the number of distinct paired reads containing a particular mutation in the plasma was at least 0.25% of the total distinct read pairs (if the nucleotide change and amino acid change are not a frequent alteration in COSMIC database or reported as a cancer hotspot previously); 3) the variant is not present in public databases of common germline variants, including 1000 genomes, ExAC, gnomAD, and KAVIAR, with population allele frequency >0.5%; 4) the variant is not present in matched PBMC samples (unpublished data, manuscript in preparation).

Candidate somatic mutations were further filtered based on gene annotation to identify those occurring in protein-coding regions. Intronic and silent changes were excluded, and mutations resulting in missense mutations, nonsense mutations, frameshifts, or splice site alterations were retained. Mutations annotated as benign or likely benign in ClinVar database were also filtered.

Evaluation

Clinical outcome was evaluated as disease-free survival (DFS) and overall survival (OS). Disease-free survival (DFS) was defined as the interval between surgery and time

of recurrence for relapsed patients so that patients with stage IV were not included. OS was defined as the time from diagnosis to the date of death or last follow-up. According to Response Evaluation Criteria in Solid Tumors version 1.1 guidelines,²⁵ we evaluated the response assessment by a computed tomography scan or magnetic resonance imaging every 6–12 weeks or as the patient's condition deteriorated.

Statistical Analysis

SPSS software version 20 was used to analyze the *TP53* status and categorical patient characteristics. DFS and OS were estimated by the Kaplan–Meier method and comparisons between groups were conducted by the log rank test. P values <0.05 were considered significant. For multivariable analysis, Cox proportional hazards method was used to evaluate clinical outcome. The association between the *TP53* status and clinical characteristics was examined using the Chi-square test.

Results

Patient Characteristics

Of 187 patients, 79 carried *TP53* mutations and 108 had wild-type *TP53*. Detailed baseline clinical information of all patients is shown in Table 1. The median age in the *TP53* mutated group was 48 years (range: 27–69 years old) versus 46 years of age in the *TP53* wild-type group (range: 26–80 years old) ($P = 0.702$). We also found that 73.4% (58/79) of *TP53*-mutated patients and 86.1% (93/108) of *TP53* wild-type patients were HER2 negative ($P=0.030$).

In univariate analysis of DFS (Table 2), HER2 status ($P=0.024$) and HR status ($P=0.000$) were significant predictors in *TP53* wild-type patients and *TP53*-mutated patients, respectively, and Ki67 status was also a significant predictor for *TP53* wild-type patients ($P=0.001$) and *TP53*-mutated patients ($P=0.022$). After multivariable analysis of DFS (Table 2), Ki67 status ($P=0.003$) and HR status ($P=0.000$) in *TP53* mutated group remained significant predictors and patients with stage III had a higher risk of relapse after surgery than stage I–II ($p=0.030$) in *TP53* wild-type cohort.

Characteristics of *TP53*-Mutated Patients

A total of 87 somatic *TP53* mutations were identified in the 79 *TP53*-mutated patients. Sixty-seven of these (77.0%) were located in exons 5–8, which span the DBD of the protein (Supplementary Table S1). Codons 175, 220, and 248 within the DBD were the locations of 4.6% of all mutations, respectively, which were all missense

Table I Baseline Clinical Characteristics of *TP53* Wild-Type and -Mutated Metastatic Breast Cancer Patients (n=187)

Characteristics	<i>TP53</i> Status		p value
	Wild-Type (n=108)	Mutated (n=79)	
Age at diagnosis (years)			
Median age(range)	46(26–80)	48(27–69)	0.702
≤50	64(59.3%)	49(62.0%)	
>50	44(40.7%)	30(38.0%)	
Family history			
Breast/ovarian cancer	7(6.5%)	3(3.8%)	0.688
Other cancers	17(15.7%)	14(17.7%)	
No	84(77.8%)	62(78.5%)	
Stage at diagnosis			
I–II	52(48.1%)	29(36.7%)	0.136
III	22(20.4%)	22(27.9%)	
IV	18(16.7%)	20(25.3%)	
Unknow	16(14.8%)	8(10.1%)	
Grade			
I–II	53(49.1%)	41(51.9%)	0.563
III	13(12.0%)	13(16.5%)	
Unknow	42(38.9%)	25(31.6%)	
Ki67			
I~20%	35(32.4%)	35(44.3%)	0.245
>20%	55(50.9%)	38(48.1%)	
Unknow	18(16.7%)	6(7.6%)	
HR status			
Positive	85(78.7%)	53(67.1%)	0.074
Negative	23(21.3%)	26(32.9%)	
HER2 status			
Positive	15(13.9%)	21(26.6%)	0.030
Negative	93(86.1%)	58(73.4%)	
Lymph node status			
Positive	65(60.2%)	44(55.7%)	0.554
Negative	42(38.9%)	34(43.0%)	
Unknow	1(0.9%)	1(1.3%)	
Disease involvement			
Visceral	64(59.3%)	46(58.2%)	0.848
Non-visceral	42(38.9%)	32(40.5%)	
Unknow	2(1.9%)	1(1.3%)	
Adjuvant endocrine therapy			
Tamoxifen/toremifene	41(37.9%)	23(29.1%)	0.238
Aromatase inhibitor	14(13.0%)	10(12.6%)	
Tamoxifen+ aromatase inhibitor	3(2.8%)	3(3.8%)	
Others	1(0.9%)	1(1.3%)	
No	49(45.4%)	42(53.2%)	

(Continued)

Table I (Continued).

Characteristics	TP53 Status		p value
	Wild-Type (n=108)	Mutated (n=79)	
Adjuvant chemotherapy			0.134
Paclitaxel	9(8.4%)	12(15.2%)	
Anthracycline	23(21.3%)	7(8.9%)	
Paclitaxel+ anthracycline	36(33.3%)	25(31.6%)	
Others	4(3.7%)	4(5.1%)	
No	36(33.3%)	31(39.2%)	
Adjuvant targeted therapy			0.223
Yes	2(1.8%)	4(5.1%)	
No	99(91.7%)	73(92.4%)	
Unknown	7(6.5%)	2(2.5%)	

Abbreviations: HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; DFS, disease free survival.

mutations (Figure 2). Of the 87 mutations, there were 46 missense mutations (43 was in DBD, 1 was in TD, 1 was in TAD, and 1 was outside the p53 protein domain) and 41 non-missense mutations (18 nonsense mutations, 3 splicing mutations, 16 frameshift mutations, 4 in-frame mutations).

