Clinical relevance of *TP53* mutation and its characteristics in breast cancer with long-term follow-up data

Seung Hyun Hwang

The Graduate School
Yonsei University
Department of Medicine

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This certifies that the Dissertation of Seung Hyun Hwang is approved

Thesis Supervisor	Joon Jeong
Thesis Committee Member	Joohyuk Sohn
Thesis Committee Member	Sang Uk Woo
Thesis Committee Member	Kyung A Lee
Thesis Committee Member	Ja Seung Koo

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ABSTRACT

Clinical relevance of *TP53* mutation and its characteristics in breast cancer with long-term follow-up data

Introduction

TP53 mutations is one of the most frequently identified mutations in human cancers. Generally, breast cancers with *TP53* mutations are known to have a poor prognosis, though there are also controversial findings. Therefore, we aimed to verify the clinical relevance of *TP53* mutations in breast cancer including all subtypes and treatments with long-term follow-up data.

Methods

We retrospectively collected data of patients who underwent *TP53* mutation testing after being diagnosed with breast cancer. Stratified log-rank tests and Cox regression analysis was performed to compare oncologic outcomes based on the *TP53* mutation status and characteristics of *TP53* mutations, such as mutation type and locations. In this study, polymerase chain reaction-denaturing high performance liquid chromatography (PCR-DHPLC) and direct sequencing was used to identify *TP53* mutations in exons 5-9. All statistical significance set as $p \leq 0.05$.

Results

Between January 2007 and December 2015, 650 breast cancer patients underwent *TP53* mutation testing. Among them, there were 172 patients (26.5%) who were detected *TP53* mutations. Of the 172 cases with *TP53* mutations, 34 (19.8%) had missense hotspot mutations. Patients with *TP53* mutations (*TP53*-mutated group) had worse prognosis (10-

year recurrence free survival (RFS), 83.5% vs. 86.6%, HR, 1.67; 95% CIs, 1.06-2.64; p = 0.026 and 10-year overall survival (OS), 88.1% vs. 91.0%, HR, 3.02; 95% CIs, 1.43-6.70, p = 0.003). However, within the *TP53*-mutated group, subgroup analyses based on characteristics of mutation did not reveal any significant differences in oncologic outcomes.

Conclusion

We found that *TP53* mutations are associated with worse prognosis in breast cancer including all subtypes and treatments with long-term follow-up data. But, within the *TP53*-mutated group, there were no differences in oncological outcomes based on the characteristics of *TP53* mutations such as mutation type and location.

Keywords: *TP53* mutation, Missense mutation, DNA-binding domain, Hotspots mutation, Recurrence-free survival (RFS), Overall survival (OS)

I. INTRODUCTION

TP53 gene, coding for the tumor-suppressor p53, is the most frequently mutated gene in human cancers.[1] *TP53*, situated on chromosome 17p13.1, comprises 11 exons, 10 introns, and 393 amino acid residues, encodes the p53 protein which functions as a transcription factor with distinct amino-terminal, DNA-binding, and carboxy-terminal domains.[2] *TP53*-activated pathway exerts its tumor suppressive functions by regulating DNA repair, cell-cycle arrest, senescence, and apoptosis, thereby inhibiting early tumorigenesis, tumor growth and development.[3-5] Given these circumstances, the activation of p53 in normal tissues is imperative to protect themselves from tumorigenesis. However, tumor with *TP53* mutations not only lose the functions necessary for tumor suppression but may also harbors gain-of-functions that promote tumor growth.[6,7] Consequently, tumors with *TP53* mutations typically have a poor prognosis because of its rapid progression and resistance to treatment.[8-10]

According to the International Agency for Research on Cancer (IACR) database, over 75% of *TP53* mutations were missense mutations, and approximately 97% were situated in exons encoding for the DNA-binding domain (DBD) (residues 98-292). Six codons (175, 220, 245, 248, 273, and 282) with a frequency of more than 2% among all missense mutations are well-known missense hotspots (https://www.cbioportal.org/). Theses single nucleotide substitution mutations affect the 3D structure of the p53 protein or its contact with DNA, leading to a loss-of-function.[11]

Mutations of the *TP53* gene are identified in nearly 30% of all breast cancers.[12,13] Many preclinical and clinical trials have explored the clinical significance of *TP53* mutations in breast cancer, and it is generally associated with poor prognosis.[14-17] However, there were some studies that reported neutral[18,19] or even positive outcomes,[20] making the clinical relevance of *TP53* mutations still controversial. Furthermore, there are few studies that assess the prognosis based on the characteristics of *TP53* mutations, such as mutation type or location of mutation. Given these circumstances, we investigated the association between *TP53* mutations and prognosis in breast cancer patients using long follow-up data. Additionally, we explored the clinical relevance of the characteristics of *TP53* mutations within the patients with *TP53* mutation.

II. METHODS

2.1 Data collection

The study protocol received approval from the Institutional Review Boards of Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea (IRB no. 3-2024-0197) and adhered to the principles of the Declaration of Helsinki. The need for written informed consent was waived because of retrospective study design.

We retrospectively identified patients diagnosed with breast cancer who underwent *TP53* mutation testing at our institution from January 2007 to December 2015. Clinicopathological data were collected from electronic medical records including age at diagnosis, histologic subtype, histologic grade, estrogen receptor (ER) and progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status, lymphovascular invasion (LVI), Ki-67 index, T stage, N stage, and implementation of (neo)adjuvant systemic treatment and/or radiotherapy. We also collected genetic information about *TP53* mutation status and characteristics of *TP53* mutation. Patients diagnosed with recurrent breast cancer, or de novo metastatic breast cancer were excluded.

T and N stage were determined using surgical specimens according to the American Joint Committee on Cancer Guidelines (AJCC) (8th edition). ER and PR status were also determined from surgical specimen using immunohistochemistry (IHC). Positive for ER and PR were defined as those in which more than 1% of tumor nuclei in the sample were stained. HER2 status was assessed following the recommendation of the 2013 American Society of Clinical Oncology (ASCO)/College of American Pathologist (CAP). In this study, we defined high Ki67 as a value more than 20%. Neoadjuvant or adjuvant systemic therapies and/or radiotherapy were administered in accordance with established guidelines based on the age at diagnosis, tumor characteristics, and axillary lymph node status.

2.2 Mutational analysis of *TP53* gene

Mutational analysis of exon 5-9 of the *TP53* gene was performed using polymerase chain reaction-denaturing high performance liquid chromatography (PCR-DHPLC) and direct sequencing. Approximately 1mg of samples from either biopsies or surgical specimens, freshly frozen or

paraffin-embedded, were cut into pieces, and DNA was extracted using the Easy-DNA™ kit (Invitrogen, Carlsbad, CA, USA) with 100 ng/μL of DNA used for each PCR reaction, where each PCR was performed in a 20 μL reaction mixture containing 100 ng of DNA, 20 μM of forward and reverse primers, 2 μL of Taq buffer (10x), 2.5 mM of deoxyribonucleotide triphosphates (dNTPs), 2.5 mM of MgCl2, and 0.7 U of Taq DNA polymerase, under conditions of 95 ºC for 5 minutes, followed by 50 cycles of 94 ºC for 10 seconds, 62 ºC for 10 seconds, 72 ºC for 15 seconds, and a final extension at 72 ºC for 5 minutes in a DNA terminal cycler (Perkin-Elmer, GeneAmp PCR System 2400, USA), after which the PCR products were kept at 4 ºC until further analysis, initially screened for mutations by denaturing high-performance liquid chromatography (DHPLC) (WAVE; Transgenomic, Omaga, Nebraska, USA), followed by sequence analysis if heteroduplex formation was detected, with DHPLC performed by mixing 20 μL of each exon PCR product with an equal amount of the corresponding wild-type PCR product, incubating at 95 ºC for 5 minutes, and then at room temperature, and separating heteroduplex and homoduplex strands using triethylammonium acetate (TEAA) absorbed into the surface of the DNASep cartridge (Transgenomic, USA) through an association with the negatively charged phosphate backbone of DNA, with elution using acetonitrile (ACN), in a gradient solution of buffer A (0.1 M TEAA solution, pH 7.0) and buffer B (0.1 M TEAA and 25% ACN, pH 7.0), with buffer C (8% ACN (syringe washing solution)) and buffer D (75% ACN (DNASep Cartridge UltraClean and Storage Solution)) used for cleansing, while the stationary phase involved the DNASep Cartridge (Transgenomic, USA) column in an alkylated nonporous poly(styrene-divinylbenzene) form, washed with buffer D at 0.9 mL/min for 60 minutes, and the detection of separated DNA checked for purity by injecting 0.5 μL of the non-denatured specimen into the column at 0.9 mL/min at 50 ºC, with the temperature elevated to 63 ºC and the eluted DNA detected using an ultraviolet light detector at 260 nm, with analysis showing heteroduplexes eluted more rapidly than homoduplexes and appearing as separate forms in the chromatogram, and the DHPLC device operated per the manufacturer's instructions, with denatured PCR products at 95 ºC for 5 minutes, annealed at 55 ºC for about 40 minutes, and monitored as a chromatogram, where heterogenous molecules typically displayed an additional peak compared to homozygous molecules, which had only one peak, and sequence analysis was performed using commercial reagents and an automated sequencer (ABI Prism BigDye Terminator v3.1 cycles sequencing kit and ABI 310 Genetic Analyzer; Applied Biosystems, Foster City, USA), with both forward and reverse strands sequenced to confirm nucleotide alterations.

2.3 Definition of *TP53* mutation characteristics and oncologic outcomes

In this study, we classified cases with mutations identified in exons 5-9 through DNA sequencing, as previously described,[21,22] into the *TP53*-mutated group and cases with no mutations detected into the *TP53* wild-type group. To validate the clinical relevance of the characteristics of *TP53* mutation, we subcategorized the *TP53*-mutated group into some categories. Since most *TP53* mutations are missense mutations and are predominantly found in the DBD, we performed a subgroup analysis by subdividing the *TP53*-mutated group into missense mutation vs. other mutations and DBD vs. other locations. Additionally, we distinguished and analyzed cases with missense hotspot mutations (missense mutations situated at codon 175, 220, 245,248, 273, and 282) separately from other cases. The types and locations of *TP53* mutations in patients within the *TP53*-mutated group were summarized in Supplementary Figure 1.

Recurrence-free survival (RFS) was defined as the time from treatment of breast cancer (surgery or neoadjuvant chemotherapy) to recurrence or death from any cause. Recurrent tumor occurring in the parenchyma of the ipsilateral breast affected the primary cancer was defined as local recurrence (LR) and metastasis to ipsilateral axillary lymph node, internal mammary node, and supraclavicular node were classified as regional recurrence (RR). Metachronous breast cancer (recurrence affecting the contralateral breast diagnosed after 1 year from the first cancer diagnosis[23]) was also defined as regional recurrence in this study. Metastasis to all other organs was defined as distant metastasis (DM). Overall survival (OS) was defined as the time from the treatment to death from any cause.

2.4 Statistical analysis

We utilized the chi-square test or Fisher's exact test to compare the proportion of demographic and clinicopathological variables between the two groups based on *TP53* mutation status. Comparisons among *TP53*-mutated subgroups, based on characteristics of *TP53* mutation including mutation types and locations, were also conducted. Oncologic outcomes between the two groups, classified according to *TP53* mutation status and characteristics, were compared using a stratified log-rank test at a two-sided significance level of 0.05. A stratified Cox regression analysis was performed to estimate hazard ratio (HR) and 95% confidence intervals (CIs) for oncologic outcomes. To estimate HR of each clinicopathological variables and *TP53* mutation status for RFS and OS, we performed Cox proportional hazards model. Multivariable Cox analyses were performed using all variables

with *p*-value ≤ 0.05. All statistical significance was set as $p \leq 0.05$. All data analysis were conducted with SPSS software version 26.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism software version 10.0 (GraphPad software Inc., Boston, MA, USA).