TP53 Status in Different Subtypes

We found that the median DFS of *TP53*-mutated patients was significantly shorter at 33.0 months (95% confidence interval [CI]=21.4–44.6) than that of *TP53* wild-type patients at 51.0 months (95% CI=39.1–60.9) (hazard ratio=1.89, 95% CI=1.31–2.71, $P=0.001$) (Figure 3A). Similarly, the median OS of *TP53*-mutated patients was significantly shorter at 67.0 months (95% CI=44.4–89.6) than that of *TP53* wild-type patients at 140.0 months (95% CI=119.5–160.5) (hazard ratio=1.99, 95% CI=1.21–3.26, $P=0.006$) (Figure 3B).

In the HER2+ cohort ($n=36$, 21 of whom were *TP53*-mutated patients), there was no significant difference regarding *TP53* status with respect to DFS (34.0 vs 21.0 months, $P=0.822$) (Figure 3C) or OS (91.0 vs 65.0 months, $P=0.080$) (Figure 3D).

In the HR+/HER2- cohort ($n=113$, 40 of whom were *TP53*-mutated patients), the median DFS of *TP53* mutated patients of 44.0 months (95% CI=35.9–52.1) was significantly shorter than the 58.0 months (95% CI=46.2–69.8) of *TP53* wild-type patients (hazard ratio=1.57, 95% CI=0.97–2.54, $P=0.038$) (Figure 3E). No significant difference was observed for OS ($P=0.606$) (Figure 3F).

In the TNBC cohort ($n=38$, 18 of whom were *TP53*-mutated patients), the median DFS of *TP53*-mutated

patients of 16.0 months (95% CI=7.8–24.2) was significantly shorter than the 26.0 months (95% CI=16.6–35.4) of *TP53* wild-type patients (hazard ratio=2.17, 95% CI=0.96–4.90, $P=0.023$) (Figure 3G). There was no significant difference regarding *TP53* status with respect to OS (137.0 vs 54.0 months, $P=0.117$) (Figure 3H).

DBD Missense Mutations Were Associated with Improved Survival

We next classified the 187 patients into three groups by mutation domain: *TP53* mutations in the DBD, *TP53* mutations in the non-DBD, and *TP53* wild-type groups. The median DFS for these patients was 36.6 (95% CI=25.3–42.7), 22 (95% CI=16.1–25.9), and 51 (95% CI=39.1–60.9) months, respectively, while the median OS was 80 (95% CI=46.3–113.7), 51 (95% CI=41.2–60.8), and 140 (95% CI=119.5–160.5) months, respectively.

TP53 wild-type patients had a significantly better clinical outcome than those with *TP53* mutations in the DBD with respect to DFS ($P=0.008$, Figure 4A) and OS ($P=0.003$, Figure 4B). Similarly, *TP53* wild-type patients had a significantly better clinical outcome than those with *TP53* mutations in the non-DBD with respect to DFS ($P<0.001$, Figure 4A) and OS ($P=0.001$, Figure 4B). There were no significant differences in DFS or OS between patients with *TP53* mutations in the DBD compared with those in the non-DBD.

And then, we divided patients into three groups: *TP53* wild-type group; protein stable mutations group (non-truncating and non-frame altering mutations outside of the p53 tetramerization domain); protein non-stable

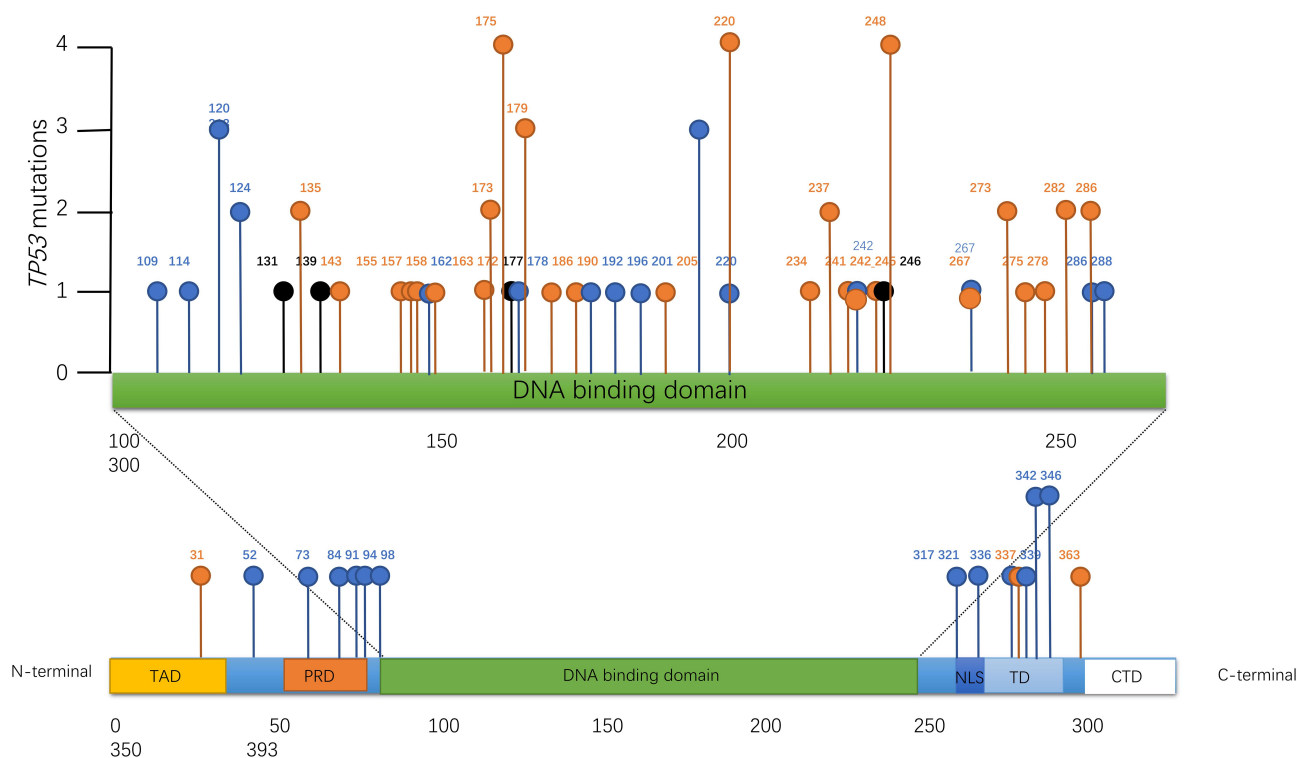


Figure 2 The mutational spectra of TP53 in TP53-mutated patients. (●) Missense (●) Truncating (●) Inframe.

Notes: According to <http://www.cbioportal.org>, truncating mutations included nonsense mutations, frameshift mutations and splicing mutations.

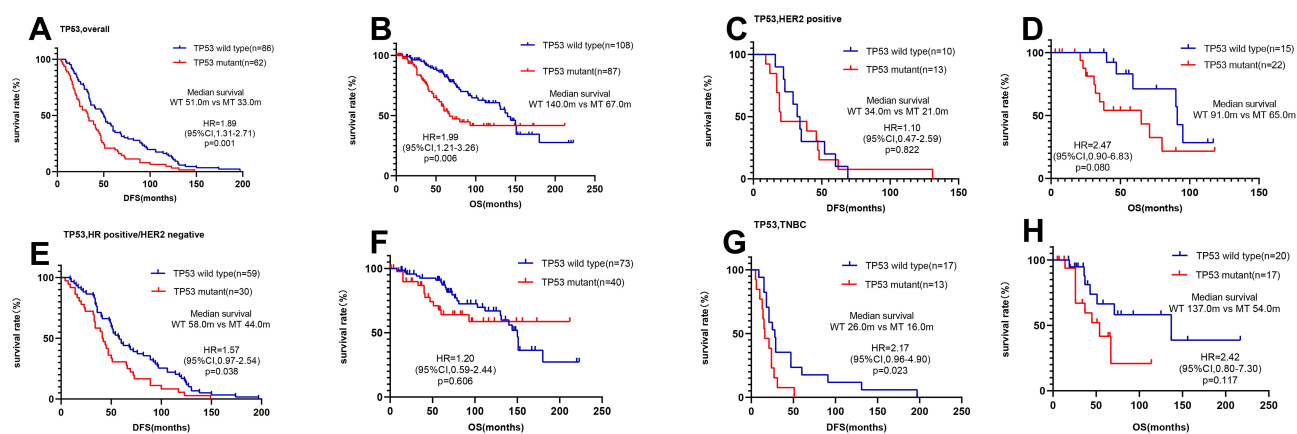


Figure 3 Survival analyses by Kaplan-Meier according to TP53 status in MBC patients. (A and B) TP53 wild-type patients had a significantly better clinical outcome than TP53-mutated patients. (C and D) there were no significant differences between TP53 wild-type and -mutated patients in the HER2-positive cohort. (E and F) TP53 wild-type patients had a significantly longer median DFS and OS than TP53-mutated patients in the HR+/HER2- cohort. (G and H) TP53 wild-type patients had a significantly longer median DFS than TP53-mutated patients in the TNBC cohort.

mutations group (all truncating and frame-altering mutations, and mutations in the tetramerization domain).

Patients with protein non-stable mutations had significantly shorter DFS (21.0 months vs 49.0 months, respectively, hazard ratio=2.82, 95% CI=1.63–4.87, $P<0.001$, Figure 4C) and OS (57.0 months vs 140.0 months, respectively, hazard ratio=4.05, 95% CI=1.95–8.40, $P<0.001$, Figure 4D) than TP53 wild-type patients. Moreover, the

median DFS of protein stable mutations was 43.5 months, longer than protein non-stable mutations (hazard ratio=0.54, 95% CI=0.31–0.93, $P=0.025$, Figure 4C). There were no significant differences in DFS or OS between patients with protein stable mutations and TP53 wild type.

Furthermore, we wanted to study mutations in DBD so that we classified them into missense ($n=43$) and non-missense mutations ($n=24$, including nonsense mutations,

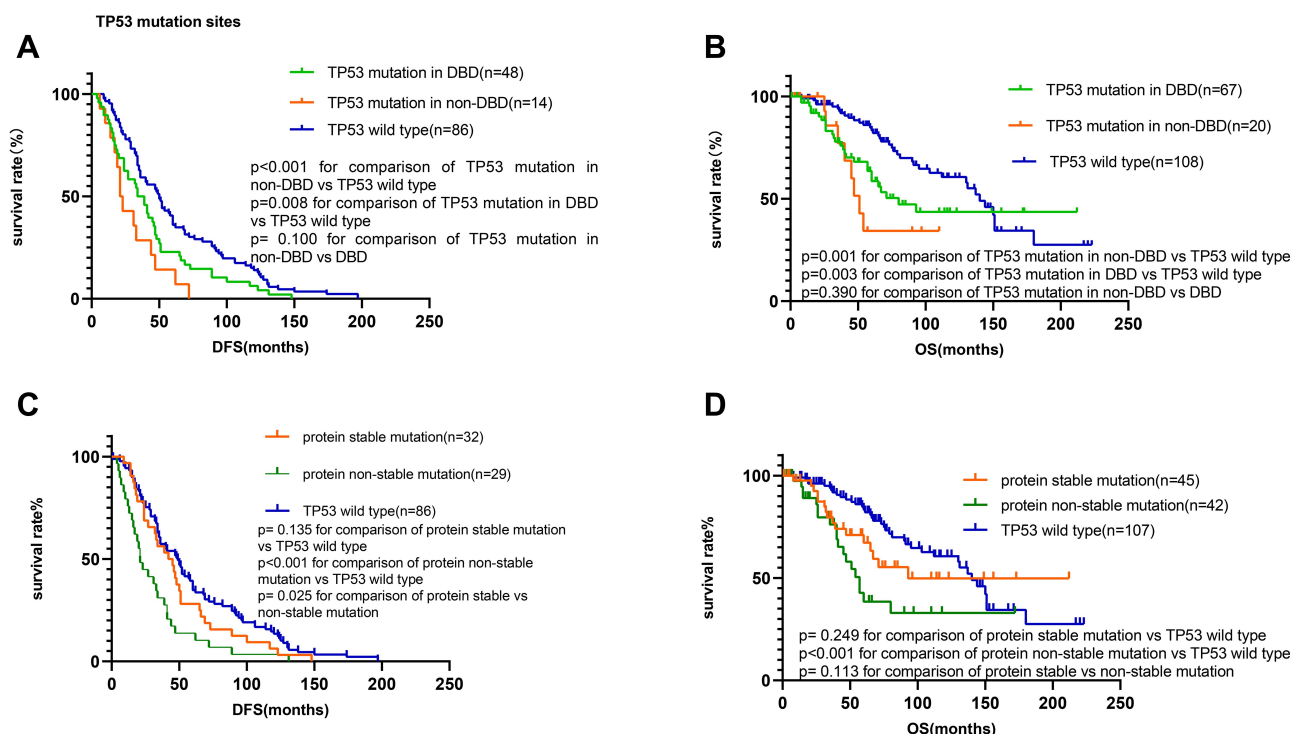


Figure 4 Survival analyses by Kaplan–Meier according to TP53 mutation sites in MBC patients. **(A)** Patients with a mutation in the non-DNA binding domain had a significantly shorter median DFS than TP53 wild-type patients and those with mutations in the DNA-binding domain. **(B)** Patients with a mutation in the non-DNA binding domain had shorter median OS than TP53 wild-type patients and those with mutations in the DNA-binding domain. **(C)** Patients with protein non-stable mutation had shortest median DFS than patients with protein stable mutation and TP53 wild-type patients. **(D)** Patients with protein non-stable mutation had shortest median OS than patients with protein stable mutation and TP53 wild-type patients.