III. RESULTS

3.1 Baseline patients' characteristics

Between January 2007 and December 2015, there were 650 patients who underwent *TP53* mutation testing on preoperative biopsies or surgical specimens in Gangnam Severance Hospital. In total, there were 172 (26.5%) patients who were detected *TP53* mutations. Among the 172 patients with confirmed *TP53* mutations, 34 (19.8%) had missense hotspot mutations (Figure 1).

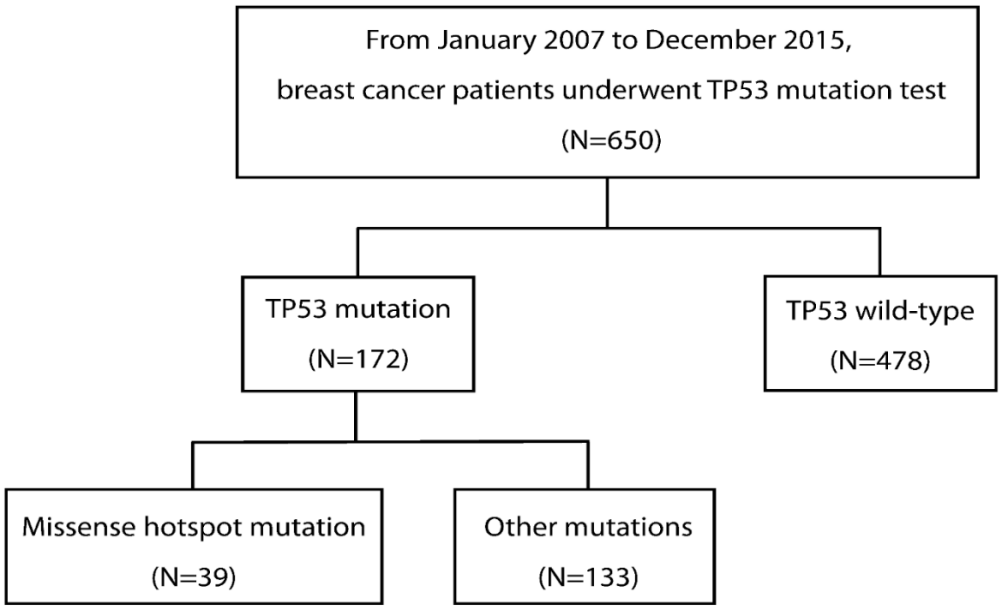


Figure 1. Consort diagram

Table 1 presented demographic and clinicopathological characteristics of patients according to *TP53*

mutation status. The median age of patients in both groups was 52 years. Compared to *TP53* wild-type group, the *TP53*-mutated group exhibited a higher proportion of ductal-type breast cancer (86.0% vs. 76.6%, $p = 0.016$), high histologic grade (61.6% vs. 28.9%, $p < 0.001$), and increased rates of LVI (34.5% vs. 17.4%, $p < 0.001$) and high Ki67 index (73.8% vs. 31.4%, $p < 0.001$). Furthermore, the *TP53*-mutated group had a higher incidence of negative ER tumor (64.5% vs. 39.2%, $p < 0.001$) and negative PR tumor (74.7% vs. 46.1%, $p < 0.001$), along with higher frequency of positive HER2 tumor (44.2% vs. 26.8%, $p < 0.001$). In summary, the *TP53*-mutated group had a higher proportion of HER2-positive breast cancer (44.2% vs. 29.4%) and TNBC (37.8% vs. 19.7%), and a lower proportion of HR-positive, HER2-negative breast cancer (18.0% vs. 50.9%) compared to the *TP53* wild-type group ($p < 0.001$). After excluding patients who underwent neoadjuvant chemotherapy, the distribution of T stage in the *TP53* wild-type group was 54.6% (253/463) for T1, 42.1% (195/463) for T2, and 3.2% (15/463) for T3-4, whereas in the *TP53*-mutated group, it was 42.9% (69/161) for T1, 53.4% (86/161) for T2, and 3.7% (6/161) for T3-4. At the time of data cut-off of this study, median follow-up period was 86.2 months (IQR, 60.3-111.8) in *TP53*-mutated group and 97.4 months (IQR, 63.6-134.4) in *TP53* wild-type group.

Table 1. Baseline patient characteristics according to *TP53* mutation status

N (%)	*TP53*-mutated (N=172)	*TP53* wild-type (N=478)	*p*
Age, median [range]	52 [27-78]	52 [51-87]	0.284
Age distribution			0.2
≤ 50 years	78 (45.3)	244 (51.0)	
> 50 years	94 (54.7)	234 (49.0)	
Tumor subtype			0.016
Ductal	148 (86.0)	366 (76.6)	
Lobular	2 (1.2)	23 (4.8)	
Others and Mixed	22 (12.8)	89 (18.6)	
Histologic grade			< 0.001
Grade I-II	66 (38.4)	340 (71.1)	
Grade III	106 (61.6)	138 (28.9)	
ER status #			< 0.001
Positive	60 (35.1)	253 (60.8)	
Negative	111 (64.9)	163 (39.2)	
PR status #			< 0.001
Positive	43 (25.3)	226 (53.9)	

Negative	127 (74.7)	193 (46.1)	
HER2 status			< 0.001
Positive	76 (44.2)	128 (26.8)	
Negative	96 (55.8)	350 (73.2)	
Molecular subtype #			< 0.001
HR-positive, HER2-negative	31 (18.0)	222 (50.9)	
HER2-positive	76 (44.2)	128 (29.4)	
Triple-negative	65 (37.8)	86 (19.7)	
LVI #			< 0.001
Positive	59 (34.5)	83 (17.4)	
Negative	112 (65.5)	395 (82.6)	
Ki67 index (cut-off 20%)			< 0.001
High	127 (73.8)	150 (31.4)	
Low	45 (26.2)	328 (68.6)	
Neoadjuvant chemotherapy			0.062
Yes	11 (6.4)	15 (3.1)	
No	161 (93.6)	463 (96.9)	
T stage*			0.035
T1	69 (42.9)	253 (54.6)	
T2	86 (53.4)	195 (42.1)	
T3-4	6 (3.7)	15 (3.2)	
N stage*			0.922
N0	98 (60.9)	276 (59.7)	
N1	49 (30.4)	141 (30.5)	
N2-3	14 (8.7)	45 (9.7)	
Adjuvant chemotherapy*			< 0.001
Yes	140 (87.0)	314 (67.8)	
No	21 (13.0)	149 (32.2)	

Patients without definite data was excluded.

* Patients who underwent neoadjuvant chemotherapy was excluded.

Abbreviation, ER; estrogen receptor, PR: progesterone receptor, HER2; human epidermal growth factor receptor 2, HR; hormone receptor, LVI; lymphovascular invasion

3.2 Oncologic outcomes according to *TP53* mutation status

With an extended follow-up period, we assessed 5-year and 10-year oncologic outcomes and HR by using Kaplan-Meier analysis and Cox regression analysis. The RFS rates at 5-year were 88.1% (95% CIs, 84.1-91.1) in *TP53*-mutated group, 93.7% (95% CIs, 91.0-95.7) in *TP53* wild-type group, and the 10-year RFS rates were 83.5% (95% CIs, 76.2-88.8) in *TP53*-mutated group, 86.6% (95% CIs, 80.2-91.1) in *TP53* wild-type group, showing a statistically significant difference between the two groups (HR, 1.67; 95% CIs, 1.06-2.64; p = 0.026; Figure 2A). The OS rates at 5-year were 89.8% (95% CIs, 83.8-93.6) in *TP53*-mutated group and 95.3% (95% CIs, 92.8-97.0) in *TP53* wild-type group, while the 10-year OS rates were 88.1% (95% CIs, 81.7-92.4) in *TP53*-mutated group and 91.0% (95% CIs, 87.3-93.6) in *TP53* wild-type group, indicating that the *TP53*-mutated group had a worse prognosis compared to *TP53* wild-type group (HR, 3.02; 95% CIs, 1.43-6.70; p = 0.003; Figure 2B).

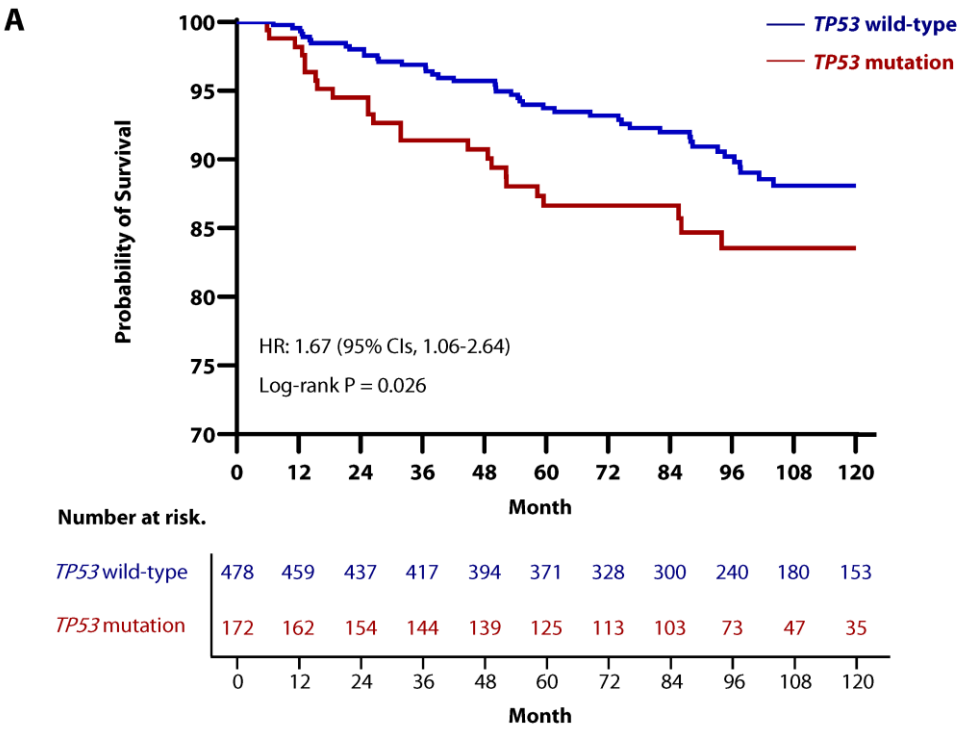


Figure 2A. Kaplan-Meier curve for RFS in patients stratified by *TP53* mutation status. Stratified

log-rank test and Cox regression analysis showed a significant difference between the two groups (HR, 1.67; 95% CIs, 1.06-2.64; $p = 0.026$). (The 5-year RFS rate: 88.1% (95% CIs, 84.1-91.1) in *TP53* mutation group vs. 93.7% (95% CIs, 91.0-95.7) in *TP53* wild-type group, the 10-year RFS rate: 83.5% (95% CIs, 76.2-88.8) in *TP53* mutation group vs. 86.6% (95% CIs, 80.2-91.1) in *TP53* wild-type group)