Notes: Protein stable mutations would include non-truncating and non-frame altering mutations outside of the p53 tetramerization domain, and protein non-stable mutations would include all truncating and frame-altering mutations, as well as mutations in the tetramerization domain.

splicing mutations, frameshift mutations and in-frame mutations). Patients with non-missense mutations in the DBD had significantly shorter DFS (20.0 months vs 51.0 months, respectively, hazard ratio=3.26, 95% CI=1.58–6.71, $P=0.001$, Figure 5A) and OS (57.0 months vs 140.0 months, respectively, hazard ratio=10.45, 95% CI=3.79–28.8, $P<0.001$, Figure 5B) than TP53 wild-type

patients. Moreover, the median OS of patients with non-missense mutations in the DBD was significantly shorter than those with missense mutations in the DBD (hazard ratio=2.45, 95% CI=1.05–5.09, $P=0.015$, Figure 5B). There were no significant differences in DFS or OS between patients with missense mutations in the DBD and wild-type TP53 patients.

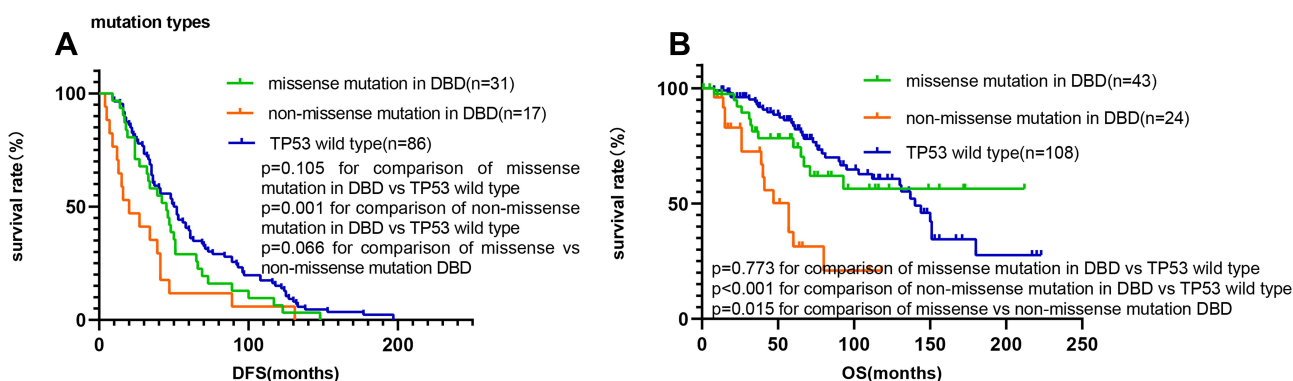


Figure 5 Survival analyses by Kaplan–Meier according to TP53 mutation type in the DNA binding domain. **(A and B)** Patients with non-missense mutations in the DNA binding domain had a significantly shorter median DFS and OS than TP53 wild-type patients and those with missense mutations in the DNA binding domain.

Table 2 Univariate and Multivariate Cox Regression Analysis of DFS in TP53 Wild-Type and -Mutated Patients

	TP53 Wild-Type Group										TP53 Mutated Group									
	Univariate						Multivariate				Univariate						Multivariate			
	N	%	DFS			HR (95% CI)	p value	N	%	mDFS (Range, Month)	HR (95% CI)	p value	N	%	mDFS (Range, Month)	HR (95% CI)	p value			
			mDFS (Range, Month)	HR (95% CI)	p value															
Age at diagnosis (years)	64	59.3	51.0(9.0–197.0)	1.00		1.00		49	62.0	28.0(4.0–148.0)	1.00					1.00				
	44	40.7	50.0(16.0–197.0)	1.09(0.70–1.70)	0.713	1.22(0.55–2.67)	0.626	30	38.0	40.0(7.0–131.0)	0.77(0.44–1.36)	0.363				0.44(0.20–0.98)	0.044			
Family history	84	77.8	52.0(9.0–197.0)	1.00		1.00		62	78.5	32.0(5.0–148.0)	1.00					1.00				
	7	6.5	30.0(10.0–124.0)	1.62(0.65–4.05)	0.300	1.20(0.14–10.20)	0.870	3	3.8	23.0(19.0–123.0)	0.75(0.23–2.46)	0.634				3.24(0.41–25.81)	0.267			
	17	15.7	46.5(9.0–125.0)	1.28(0.69–2.39)	0.435	1.52(0.43–5.32)	0.515	14	17.7	39.0(4.0–73.0)	1.12(0.54–2.32)	0.756				0.84(0.37–1.94)	0.688			
Stage at diagnosis	52	56.5	49.5(9.0–177.0)	1.00		1.00		29	40.8	21.5(4.0–72.0)	1.00					1.00				
	22	23.9	36.0(9.0–197.0)	1.11(0.66–1.87)	0.703	2.20(1.08–4.48)	0.030	22	31.0	28.0(5.0–148.0)	0.70(0.39–1.27)	0.241				0.78(0.38–1.58)	0.487			
	18	19.6	NA	NA	NA	NA	NA	20	28.2	NA	NA	NA				NA	NA			
	53	80.3	49.0(9.0–177.0)	1.00		1.00		41	75.9	32.0(9.0–148.0)	1.00					1.00				
Grade	13	19.7	41.0(17.0–197.0)	0.69(0.34–1.40)	0.310	1.04(0.20–5.49)	0.966	13	24.1	21.5(4.0–89.0)	1.52(0.77–3.01)	0.227				0.70(0.12–4.17)	0.699			
	35	38.9	60.5(16.0–177.0)	1.00		1.00		35	47.9	43.0(7.0–148.0)	1.00					1.00				
	55	61.1	34.0(9.0–133.0)	2.40(1.46–3.96)	0.001	1.90(0.99–3.64)	0.054	38	52.1	21.0(4.0–131.0)	1.96(1.10–3.49)	0.022				3.47(1.53–7.89)	0.003			
HR status	85	78.7	56.0(9.0–197.0)	1.00		1.00		53	67.1	46.0(4.0–148.0)	1.00					1.00				
	23	21.3	26.0(9.0–197.0)	1.58(0.94–2.66)	0.087	2.09(0.97–4.49)	0.059	26	32.9	19.0(5.0–51.0)	4.31(2.24–8.27)	0.000				4.40(1.97–9.84)	0.000			
	15	13.9	34.0(17.0–71.0)	1.00		1.00		21	26.6	21.0(9.0–131.0)	1.00					1.00				
HER2 status	93	86.1	52.0(9.0–197.0)	0.45(0.23–0.90)	0.024	0.48(0.19–1.20)	0.117	58	73.4	33.0(4.0–148.0)	0.85(0.45–1.59)	0.600				1.18(0.49–2.83)	0.710			
	42	39.3	51.0(9.0–197.0)	1.00		1.00		34	43.6	31.0(4.0–131.0)	1.00					1.00				
Lymph node status	65	60.7	51.0(14.0–197.0)	1.10(0.71–1.70)	0.663	0.98(0.23–4.22)	0.976	44	56.4	39.0(5.0–148.0)	0.85(0.50–1.44)	0.538				1.00(0.28–3.55)	1.00			
	42	39.6	40.5(9.0–153.0)	1.00		1.00		32	40.5	29.5(5.0–148.0)	1.00					1.00				
Disease involvement	64	60.4	52.0(14.0–197.0)	0.70(0.45–1.10)	0.124	1.43(0.68–3.01)	0.343	46	59.5	36.0(4.0–123.0)	0.87(0.49–1.54)	0.640				1.65(0.74–3.69)	0.222			

(Continued)

Table 2 (Continued).