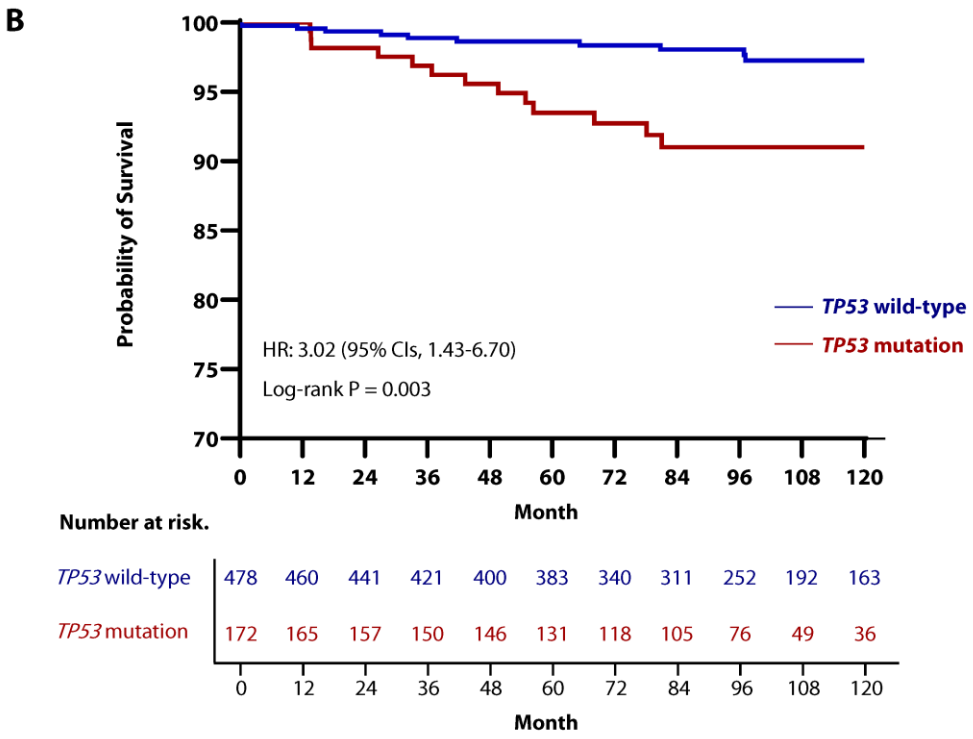


Figure 2B. Kaplan-Meier curve for OS in patients stratified by *TP53* mutation status. Stratified log-rank test and Cox regression analysis showed a significant difference between the two groups (HR, 3.02; 95% CIs, 1.43-6.70; $p = 0.003$). (The 5-year OS rate: 89.8% (95% CIs, 83.8-93.6) in *TP53* mutation group vs. 95.3% (95% CIs, 92.8-97.0) in *TP53* wild-type group, the 10-year OS rate: 88.1% (95% CIs, 81.7-92.4) in *TP53* mutation group vs. 91.0% (95% CIs, 87.3-93.6) in *TP53* wild-type group)

However, when recurrence events were analyzed by sites, there were no differences between the two

groups in terms of local recurrence-free survival (LRFS), regional recurrence-free survival (RRFS), and distant metastasis-free survival (DMFS) (Supplementary Figure 2).

Univariable Cox analysis showed that *TP53* mutation was significantly associated with a shorter period of RFS (HR, 1.669; 95% CIs, 1.058-2.635; *p*-value = 0.028; Table 2A) and OS (HR, 3.092; 95% CIs, 1.427-6.698; *p*-value = 0.004; Table 3A). In multivariable Cox analysis, which included all predictors with a *p*-value ≤ 0.05 from the univariable Cox analysis, *TP53* mutation remained an independent predictor of worse RFS (HR, 1.29; 95% CIs, 1.008-1.832; p-value = 0.046; Table 2B) and OS (HR, 2.488; 95% CIs, 1.407-3.788, *p*-value = 0.044; Table 3B). Additionally, the multivariable Cox analysis indicated that the presence of LVI and a high Ki-67 index were significantly associated with worse RFS (Table 2B), and the presence of LVI was also an independent predictor of worse OS (Table 3B).

Table 2A. Univariable analyses for RFS

Variables		Univariable		
		HR	95% CIs	*p*-value
Age	> 50 years	ref.		
	≤ 50 years	1.24	0.813-1.893	0.318
TP53 status	Wild-type	ref.		
	Mutation	1.669	1.058-2.635	0.028
Histologic type	Ductal	ref.		
	Lobular	1.236	0.277-5.525	0.781
	Others and Mixed	1.906	0.468-7.768	0.368
Histologic grade	I-II	ref.		
	III	1.123	0.730-1.730	0.597
ER status #	Negative	ref.		
	Positive	1.25	0.797-1.961	0.331
PR status #	Negative	ref.		
	Positive	1.02	0.652-1.597	0.930
HER2 status	Negative	ref.		
	Positive	0.718	0.439-1.172	0.185
LVI #	No	ref.		
	Yes	2.604	1.686-4.021	< 0.001
Ki-67 index	Low (< 20%)	ref.		

	High ((≥ 20%)	1.829	1.198-2.790	0.005
Tumor size *	≤ 2cm	ref.		
	> 2cm	1.659	1.063-2.587	0.026
Nodal status *	Negative	ref.		
	Positive	1.343	0.874-2.064	0.178

#Patients without definite data was excluded.
*Patients who underwent neoadjuvant chemotherapy was excluded.
Abbreviation, HR; hazard ratio, CIs; confidence intervals, ER; estrogen receptor, PR; progesterone receptor, HER2; human epidermal growth factor receptor 2, LVI; lymphovascular invasion, ref; reference

Table 2B. Multivariable analysis for RFS

Variables		Multivariable		
		HR	95% CIs	*p*-value
TP53 status	Wild-type	ref.		
	Mutation	1.29	1.008-1.832	0.046
LVI #	No	ref.		
	Yes	2.366	1.495-3.747	< 0.001
Ki-67 index	Low (< 20%)	ref.		
	High ((≥ 20%)	1.607	1.030-2.506	0.037
Tumor size *	≤ 2cm	ref.		
	> 2cm	1.29	0.805-2.607	0.291

Variables that were not significant in the univariable analysis were excluded from the multivariable analysis.
#Patients without definite data was excluded.
*Patients who underwent neoadjuvant chemotherapy was excluded.
Abbreviation, HR; hazard ratio, CIs; confidence intervals, LVI; lymphovascular invasion, ref; reference

Table 3A. Univariable analyses for OS

Variables		Univariable		
		HR	95% CIs	*p*-value
Age	> 50 years	ref.		
	≤ 50 years	0.812	0.375-1.757	0.597
TP53 status	Wild-type	ref.		
	Mutation	3.092	1.427-6.698	0.004
Histologic type	Ductal	ref.		

	Lobular and Others Mixed	undefined undefined		
Histologic grade	I-II	ref.		
	III	0.909	0.405-2.038	0.816
ER status #	Negative	ref.		
	Positive	0.515	0.225-1.178	0.116
PR status #	Negative	ref.		
	Positive	0.758	0.340-1.689	0.498
HER2 status	Negative	ref.		
	Positive	1.017	0.442-2.339	0.968
LVI #	No	ref.		
	Yes	2.889	1.326-6.294	0.008
Ki-67 index	Low (< 20%)	ref.		
	High ((≥ 20%)	2.419	1.096-5.340	0.029
Tumor size *	≤ 2cm	ref.		
	> 2cm	2.788	1.082-7.186	0.034
Nodal status *	Negative	ref.		
	Positive	1.999	0.876-4.558	0.1

#Patients without definite data was excluded.
*Patients who underwent neoadjuvant chemotherapy was excluded.
Abbreviation, HR; hazard ratio, CIs; confidence intervals, ER; estrogen receptor, PR; progesterone receptor, HER2; human epidermal growth factor receptor 2, LVI; lymphovascular invasion, ref; reference

Table 3B. Multivariable analysis for RFS

Variables		Multivariable		
		HR	95% CIs	*p*-value
TP53 status	Wild-type	Ref.		
	Mutation	2.488	1.407-3.788	0.044
LVI #	No	Ref.		
	Yes	2.659	1.113-6.357	0.028
Ki-67 index	Low (< 20%)	Ref.		
	High ((≥ 20%)	2.35	0.966-5.717	0.06
Tumor size *	≤ 2cm	Ref.		
	> 2cm	2.089	0.776-5.627	0.145

Variables that were not significant in the univariable analysis were excluded from the

multivariable analysis.
#Patients without definite data was excluded.
*Patients who underwent neoadjuvant chemotherapy was excluded.
Abbreviation, HR; hazard ratio, CIs; confidence intervals, LVI; lymphovascular invasion, ref; reference

3.3 Subgroup analysis based on mutation types within the *TP53*-mutated group

Since most *TP53* mutations are known to be missense mutations, we conducted a subgroup analysis to determine if there were differences in oncologic outcomes between the missense type and other mutation types. Among the 172 cases with confirmed *TP53* mutations, 96 (55.8%) had missense mutations and 76 (44.2%) had other types of mutations. There were no significant differences in demographic and clinicopathological variables between the two groups, except for a higher prevalence of T1 stage tumor in the missense mutation group (50.6% vs. 33.3%, $p = 0.026$) when patient who received neoadjuvant chemotherapy were excluded. Table 4 presented detailed information.

Table 4. Patients' characteristics based on *TP53* mutation type within the *TP53*-mutated group

N (%)	Missense mutation (N=96)	Other mutations (N=76)	*p*
Age, median [range]	52 [34-76]	52 [27-87]	0.383
Age distribution			0.285
≤ 50 years	47 (49.0)	31 (40.8)	
> 50 years	49 (51.0)	45 (59.2)	
Tumor subtype			0.201
Ductal	79 (82.3)	69 (90.8)	
Lobular	2 (2.1)	0	
Others and Mixed	15 (15.6)	7 (9.2)	
Histologic grade			0.495
Grade I-II	39 (40.6)	27 (35.5)	
Grade III	57 (59.4)	49 (64.5)	
ER status #			0.455
Positive	36 (37.5)	24 (32.0)	
Negative	60 (62.5)	51 (68.0)	
PR status #			0.730
Positive	25 (26.3)	18 (24.0)	

Negative	70 (73.7)	57 (76.0)	
HER2 status			0.625
Positive	44 (45.8)	32 (42.1)	
Negative	52 (54.2)	44 (57.9)	
Molecular subtype			0.554
HR-positive, HER2-negative	19 (19.8)	12 (15.8)	
HER2-positive	44 (45.8)	32 (42.1)	
Triple-negative	33 (34.4)	32 (42.1)	
LVI [#]			0.943
Positive	33 (34.7)	26 (34.2)	
Negative	62 (65.3)	50 (65.8)	
Ki67 index (cut-off 20%)			0.175
High	67 (69.8)	60 (78.9)	
Low	29 (30.2)	16 (21.1)	
Neoadjuvant chemotherapy			0.589
Yes	7 (7.3)	4 (5.3)	
No	89 (92.7)	72 (94.7)	
T stage[*]			0.026
T1	45 (50.6)	24 (33.3)	
T2	43 (48.3)	43 (59.7)	
T3-4	1 (1.1)	5 (6.9)	
N stage[*]			0.113
N0	52 (58.4)	46 (63.9)	
N1	32 (36.0)	17 (23.6)	
N2-3	5 (5.6)	9 (12.5)	
Adjuvant chemotherapy*			0.110
Yes	74 (83.1)	66 (91.7)	
No	15 (16.9)	6 (8.3)	

[#] Patients without definite data was excluded.
[*] Patients who underwent neoadjuvant chemotherapy was excluded.
Abbreviation, ER; estrogen receptor, PR: progesterone receptor, HER2; human epidermal growth factor receptor 2, HR; hormone receptor, LVI; lymphovascular invasion

With a median follow-up period of 86.1 months (IQR, 54.1-110.8), there was no significant differences of RFS and OS between two groups (Figure 3).

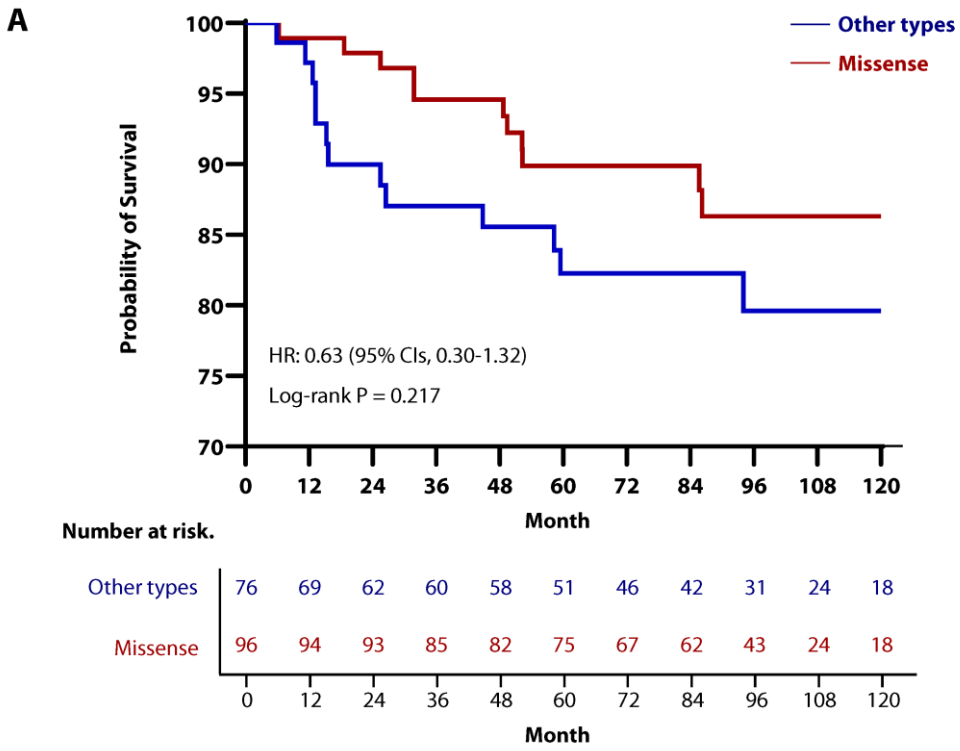


Figure 3A. Kaplan-Meier curve for RFS in patients with *TP53* mutation, stratified by type of mutation. Stratified log-rank test and Cox regression analysis showed that there was no significant difference between the two groups (HR, 0.63; 95% CIs, 0.30-1.32; $p = 0.217$). (The 5-year RFS rate: 89.9% (95% CIs, 81.4-94.6) in missense mutation group vs. 82.3% (95% CIs, 70.8-89.5) in other mutations group, the 10-year RFS rate: 86.3% (95% CIs, 76.3-92.3) in missense mutation group vs. 79.6% (95% CIs, 67.0-87.8) in other mutations group)

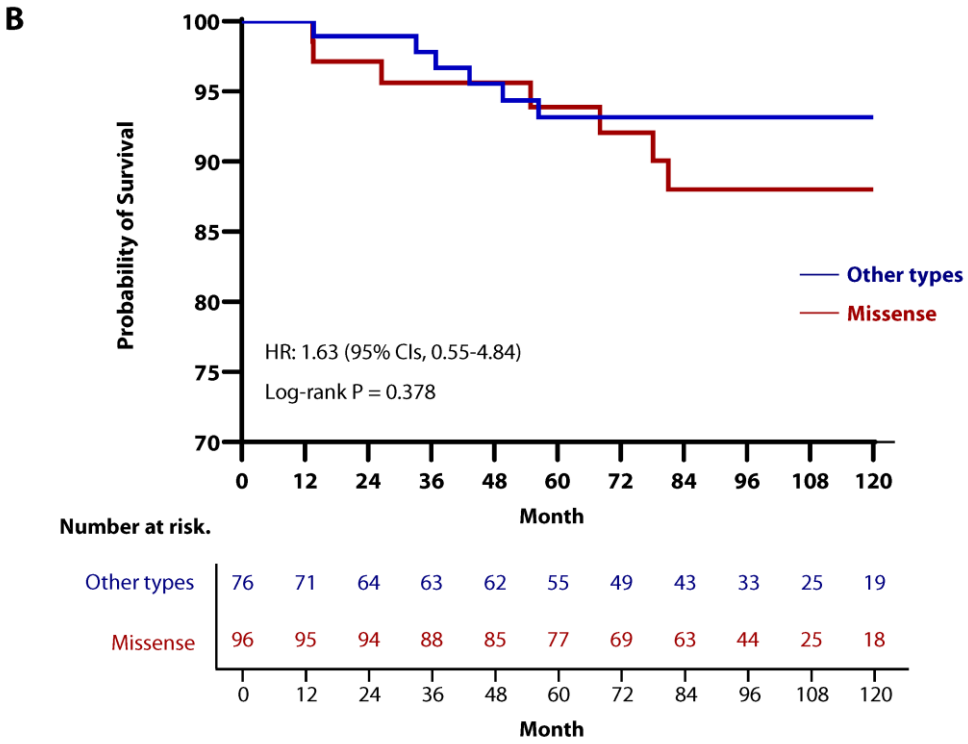


Figure 3B. Kaplan-Meier curve for OS in patients with *TP53* mutation, stratified by type of mutation. Stratified log-rank test and Cox regression analysis showed that there was no significant difference between the two groups (HR, 1.63; 95% CIs, 0.55-4.84; $p = 0.378$). (The 5-year OS rate: 93.9% (95% CIs, 84.5-97.7) in missense mutation group vs. 93.2% (95% CIs, 85.4-96.9) in other mutations group, the 10-year OS rate: 88.0% (95% CIs, 76.3-94.2) in missense mutation group vs. 93.2% (95% CIs, 85.4-96.9) in other mutations group)

The 5-year RFS rates were 89.9% (95% CIs, 81.4-94.6) in missense mutation group and 82.3% (95% CIs, 70.8-89.5) in other mutations group, while the rates of RFS at 10 years were 86.3% (95% CIs, 76.3-92.3) in missense mutation group and 79.6% (95% CIs, 67.0-87.8) in other mutations group (HR, 0.63; 95% CIs, 0.30-1.32; $p = 0.217$). The 5-year OS rates were 93.9% (95% CIs, 84.5-97.7) in missense mutation group and 93.2% (95% CIs, 85.4-96.9) in other mutations group, while the 10-year OS rates were 88.0% (95% CIs, 76.3-94.2) in missense mutation group and 93.2% (95% CIs, 85.4-96.9) in other mutations group (HR, 1.63; 95% CIs, 0.55-4.84; $p = 0.378$). Additionally, LRFS,

RRFS, and DMFS did not differ significantly between two groups (Supplementary Figure 3).

3.4 Subgroup analysis based on locations of mutation within the *TP53*-mutated group

Next, within the patient with *TP53* mutation, we conducted a subgroup analysis to investigate oncologic outcomes based on the locations of *TP53* mutations. First, we classified the location of *TP53* mutations into the DBD and other locations. In total, there were 151 cases (87.8%) in DBD group and 21 cases (12.2%) in other locations group. Compared to the other locations group, the DBD group had a higher proportion of TNBC and lower proportion of HER2-positive tumors (TNBC; 41.7% vs. 9.5%, HER2-positive; 41.7% vs. 61.9%; p = 0.016). No other significant differences were observed between the two groups (Table 5).

Table 5. Patients' characteristics based on location of *TP53* mutations in *TP53*-mutated group

N (%)	DNA-binding domain (N=151)	Other locations (N=21)	*P*
Age, median [range]	54 [27-87]	52 [37-75]	0.819
Age distribution			0.824
≤ 50 years	68 (45.0)	10 (47.6)	
> 50 years	83 (55.0)	11 (52.4)	
Tumor subtype			0.140
Ductal	127 (84.1)	21 (100)	
Lobular	2 (1.3)	0	
Others and Mixed	22 (14.6)	0	
Histologic grade			0.352
Grade I-II	56 (37.1)	10 (47.6)	
Grade III	95 (62.9)	11 (52.4)	
ER status #			0.013
Positive	48 (31.8)	12 (60.0)	
Negative	103 (68.2)	8 (40.0)	
PR status #			0.031
Positive	34 (22.7)	9 (45.0)	
Negative	116 (77.3)	11 (55.0)	
HER2 status			0.081
Positive	63 (41.7)	13 (61.9)	
Negative	88 (58.3)	8 (38.1)	

Molecular subtype			0.016
HR-positive, HER2-negative	25 (16.6)	6 (28.6)	
HER2-positive	63 (41.7)	13 (61.9)	
Triple-negative	63 (41.7)	2 (9.5)	
LVI [#]			0.542
Positive	53 (35.3)	6 (28.6)	
Negative	97 (64.7)	15 (71.4)	
Ki67 index (cut-off 20%)			0.063
High	115 (76.2)	12 (57.1)	
Low	36 (23.8)	9 (42.9)	
Neoadjuvant chemotherapy			> 0.999
Yes	10 (6.6)	1 (4.8)	
No	141 (93.4)	20 (95.2)	
T stage[*]			0.736
T1	62 (44.0)	7 (35.0)	
T2	74 (52.5)	12 (60.0)	
T3-4	5 (3.5)	1 (5.0)	
N stage[*]			0.440
N0	88 (62.4)	10 (50.0)	
N1	42 (29.8)	7 (35.0)	
N2-3	11(7.8)	3 (15.0)	
Adjuvant chemotherapy*			0.078
Yes	120 (85.1)	20 (100)	
No	21 (14.9)	0	

[#] Patients without definite data was excluded.

[*] Patients who underwent neoadjuvant chemotherapy was excluded.

Abbreviation, ER; estrogen receptor, PR: progesterone receptor, HER2; human epidermal growth factor receptor 2, HR; hormone receptor, LVI; lymphovascular invasion

As with *TP53* mutation type, there were no differences in oncologic outcomes between the two groups based on mutation location (Figure 4).