	TP53 Wild-Type Group										TP53 Mutated Group									
	Univariate					Multivariate					Univariate					Multivariate				
	N	%	DFS			HR (95% CI)	p value	mDFS (Range, Month)	HR (95% CI)	p value	N	%	DFS			HR (95% CI)	p value	mDFS (Range, Month)	HR (95% CI)	p value
			mDFS (Range, Month)	HR (95% CI)	p value								mDFS (Range, Month)	HR (95% CI)	p value					
Adjuvant endocrine therapy TAM/TOR AI TAM plus AI Others	41	67.2	52.0(9.0–197.0)	1.00		1.00					23	62.2	41.5(4.0–148.0)	1.00		1.00				
	14	23.0	53.0(16.0–177.0)	0.92(0.49–1.73)	0.792	0.52(0.13–2.06)	0.354				10	27.0	28.5(7.0–131.0)	1.30(0.61–2.78)	0.501	1.53(0.43–5.42)	0.508			
	3	4.9	100.5(63.0–138.0)	0.50(0.12–2.10)	0.343	1.00(0.12–8.68)	0.998				3	8.1	50.0(32.0–72.0)	0.95(0.28–3.21)	0.932	1.05(0.09–12.54)	0.967			
	3	4.9	153.0(35.0–197.0)	0.32(0.10–1.09)	0.069	NA	NA				1	2.7	NA	0.36(0.05–2.78)	0.330	NA	NA			
Adjuvant chemotherapy PTX Anthracycline PTX plus anthracycline Others	9	12.5	60.0(23.0–122.0)	1.00		1.00					12	25.0	39.0(23.0–123.0)	1.00		1.00				
	23	31.9	55.5(18.0–197.0)	0.77(0.35–1.69)	0.507	3.29(0.78–13.89)	0.106				7	14.6	34.0(5.0–66.0)	1.72(0.66–4.48)	0.265	1.38(0.18–10.32)	0.754			
	36	50.0	48.0(9.0–177.0)	1.03(0.49–2.18)	0.932	0.78(0.24–2.59)	0.688				25	52.1	24.0(9.0–131.0)	1.43(0.71–2.88)	0.323	1.59(0.35–7.22)	0.550			
	4	5.6	44.0(30.0–84.0)	1.30(0.40–4.25)	0.661	2.26(0.19–27.58)	0.523				4	8.3	72.0(9.0–117.0)	0.73(0.20–2.63)	0.629	0.47(0.02–12.22)	0.650			

Abbreviations: HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; mDFS, median disease free survival; HR, hazard ratio; CI, confidence interval; NA, unknown; AI, aromatase inhibitor; TAM, tamoxifen; TOR, toremifene; PTX, paclitaxel.

TP53 Status Was Associated with Adjuvant Endocrine Therapy Response

A total of 96 patients who received adjuvant endocrine therapy were selected to evaluate the relationship between *TP53* mutation status and the response to endocrine therapy. As shown in Table 3, we found that 84.7% (50/59) of patients accepted adjuvant chemotherapy in *TP53* wild-type group, whereas 78.4% (29/37) of patients accepted adjuvant chemotherapy treatment in *TP53* mutant patients. There was no significant difference between *TP53* status and adjuvant chemotherapy (P=0.467). As well known, ESR1 mutations are associated with acquired endocrine resistance in breast cancer so that we took ESR1 mutation rate into consideration in Table 3, but there were no significant differences in ESR1 mutation rate (p=0.558) between the two groups.

To further explore the relationship between *TP53* status and treatment response, we classified patients into the adjuvant endocrine therapy-resistant group and the adjuvant endocrine therapy sensitive group. Interestingly, we found that in the adjuvant endocrine therapy sensitive group, patients with *TP53* mutations had a significantly shorter DFS than *TP53* wild-type patients (69.0 months vs 108.0 months, respectively, hazard ratio=3.22, 95% CI=0.70–14.77, P=0.008) (Figure 6B). No significant DFS differences between *TP53*-mutated and *TP53* wild-type patients were seen in the endocrine therapy-resistant group (34.0 months vs 40.0 months, respectively, P=0.903) (Figure 6A).

Discussion

In our study, we used NGS to detect *TP53* mutations in the cfDNA, which might affect tumor temporal and spatial heterogeneity, of 187 Chinese MBC patients. Our results indicated that *TP53* mutations could be used as a prognostic marker for worse outcome in MBC and for the response of adjuvant endocrine therapy.

We established genomic profiles of patients which revealed a *TP53* mutation frequency of 42.2%, similar to that seen in the Guangdong Provincial People’s Hospital cohort (45.0%) but higher than in the TCGA breast cancer cohort (30.0%).²⁶ Another recent study on cfDNA molecular profiling in Chinese patients with MBC reported a *TP53* mutation rate of 64.1% compared with 52% in Caucasian patients.^{27,28} These discrepancies could reflect differences between patient ethnicities, such as in the median age of breast cancer patients with *TP53* mutations in our study of 48 years compared with 55.2 years in Caucasians.²⁹

Table 3 Clinical Characteristics of Patients Receiving Adjuvant Endocrine Therapy (n=96)