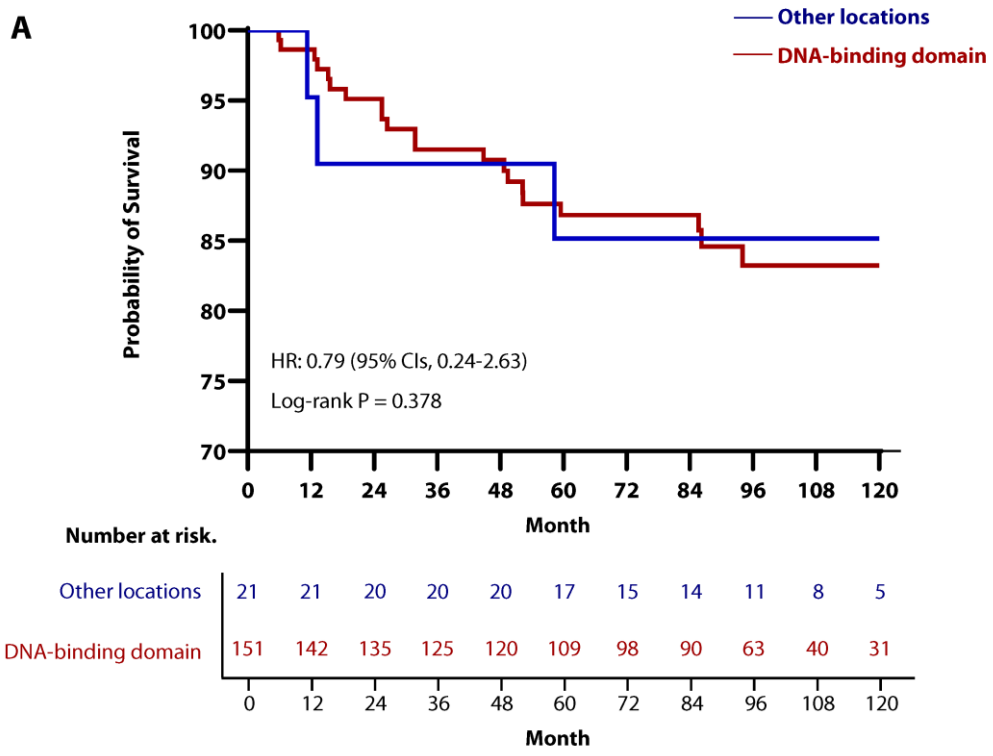


Figure 4A. Kaplan-Meier curve for RFS in patients with *TP53* mutation, stratified by locations of mutation. Stratified log-rank test and Cox regression analysis showed that there was no significant difference between the two groups (HR, 0.79; 95% CIs, 0.24-2.63; $p = 0.378$). (The 5-year RFS rate: 86.8% (95% CIs, 79.9-91.5) in DBD group vs. 85.2% (95% CIs, 60.6-95.0) in other locations group, the 10-year RFS rate: 83.2% (95% CIs, 75.2-88.9) in DBD group vs. 85.2% (95% CIs, 60.6-95.0) in other locations group)

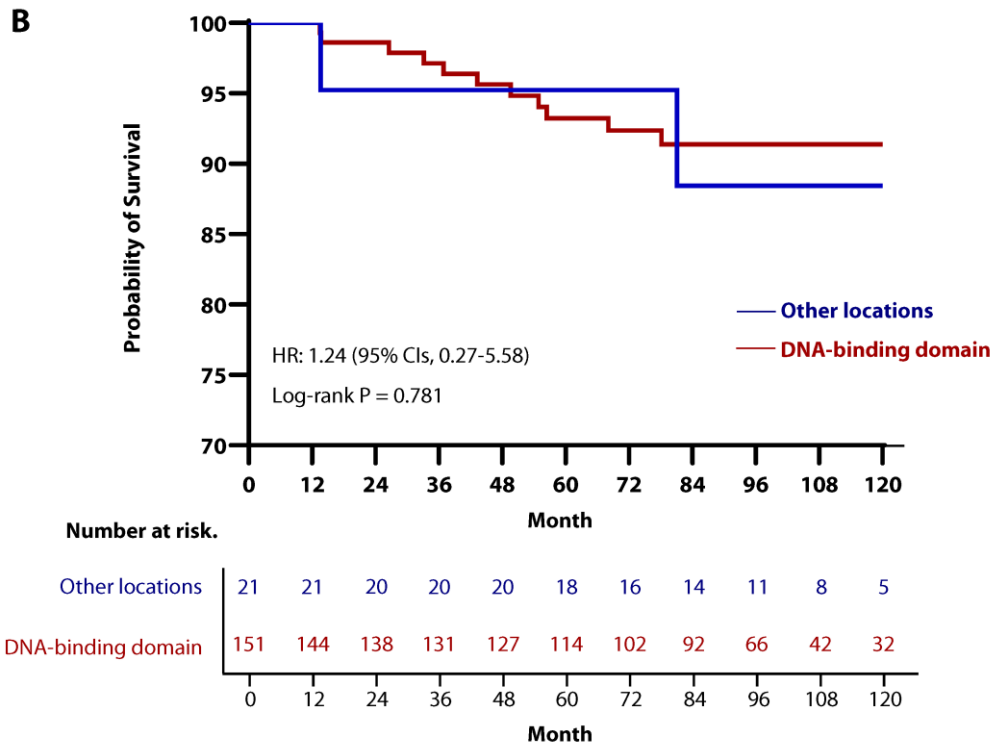


Figure 4B. Kaplan-Meier curve for OS in patients with *TP53* mutation, stratified by locations of mutation. Stratified log-rank test and Cox regression analysis showed that there was no significant difference between the two groups (HR, 1.24; 95% CIs, 0.27-5.58; $p = 0.781$). (The 5-year OS rate: 93.2% (95% CIs, 87.4-96.4) in DBD group vs. 95.2% (95% CIs, 70.7-99.3) in other locations group, the 10-year OS rate: 91.4% (95% CIs, 84.9-95.2) in DBD group vs. 88.4% (95% CIs, 60.3-97.1) in other locations group)

The 5-year RFS rates were 86.8% (95% CIs, 79.9-91.5) in DBD group, 85.2% (95% CIs, 60.6-95.0) in other locations group, and the 10-year RFS rates were 83.2% (95% CIs, 75.2-88.9) in DBD group, 85.2% (95% CIs, 60.6-95.0) in other locations group (HR, 0.79; 95% CIs, 0.24-2.63; $p = 0.378$). The OS rates at 5 years were 93.2% (95% CIs, 87.4-96.4) in DBD group and 95.2% (95% CIs, 70.7-99.3) in other locations group, while the 10-year OS rates were 91.4% (95% CIs, 84.9-95.2) in DBD group and 88.4% (95% CIs, 60.3-97.1) in other locations group (HR, 1.24; 95% CIs, 0.27-5.58; $p = 0.781$). LRFS, RRFS, and DMFS did not differ significantly between two groups (Supplementary

Figure 4).

3.5 Subgroup analysis based on missense hotspot mutations or not within the *TP53*-mutated group

Lastly, we analyzed oncologic outcomes by distinguishing between cases with missense hotspot domains and those without within the patients with *TP53* mutation. Majority of mutations identified at hotspot codons were missense mutations (39/44, 88.6%). Although no statistically significant differences in patients' characteristics were observed between the two groups, the missense hotspot mutations group had a higher proportion of T1 tumors and relatively fewer T2 tumors (T1 tumors; 70.0% vs. 36.6%, T2 tumors; 26.7% vs. 59.5%, $p = 0.003$) (Table 6).

Table 6. Patients' characteristics between missense hotspot mutations and other mutations

N (%)	Missense hotspot mutations (N=34)	Other mutations (N=138)	*p*
Age, median [range]	53 [34-76]	53 [27-87]	0.953
Age distribution			0.823
≤ 50 years	16 (47.1)	62 (44.9)	
> 50 years	18 (52.9)	76 (55.1)	
Tumor subtype			0.448
Ductal	32 (94.1)	116 (84.1)	
Lobular	0	2 (1.4)	
Others and Mixed	2 (5.9)	20 (14.5)	
Histologic grade			0.680
Grade I-II	12 (35.3)	54 (39.1)	
Grade III	22 (64.7)	84 (60.9)	
ER status #			0.667
Positive	13 (38.2)	47 (34.3)	
Negative	21 (61.8)	90 (65.7)	
PR status #			0.537
Positive	10 (29.4)	33 (24.3)	
Negative	24 (70.6)	103 (75.7)	
HER2 status			0.993
Positive	15 (44.1)	61 (44.2)	
Negative	19 (55.9)	77 (55.8)	
Molecular subtype			0.894

HR-positive, HER2-negative	7 (20.6)	24 (17.4)	
HER2-positive	15 (44.1)	61 (44.2)	
Triple-negative	12 (35.3)	53 (38.4)	
LVI [#]			0.331
Positive	9 (27.3)	50 (36.2)	
Negative	24 (72.7)	88 (63.8)	
Ki67 index (cut-off 20%)			0.697
High	26 (76.5)	101 (73.2)	
Low	8 (23.5)	37 (26.8)	
Neoadjuvant chemotherapy			0.231
Yes	4 (11.8)	7 (5.1)	
No	30 (88.2)	131 (94.9)	
T stage[*]			0.003
T1	21 (70.0)	48 (36.6)	
T2	8 (26.7)	78 (59.5)	
T3-4	1 (3.3)	5 (3.8)	
N stage[*]			0.429
N0	18 (60.0)	80 (61.1)	
N1	11 (36.7)	38 (29.0)	
N2-3	1 (3.3)	13 (9.9)	
Adjuvant chemotherapy*			0.168
Yes	28 (80.0)	112 (88.9)	
No	7 (20.0)	14 (11.1)	

[#] Patients without definite data was excluded.
[*] Patients who underwent neoadjuvant chemotherapy was excluded.
Abbreviation, ER; estrogen receptor, PR: progesterone receptor, HER2; human epidermal growth factor receptor 2, LVI; lymphovascular invasion

The median follow-up period was 95.1 months (IQR, 81.9-98.8) in missense hotspot mutation group, 83.7 months (IQR, 75.6-89.3) in other mutations group. At 5 years, RFS rates were 100% in missense hotspot mutation group and 84.5% (95% CIs, 75.6-88.8) in other mutations group. The Kaplan-Meier estimates of 10-year RFS rate was 100% in missense hotspot mutation group and 79.6% (95% CIs, 70.8-86.0) in other mutations group, indicating missense hotspot mutation group had a better oncologic outcome than other mutations group (HR, 0.15; 95% CIs, 0.06-0.39; $p = 0.033$). However, there was no significant difference in OS between the two groups (HR, 0.70; 95% CIs, 0.18-2.66; $p = 0.636$) (Figure 5).

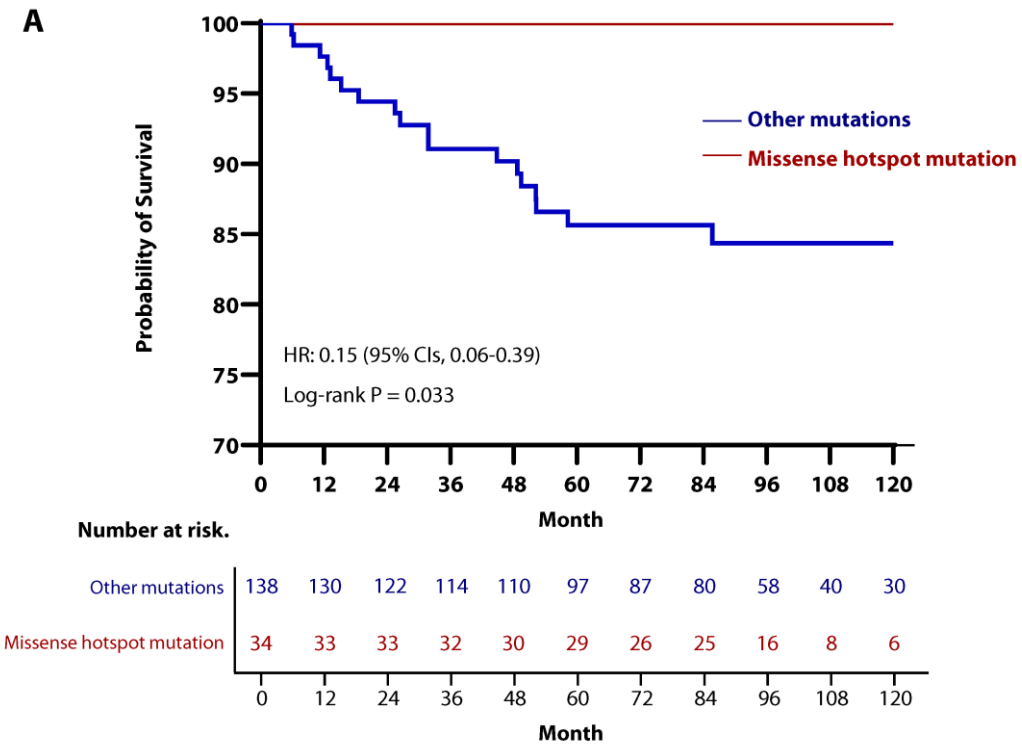


Figure 5A. Kaplan-Meier curve for RFS in patients with *TP53* mutation, stratified by the presence or absence of missense hotspot mutation. Compared to patients without missense hotspot mutation, patients with missense hotspot mutation had a longer RFS period (HR, 0.15; 95% CIs, 0.06-0.39; $p = 0.033$). (The 5-year RFS rate: 100% in missense hotspot mutation group vs. 83.4% (95% CIs, 75.6-88.8) in other mutations group, the 10-year RFS rate: 100% in missense hotspot mutation group vs. 79.6% (95% CIs, 70.8-86.0) in other mutations group)

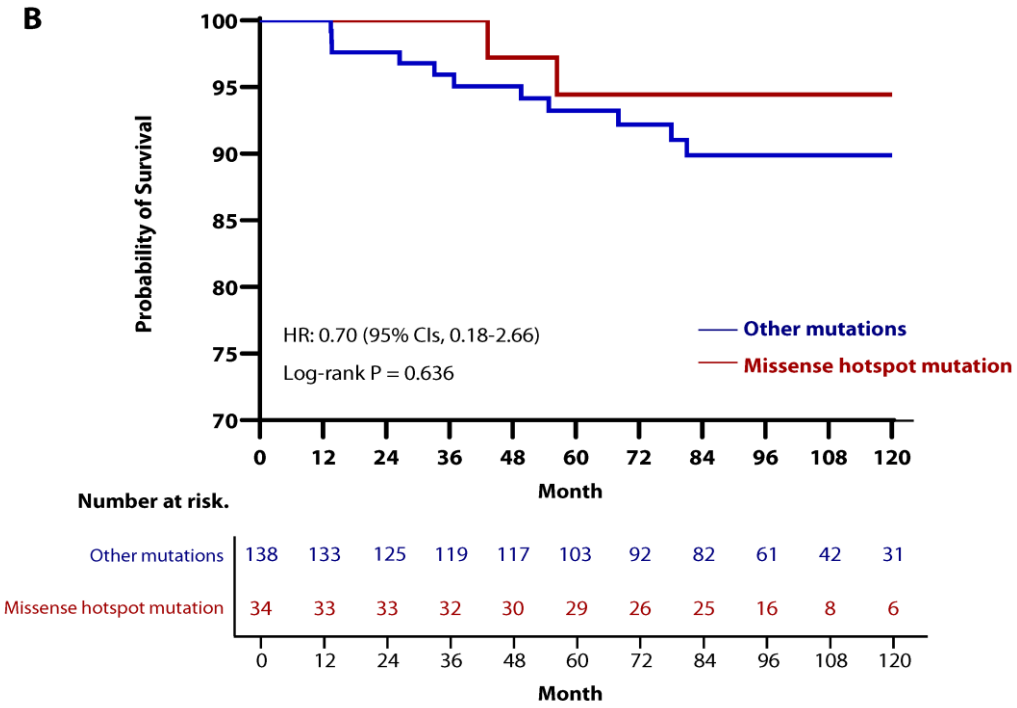


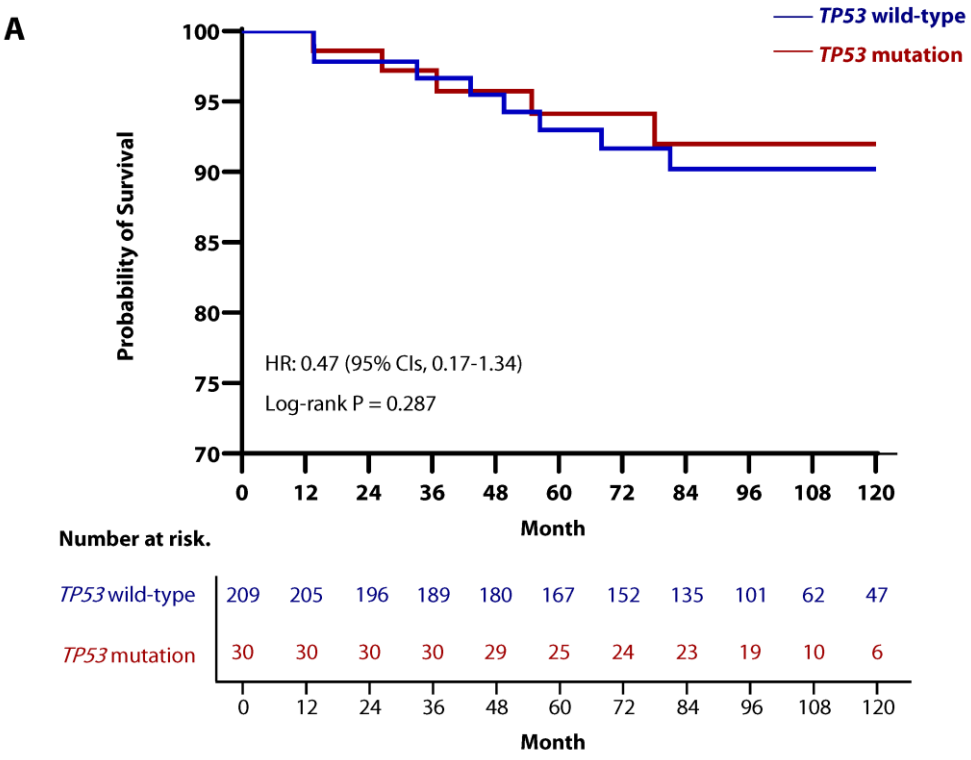
Figure 5B. Kaplan-Meier curve for OS in patients with *TP53* mutation, stratified by the presence or absence of missense hotspot mutation. Stratified log-rank test and Cox regression analysis showed that there was no significant difference between two groups. (HR, 0.70; 95% CIs, 0.18-2.66; $p = 0.636$). (The 5-year OS rate: 93.3% (95% CIs, 75.9-98.3) in missense hotspot mutation group vs. 93.6% (95% CIs, 87.6-96.7) in other mutations group, the 10-year OS rate: 93.3% (95% CIs, 75.9-98.3) in missense hotspot mutation group vs. 90.4% (95% CIs, 83.3-94.6) in other mutations group)

Additionally, the RFS rates stratified by recurrence sites were not significantly different between the two groups (Supplementary Figure 5).

3.6. Clinical relevance of *TP53* within molecular subtypes of breast cancer

As part of an exploratory analysis, we examined the clinical relevance of *TP53* mutations within specific molecular subtypes. After excluding 72 patients for whom IHC-based HR status was unavailable, there were 239 patients (41.3%) with HR-positive/HER2-negative (HR+/HER2-) breast

cancer, 188 patients (32.5%) with HER2-positive breast cancer, and 151 patients (26.1%) with TNBC. The proportion of patients with confirmed *TP53* mutations in each subtype was 30 patients (12.6%) in the HR+/HER2- subtype, 75 patients (39.9%) in the HER2-positive subtype, and 65 patients (43.0%) in the TNBC group. When comparing survival outcomes based on *TP53* mutation status within each subtype using the Kaplan-Meier estimated model, there were no differences in RFS or OS in HR+/HER2- and HER2-positive breast cancer. However, in TNBC, patients with *TP53* mutation had worse RFS compared to those with *TP53* wild-type (HR, 2.13; 95% CIs, 1.01—4.50; p = 0.046; Figure 6E). Nevertheless, there was no statistical difference in OS according to *TP53* mutation status in TNBC (HR, 1.83; 95% CIs, 0.58—5.73; p = 0.295; Figure 6F).



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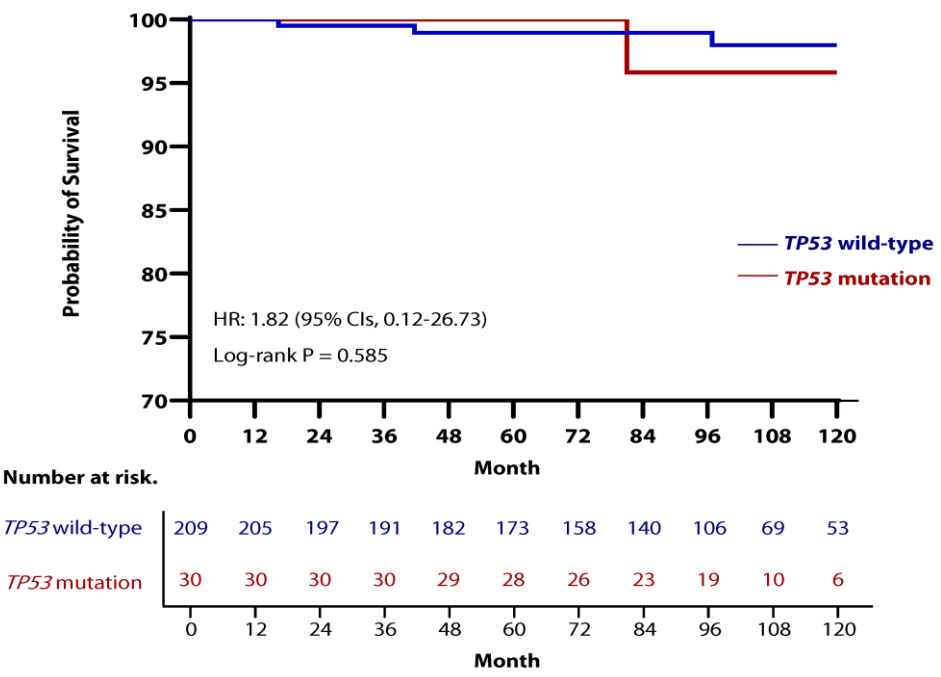
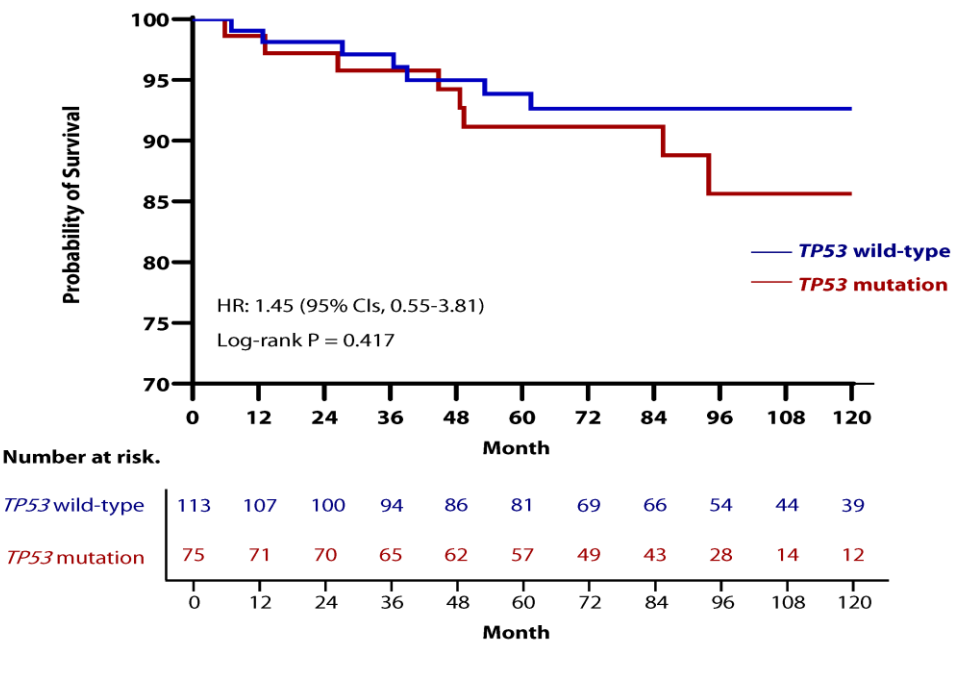


Number at risk.