Characteristics	TP53 Status		p value
	Wild-Type (n=59)	Mutated (n=37)	
Age at diagnosis (years)			
≤50	38(64.4%)	26(70.3%)	0.553
>50	21(35.6%)	11(29.7%)	
Stage at diagnosis			
I–II	36(61.0%)	19(51.4%)	0.273
III–IV	16(27.1%)	14(37.8%)	
Unknow	7(11.9%)	4(10.8%)	
Grade			
I–II	30(50.8%)	14(37.8%)	0.257
III	7(11.9%)	1(2.7%)	
Unknow	22(37.3%)	22(59.5%)	
Ki67			
I~20%	24(40.7%)	15(40.5%)	0.820
>20%	26(44.1%)	18(48.7%)	
Unknow	9(15.2%)	4(10.8%)	
Lymph node status			
Positive	37(62.7%)	22(59.5%)	0.928
Negative	21(35.6%)	13(35.1%)	
Unknow	1(1.7%)	2(5.4%)	
Disease involvement			
Visceral	38(64.4%)	23(62.2%)	0.824
Non-visceral	21(35.6%)	14(37.8%)	
Adjuvant endocrine therapy			
Tamoxifen/toremifene	41(69.5%)	23(62.2%)	0.238
Aromatase inhibitor	14(23.7%)	10(27.0%)	
Tamoxifen+ aromatase inhibitor	3(5.1%)	3(8.1%)	
Others	1(1.7%)	1(2.7%)	
Adjuvant chemotherapy			
Yes	50(84.7%)	29(78.4%)	0.467
No	8(13.6%)	7(18.9%)	
Unknow	1(1.7%)	1(2.7%)	
ESR1 mutation			
With ESR1 mutation	7(11.9%)	3(8.1%)	0.558
Without ESR1 mutation	52(88.1%)	34(91.9%)	

The p53 pathway was previously shown to rank top in the basal-like breast cancer subtype, but not in the HER2-enriched type; therefore, *TP53* mutations were not associated with poor prognosis in the HER2-enriched group.⁶ In support of this, our data indicated that the *TP53*

mutation status was an independent predictive factor of survival especially in HR+/HER2– and TNBC cohorts, but not in the HER2-positive cohort.

Several studies have shown that the DBD is the most frequently mutated *TP53* region in breast cancer. In line with this, codons 175, 220, and 248 located within the DBD were the site of many *TP53* mutations in our study, of which most were missense mutations. DBD mutations were previously reported to have prognostic value,^{30,31} while non-missense mutations were associated with a worse outcome in MBC.³² A recent study showed that missense mutation in the DNA-binding domain had dominant-negative effects (DNE).³³ There was no difference in survival between patients with dominant-negative p53 mutant tumors and those with *TP53* mutations that are predicted to be non-dominant negative.^{34,35} In our study, *TP53* missense mutations in the DBD were associated with improved survival. Further analysis showed that patients with *TP53* mutations in the non-DBD had a significantly shorter DFS than those in the *TP53* non-mutation cohort.³⁶ In order to investigate the prognostic value of p53 protein further, we divided them into *TP53* wild-type group; protein stable mutations group and protein non-stable mutations group. In our study, patients with protein non-stable mutations had significantly shorter DFS and OS than *TP53* wild-type patients. Moreover, protein non-stable mutations included all truncating and frame-altering mutations, and mutations in the tetramerization domain so that mutations in TD had a worse clinical outcome. The reasons were that mutations in TD could either abolish or reduce binding of p53 protein to DNA and transcriptional activation, and *TP53* mutation in TD domain had dominant-negative effects (DNE) that inactivate *TP53* wild type in some cases.³⁷ Other researchers also found mutations in TD domain were associated with cancer-associated development.³⁸ Not all missense mutations cause protein accumulation, while non-missense mutations are true loss-of-function mutations. Thus, missense mutations have generally been associated with higher protein expression compared with non-missense mutations.¹⁶

Some clinical trials showed us *TP53* might be the potential to be a therapeutic biomarker. Studies on the role of *TP53* mutation in breast cancer response to chemotherapy are conflicting.^{39–42} Data on the association between *TP53* mutations and endocrine therapy response were also controversial.^{43–45} When it came to the association between hormone therapy and chemotherapy, some researchers found that adding hormone therapy to chemotherapy could improve the survival for *TP53* wild-type patients not for

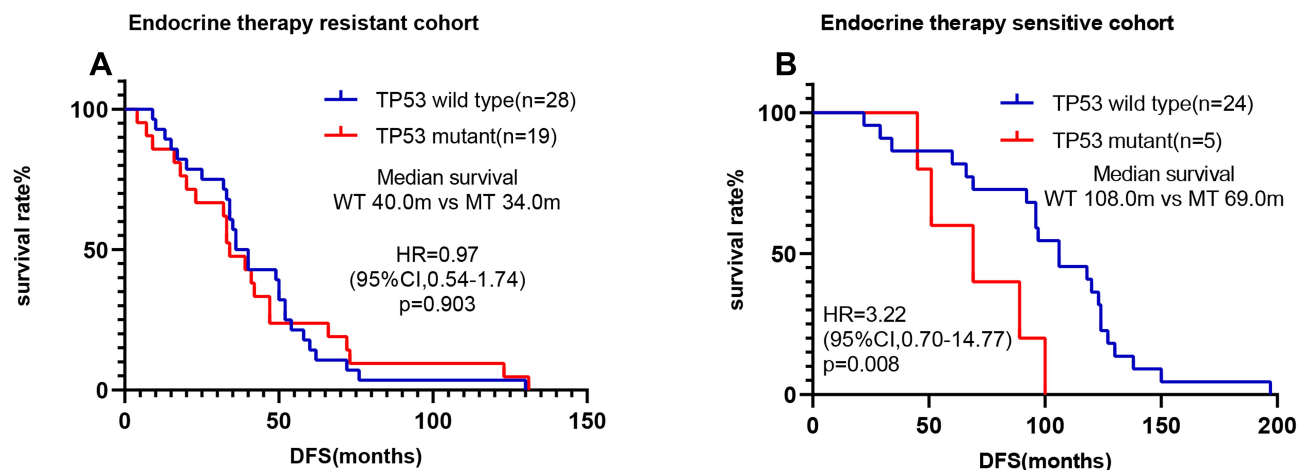


Figure 6 Survival analyses by Kaplan–Meier according to TP53 status in MBC receiving adjuvant endocrine therapy. (A) There was no significant difference in TP53 status in the endocrine therapy-resistant cohort. (B) TP53 wild-type patients had a significantly better clinical outcome than TP53-mutated patients in the endocrine therapy sensitive cohort.

TP53 mutation patients.^{46,47} While in our research, 84.7% of patients in *TP53* wild-type group and 78.4% patients in *TP53* mutant group all accepted adjuvant chemotherapy and endocrine therapy treatment in Table 3, and the distribution of patients with adjuvant chemotherapy was balanced in two groups, which did not exert an influence on the analysis of endocrine therapy and *TP53* status. In our study, we also found *TP53* mutations were associated with endocrine resistance. *TP53*-mutated patients had a shorter DFS than *TP53* wild-type patients in the adjuvant endocrine therapy sensitive group. Previously, increased expression of estrogen-related receptor (ERR) α was associated with increased levels of p53 in ER α -positive cases. ER α and ERR α share only 33% homology in their ligand-binding domains, resulting in the insensitivity of ERR α to tamoxifen.⁴⁸ Additionally, *TP53* wild-type tumors might be more responsive to endocrine therapy because this disrupts the ER α -p53 interaction and reactivates p53.⁴⁹

The retrospective nature of our study resulted in a number of limitations. DFS might have influenced the survival analysis, which was retrospectively calculated. Additionally, we lacked matched primary and recurrence samples for analysis. Finally, we did not analyze p53 protein expression to verify our results.