TP53 wild-type	209	205	197	191	182	173	158	140	106	69	53
TP53 mutation	30	30	30	30	29	28	26	23	19	10	6
	0	12	24	36	48	60	72	84	96	108	120

Month

C

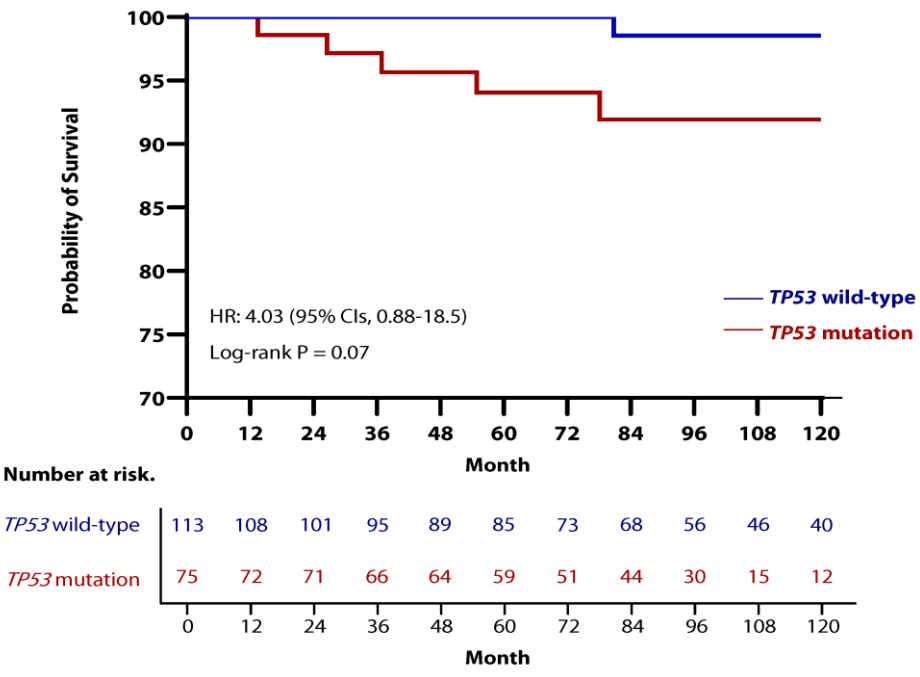


Number at risk.

TP53 wild-type	113	107	100	94	86	81	69	66	54	44	39
TP53 mutation	75	71	70	65	62	57	49	43	28	14	12
	0	12	24	36	48	60	72	84	96	108	120

Month

D



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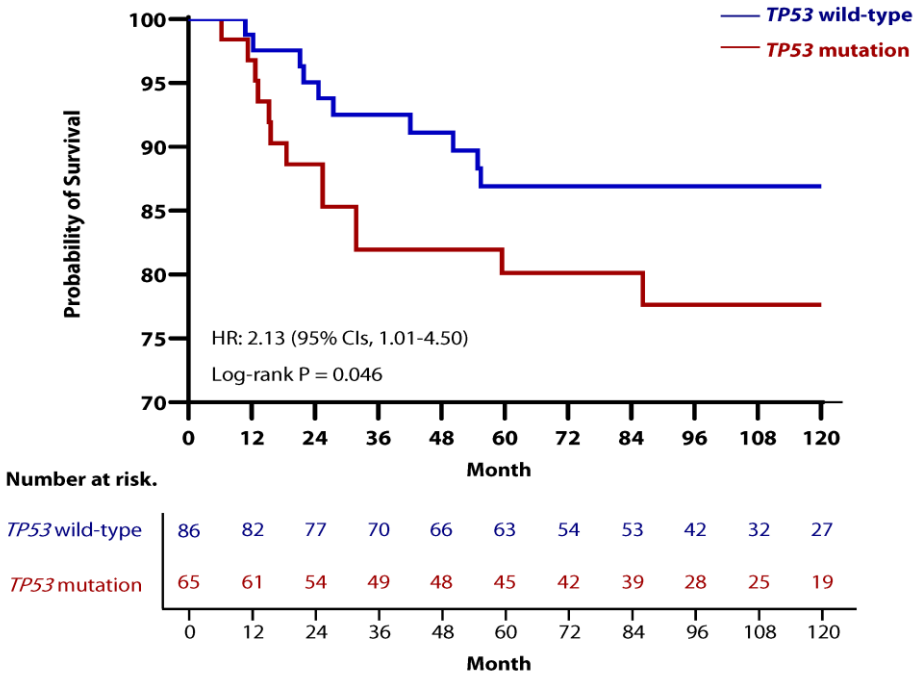

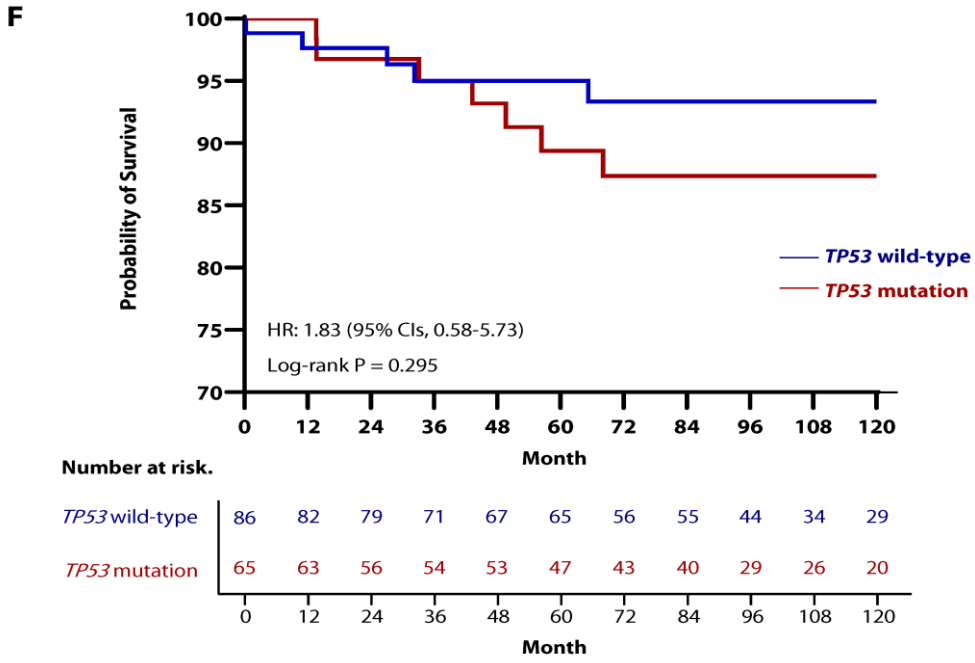

Figure 6. Kaplan-Meier curve for RFS and OS according to *TP53* mutation status in each subtype. In HR+/HER2- subtype, there was no difference in (A) RFS (HR, 0.47; 95% CIs, 0.17—1.34; p = 0.287) or (B) OS (HR, 1.82; 95% CIs; 0.12—26.73; p = 0.585) between *TP53*-muated group and *TP53* wild-type group. Similarly, in the HER2-positive subtype, no differences were observed in (C) RFS (HR, 1.45; 95% CIs; 0.55—3.81; p = 0.417) or (D) OS (HR, 4.03; 95% CIs; 0.88—18.5; p = 0.07) based on *TP53* mutation status. In the TNBC group, (E) *TP53*-mutated tumors showed worse RFS compared to the *TP53* wild-type group (HR, 2.13; 95% CIs; 1.01—4.50; p = 0.046). However, (F) although there was a trend toward worse OS in *TP53*-mutated tumors, it was not statistically significant (HR, 1.83; 95% CIs, 0.58—5.73; p = 0.295).

IV. DISCUSSION

In this retrospective cohort study, we assessed the clinical relevance of *TP53* mutations in breast cancer patients, including all subtypes and treatments, and conducted subgroup analyses based on the characteristics of *TP53* mutations within the *TP53*-mutated group. *TP53* mutations were more frequent in breast cancer with more aggressive clinicopathological variables, such as large tumor,

tumor with LVI or high tumor grade, and overexpression of HER2. Patients with *TP53* mutations had shorter RFS and OS compared to patients with *TP53* wild-type tumor. However, within the *TP53*-mutated group, the oncologic outcomes did not significantly differ between subgroups based on the characteristics of the *TP53* mutations. Missense mutation, locations within the DBD, and even missense mutation situated in hotspots, which all well-known dominant characteristics of *TP53* mutation, did not have clinical relevance compared to other types or locations of *TP53* mutations. Although patients with missense hotspot mutations in the *TP53*-mutated group had a longer RFS period compared to other patients, there was no difference in OS rate. Therefore, prognostic impact of missense hotspot mutations of *TP53* gene remains questionable.

Although *TP53* mutations are found in approximately 30% of all breast cancers,[13] the proportion of these mutations varies by tumor subtypes. Furthermore, due to the differing mechanisms of p53 protein among tumor subtypes and treatments, most studies on the clinical relevance of *TP53* mutations in breast cancer have been conducted within specific subtypes or treatments. Given that p53 regulates cell response to DNA damage, there have been several studies investigating the role of *TP53* mutations in patients undergoing chemotherapy or radiation, which induces tumor cell damage. Early preclinical trials indicated that p53 plays a role in regulating apoptosis or cell cycle arrest following the cell damage such as radiation or systemic anticancer treatments.[24-27] Subsequent studies have shown that breast cancer patients with *TP53* mutations often have higher pathologic complete response (pCR) rates following neoadjuvant chemotherapy.[28-31] Otherwise, there were studies showing neutral or negative results regarding the association between *TP53* mutations and pCR rates following neoadjuvant chemotherapy.[32-34] Most of previous studies had small sample sizes and used different chemotherapy regimens and detecting method of *TP53* mutations, making it difficult to define the clinical relevance of *TP53* mutations. Recently, a meta-analysis of 26 studies involving 3,476 breast cancer patients who underwent neoadjuvant chemotherapy found that those with *TP53* mutations had a higher pCR rate.[20] However, even this study confirmed the clinical relevance of *TP53* mutations through a sizable cohort, it also had the limitation of inconsistent *TP53* mutation detection methods across the including studies. Additionally, most cases receiving neoadjuvant chemotherapy are HER2-positive breast cancer or triple-negative breast cancer (TNBC). Therefore, it is difficult to consider these studies as having a balanced representation of all breast tumor subtypes.

ER-positive breast tumors account for approximately 70% of all breast cancers, making them the

most prevalence subtype. In ER-positive breast tumors, the frequency of *TP53* mutations is lower than in other subtypes;[31] however, when these mutations are present, they are associated with a poor prognosis. Many studies presented that *TP53* mutations could lead to alterations in the p53 protein, potentially causing endocrine resistance[35-37]. However, the relationship between *TP53* mutations and survival outcome in patients receiving only hormone therapy has been controversial.[31,36,38] This is due to several factors such as the small sample size, the detection of *TP53* mutations primarily through IHC, and the lack of information on additional treatments beyond hormone therapy. In a meta-analysis examining the clinical relevance of *TP53* mutations in patients receiving only hormone therapy, it was found that patients with *TP53* mutations had worse overall survival compared to those without *TP53* mutations.[39] Although a difference dataset with varying *TP53* mutation detection methods was utilized, we previously identified an association between *TP53* mutations and high 21-gene recurrence score (RS) in ER+HER2- breast tumors.[40] This finding aligns with prior research indicating that *TP53* mutations are associated with endocrine resistance in ER-positive breast tumors. Compared to ER-positive breast cancer, ER-negative breast cancer accounts for a smaller proportion of all breast tumors; however, the frequency of *TP53* mutations is higher in ER-negative breast cancer. *TP53* mutation rates are higher in HER2-positive and TNBC (also referred to as basal-like type) compared to luminal-type breast cancer, which are predominantly ER-positive tumors.[13,41-47] Some studies indicated that the presence of *TP53* mutations is associated with poor prognosis and might confer resistance to chemotherapy in HER2-positive cancer and TNBC.[45,48-50] However, some studies showed no difference in oncologic outcomes based on *TP53* mutation status in ER-negative tumors,[51-55] or even suggested that *TP53* mutations are associated with better prognosis.[30,56,57] This trend has become more pronounced in recent studies as chemotherapy regimens have continuously evolved and the clinical use of new drugs, such as dual HER2 blockade and immune checkpoint inhibitors, has increased. Consequently, determining the clinical significance of *TP53* mutations in ER-negative breast cancer has become even more challenging. Given these circumstances, conducting studies to determine the clinical relevance of *TP53* mutations across all subtypes and treatments have many hurdles and interpreting the results is also challenging.