Conclusion

In conclusion, *TP53* wild-type MBC patients showed better survival than *TP53*-mutated patients in HR+/HER2– and TNBC cohorts. Missense mutations in the DBD of p53 appeared to be an independent prognostic marker for short

DFS, while *TP53* mutations were associated with endocrine resistance. This indicates that alternative therapies for HR-positive patients with *TP53* mutations should be considered. Large-scale prospective studies are needed to verify our findings.

Data Sharing Statement

We can provide the original data in this manuscript upon request.

Research Ethics and Consent

The written informed consent of this research had been provided by the patients, and this study was approved by the Medical Ethics Committee of Peking University Cancer Hospital & Institute (Approval No.2016KT47) according to the Declaration of Helsinki.

Acknowledgments

We thank Sarah Williams, PhD, from Liwen Bianji, Edanz Editing China, for editing the English text of a draft of this manuscript.

Funding

There were no funding sources for this work.

Disclosure

Jianjun Yu and Shidong Jia are employees and stockholders of Huidu Shanghai Medical Sciences, Ltd. The authors declare that they have no other competing interests.

References

- Kasthuber ER, Lowe SW. Putting p53 in context. *Cell*. 2017;170(6):1062–1078. doi:10.1016/j.cell.2017.08.028
- Hainaut P, Pfeifer GP. Somatic TP53 mutations in the era of genome sequencing. *Cold Spring Harb Perspect Med*. 2016;6(11):a026179. doi:10.1101/cshperspect.a026179
- Stephens PJ, Tarpey PS, Davies H, et al. The landscape of cancer genes and mutational processes in breast cancer. *Nature*. 2012;486(7403):400–404. doi:10.1038/nature11017
- Basho RK, de Melo Gagliato D, Ueno NT, et al. Clinical outcomes based on multigene profiling in metastatic breast cancer patients. *Oncotarget*. 2016;7(47):76362–76373. doi:10.18632/oncotarget.12987
- Ellis MJ, Ding L, Shen D, et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. *Nature*. 2012;486(7403):353–360. doi:10.1038/nature11143
- Ren J, Wang B, Li J. Integrating proteomic and phosphoproteomic data for pathway analysis in breast cancer. *BMC Syst Biol*. 2018;12(Suppl S8):130. doi:10.1186/s12918-018-0646-y
- Koçak A, Heselmeyer-Haddad K, Lischka A, et al. High levels of chromosomal copy number alterations and TP53 mutations correlate with poor outcome in younger breast cancer patients. *Am J Pathol*. 2020;190(8):1643–1656. doi:10.1016/j.ajpath.2020.04.015
- Wang D, Kon N, Lasso G, et al. Acetylation-regulated interaction between p53 and SET reveals a widespread regulatory mode. *Nature*. 2016;538(7623):118–122. doi:10.1038/nature19759
- Sullivan KD, Galbraith MD, Andrysik Z, et al. Mechanisms of transcriptional regulation by p53. *Cell Death Differ*. 2018;25(1):133–143. doi:10.1038/cdd.2017.174
- Marchenko ND, Hanel W, Li D, et al. Stress-mediated nuclear stabilization of p53 is regulated by ubiquitination and importin- α 3 binding. *Cell Death Differ*. 2010;17(2):255–267. doi:10.1038/cdd.2009.173
- Parry TE. Mutagenic mechanisms in leukemia and cancer: a new concept cytosine lack could be as mutagenic as cytosine deamination. *Leuk Res*. 2006;30(9):1079–1083. doi:10.1016/j.leukres.2005.12.019
- Fernández-Cuesta L, Oakman C, Falagan-Lotsch P, et al. Prognostic and predictive value of TP53 mutations in node-positive breast cancer patients treated with anthracycline- or anthracycline/taxane-based adjuvant therapy: results from the BIG 02–98 Phase III trial. *Breast Cancer Res*. 2012;14(3):R70. doi:10.1186/bcr3179
- Meric-Bernstam F, Zheng X, Shariati M, et al. Survival outcomes by TP53 mutation status in metastatic breast cancer. *JCO Precis Oncol*. 2018;2:1–5. doi:10.1200/PO.17.00245
- Lopez G, Costanza J, Colleoni M, et al. Molecular insights into the classification of luminal breast cancers: the genomic heterogeneity of progesterone-negative tumors. *Int J Mol Sci*. 2019;20(3):510. doi:10.3390/ijms20030510
- Silwal-Pandit L, Vollen HKM, Chin S-F, et al. TP53 mutation spectrum in breast cancer is subtype specific and has distinct prognostic relevance. *Clin Cancer Res*. 2014;20(13):3569–3580. doi:10.1158/1078-0432.CCR-13-2943
- Darb-Esfahani S, Denkert C, Stenzinger A, et al. Role of TP53 mutations in triple negative and HER2-positive breast cancer treated with neoadjuvant anthracycline/taxane-based chemotherapy. *Oncotarget*. 2016;7(42):67686–67698. doi:10.18632/oncotarget.11891
- Luo Y, Huang W, Zhang H, et al. Prognostic significance of CD117 expression and TP53 missense mutations in triple-negative breast cancer. *Oncol Lett*. 2018;15(5):6161–6170. doi:10.3892/ol.2018.8104
- Pilecki V, Pop L, Braicu C, et al. Double gene siRNA knockdown of mutant p53 and TNF induces apoptosis in triple-negative breast cancer cells. *Onco Targets Ther*. 2016;9:6921–6933. doi:10.2147/OTT.S110719
- Li J-P, Zhang X-M, Zhang Z, et al. Association of p53 expression with poor prognosis in patients with triple-negative breast invasive ductal carcinoma. *Medicine*. 2019;98(18):e15449. doi:10.1097/MD.00000000000015449
- Wolff AC, Hammond MEH, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch Pathol Lab Med*. 2014;138(2):241–256. doi:10.5858/arpa.2013-0953-SA
- Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol*. 2010;28(16):2784–2795. doi:10.1200/JCO.2009.25.6529
- Brandão M, Maurer C, Ziegelmann PK, et al. Endocrine therapy-based treatments in hormone receptor-positive/HER2-negative advanced breast cancer: systematic review and network meta-analysis. *ESMO Open*. 2020;5(4):e000842. doi:10.1136/esmoopen-2020-000842
- Newman AM, Lovejoy AF, Klass DM, et al. Integrated digital error suppression for improved detection of circulating tumor DNA. *Nat Biotechnol*. 2016;34(5):547–555. doi:10.1038/nbt.3520
- Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol*. 2013;31(3):213–219. doi:10.1038/nbt.2514
- Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228–247. doi:10.1016/j.ejca.2008.10.026
- Zhang G, Wang Y, Chen B, et al. Characterization of frequently mutated cancer genes in Chinese breast tumors: a comparison of Chinese and TCGA cohorts. *Ann Transl Med*. 2019;7(8):179. doi:10.21037/atm.2019.04.23
- Rossi G, Mu Z, Rademaker AW, et al. Cell-free DNA and circulating tumor cells: comprehensive liquid biopsy analysis in advanced breast cancer. *Clin Cancer Res*. 2018;24(3):560–568. doi:10.1158/1078-0432.CCR-17-2092
- Tao Z, Li T, Feng Z, et al. Characterizations of cancer gene mutations in Chinese metastatic breast cancer patients. *Front Oncol*. 2020;10:1023. doi:10.3389/fonc.2020.01023
- Warner ET, Tamimi RM, Hughes ME, et al. Racial and ethnic differences in breast cancer survival: mediating effect of tumor characteristics and sociodemographic and treatment factors. *J Clin Oncol*. 2015;33(20):2254–2261. doi:10.1200/JCO.2014.57.1349
- Olivier M, Langer A, Carrieri P, et al. The clinical value of somatic TP53 gene mutations in 1794 patients with breast cancer. *Clin Cancer Res*. 2006;12(4):1157–1167. doi:10.1158/1078-0432.CCR-05-1029
- Liu Y, Xu F, Wang Y, et al. Mutations in exon 8 of TP53 are associated with shorter survival in patients with advanced lung cancer. *Oncol Lett*. 2019;18(3):3159–3169. doi:10.3892/ol.2019.10625
- Petitjean A, Achatz MIW, Borresen-Dale AL, et al. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. *Oncogene*. 2007;26(15):2157–2165. doi:10.1038/sj.onc.1210302
- Boettcher S, Miller PG, Sharma R, et al. A dominant-negative effect drives selection of TP53 missense mutations in myeloid malignancies. *Science*. 2019;365(6453):599–604. doi:10.1126/science.aax3649
- Shahbandi A, Jackson JG. Analysis across multiple tumor types provides no evidence that mutant p53 exerts dominant negative activity. *NPJ Precis Oncol*. 2019;3(1):1. doi:10.1038/s41698-018-0074-x
- Giacomelli AO, Yang X, Lintner RE, et al. Mutational processes shape the landscape of TP53 mutations in human cancer. *Nat Genet*. 2018;50(10):1381–1387. doi:10.1038/s41588-018-0204-y

36. Terada K, Yamaguchi H, Ueki T, et al. Full-length mutation search of the *TP53* gene in acute myeloid leukemia has increased significance as a prognostic factor. *Ann Hematol*. 2018;97(1):51–61. doi:10.1007/s00277-017-3143-2
37. Gencel-Augusto J, Lozano G. p53 tetramerization: at the center of the dominant-negative effect of mutant p53. *Genes Dev*. 2020;34(17–18):1128–1146. doi:10.1101/gad.340976.120
38. Muscolini M, Montagni E, Caristi S, et al. Characterization of a new cancer-associated mutant of p53 with a missense mutation (K351N) in the tetramerization domain. *Cell Cycle*. 2009;8(20):3396–3405. doi:10.4161/cc.8.20.9910
39. Jackson JG, Pant V, Li Q, et al. p53-mediated senescence impairs the apoptotic response to chemotherapy and clinical outcome in breast cancer. *Cancer Cell*. 2012;21(6):793–806. doi:10.1016/j.ccr.2012.04.027
40. Bertheau P, Turpin E, Rickman DS, et al. Exquisite sensitivity of *TP53* mutant and basal breast cancers to a dose-dense epirubicin–cyclophosphamide regimen. *PLoS Med*. 2007;4(3):e90. doi:10.1371/journal.pmed.0040090
41. Bertheau P, Espinosa M, Turpin E, et al. *TP53* status and response to chemotherapy in breast cancer. *Pathobiology*. 2008;75(2):132–139. doi:10.1159/000123851
42. Bonnefoi H, Piccart M, Bogaerts J, et al. *TP53* status for prediction of sensitivity to taxane versus non-taxane neoadjuvant chemotherapy in breast cancer (EORTC 10994/BIG 1-00): a randomised Phase 3 trial. *Lancet Oncol*. 2011;12(6):527–539. doi:10.1016/S1470-2045(11)70094-8
43. Kai K, Nishimura R, Arima N, et al. p53 expression status is a significant molecular marker in predicting the time to endocrine therapy failure in recurrent breast cancer: a cohort study. *Int J Clin Oncol*. 2006;11(6):426–433. doi:10.1007/s10147-006-0601-6
44. Yamashita H, Toyama T, Nishio M, et al. p53 protein accumulation predicts resistance to endocrine therapy and decreased post-relapse survival in metastatic breast cancer. *Breast Cancer Res*. 2006;8(4):R48. doi:10.1186/bcr1536
45. Bailey ST, Shin H, Westerling T, et al. Estrogen receptor prevents p53-dependent apoptosis in breast cancer. *Proc Natl Acad Sci U S A*. 2012;109(44):18060–18065. doi:10.1073/pnas.1018858109
46. Ungerleider NA, Rao SG, Shahbandi A, et al. Breast cancer survival predicted by *TP53* mutation status differs markedly depending on treatment. *Breast Cancer Res*. 2018;20(1):115. doi:10.1186/s13058-018-1044-5
47. Shahbandi A, Nguyen HD, Jackson JG. *TP53* mutations and outcomes in breast cancer: reading beyond the headlines. *Trends Cancer*. 2020;6(2):98–110. doi:10.1016/j.trecan.2020.01.007
48. Thewes V, Simon R, Schroeter P, et al. Reprogramming of the ER α and ER α target gene landscape triggers tamoxifen resistance in breast cancer. *Cancer Res*. 2015;75(4):720–731. doi:10.1158/0008-5472.CAN-14-0652
49. Konduri SD, Medisetty R, Liu W, et al. Mechanisms of estrogen receptor antagonism toward p53 and its implications in breast cancer therapeutic response and stem cell regulation. *Proc Natl Acad Sci U S A*. 2010;107(34):15081–15086. doi:10.1073/pnas.1009575107

Cancer Management and Research

Dovepress

Publish your work in this journal

Cancer Management and Research is an international, peer-reviewed open access journal focusing on cancer research and the optimal use of preventative and integrated treatment interventions to achieve improved outcomes, enhanced survival and quality of life for the cancer patient.

The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/cancer-management-and-research-journal>