Therefore, the strength of our study is its ability to assess long-term oncologic outcomes using a large cohort that encompasses all breast cancer subtypes and treatments. Excluding 42 patients whose hormone receptor status was not clearly identified, the data for this study included 253

HR+/HER2- case (41.6%), 204 HER2-positive cases (33.6%), and 172 triple-negative cases (28.3%), which means the distribution of tumor subtypes in collected data was well-balanced. To date, there have been few studies that investigated the clinical relevance of *TP53* mutations using cohorts that include all breast cancer subtypes and treatments. In most of these studies, patients with *TP53* mutations were found to have worse survival compared to the wild-type group.[58-61] However, these studies had limitations such as small sample sizes, lack of follow-up data, and inconsistent treatments even within the same subtypes. This study, leveraging a large cohort from a single center, ensured consistent treatments according to tumor subtypes and stage, thereby minimizing bias from the data. In addition, by collecting data from patients who underwent *TP53* mutation testing between 2007 and 2015, we were able to secure comprehensive long-term follow-up data. Moreover, few studies have examined surgical outcomes based on location and type of *TP53* mutations in patients with confirmed *TP53* mutations, highlighting the significant of this study. As it has been revealed that mutations causing loss of DNA-binding can be critical to the biological activity of p53,[62] there is growing interest in the characteristics of *TP53* mutations. Although some studies have suggested that mutations situated in certain domains (especially in DBD) and type (such as truncating mutation) might be considered worse prognostic predictors in breast cancer compared to mutations in other regions,[63-66] other studies have reported neutral[55] or contrary results,[64,67] leading to ongoing controversy. These studies varied in their breast cancer subtypes, treatment protocols, and methods for detecting *TP53* mutations, making it challenging to interpret the results comprehensively. In our study, when performing subgroup analysis within the *TP53*-mutated group, there were no differences in the proportions of tumor subtypes and treatments between the two groups. This consistency minimizes the limitations caused by data inconsistencies that were present in previous studies.

Although our study allowed us to assess the clinical relevance of *TP53* mutations and their characteristics within a large cohort encompassing all tumor subtypes and treatments, it still had inherent limitations. The first limitation is the sensitivity of *TP53* mutations. In our study, we identified *TP53* mutations in exons 5-9 using PCR-DHPLC and direct sequencing., Despite most *TP53* mutations occur in exons 5-9, this approach might have missed mutations occurring outside these exons, incorrectly categorizing them as wild-type.[68] In addition, somatic mutations identified by PCR-DHPLC might not always be detectable by direct sequencing, because it has a threshold of detection of approximately 15 to 20%.[69,70] To overcome this limitation, next-generation sequencing (NGS) is now used for DNA sequencing in breast cancer.[71] However, since NGS was introduced at

our institution in 2017, it was not applied for the patients retrospectively collected for this study. Additionally, we were unable to fully control for confounding variables with relatively old data. Lastly, since the data was collected before 10 years ago, the treatment protocols at that time might differ significantly from those currently used in clinical practice. Most patients in this study did not receive neoadjuvant chemotherapy, and treatments such as CDK4/6 inhibitors, immune checkpoint inhibitors, and dual HER2 blockade including pertuzumab were rarely administered at that time. Nevertheless, based on this study, we expected that we could conduct further research addressing a prognostic influence on the characteristics of *TP*53 mutations in breast cancer patients with advanced research methods and molecular studies.

V. CONCLUSION

Using consistently collected long-term follow-up data, we found that *TP53* mutations are associated with worse prognosis in breast tumors including all subtypes and treatments. Additionally, within the *TP53*-mutated group, there were no differences in surgical outcomes based on the characteristics of *TP53* mutations such as mutation type and location. Based on our study, further research could be conducted to address a prognostic influence of the types of *TP53* mutations in patients with breast cancer.

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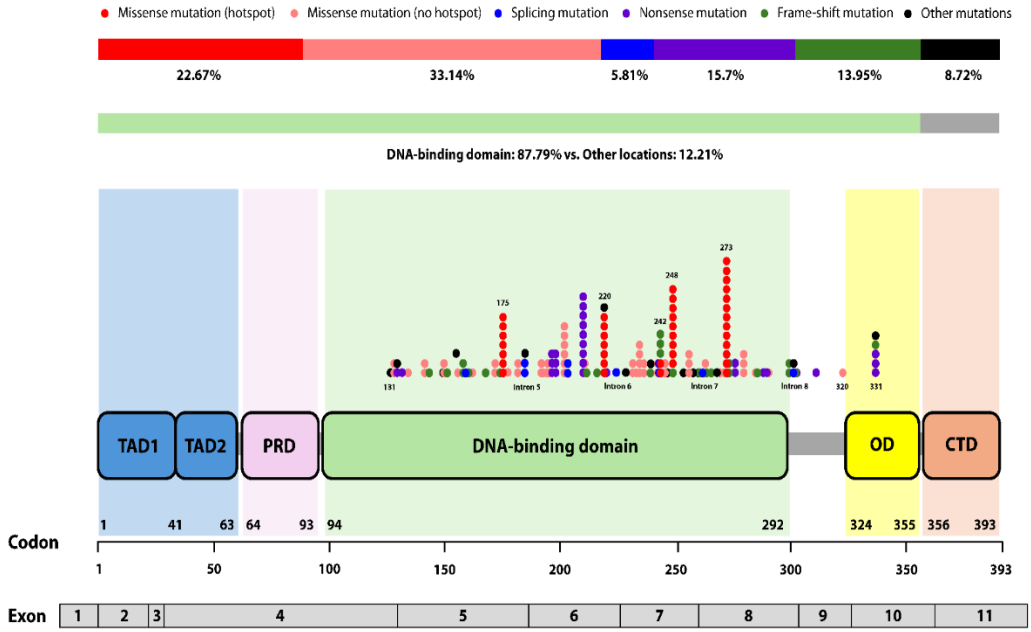
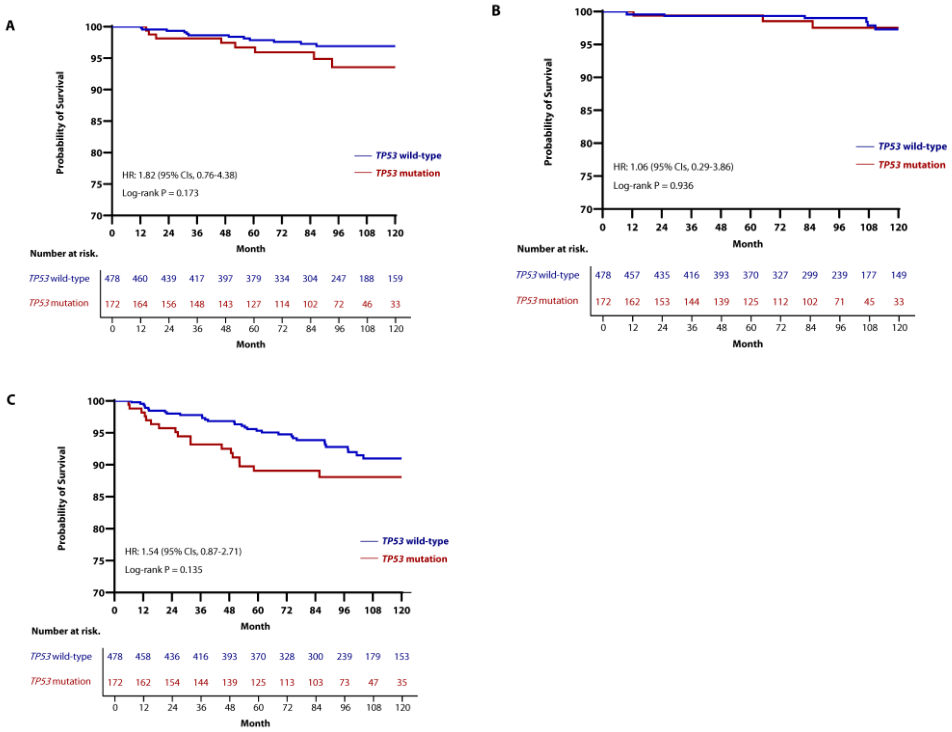
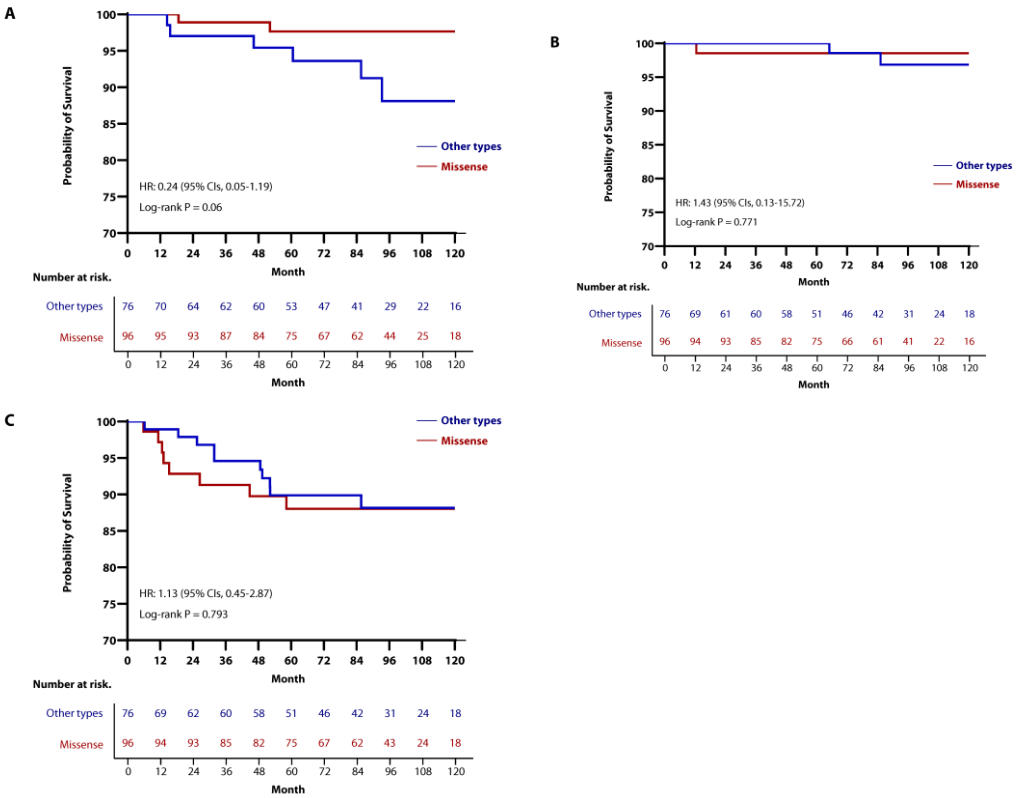


Abbreviations, TAD; transactivation domain, PRD; proline-rich domain, OD; oligomerization domain, CTD; carboxy-terminal domain

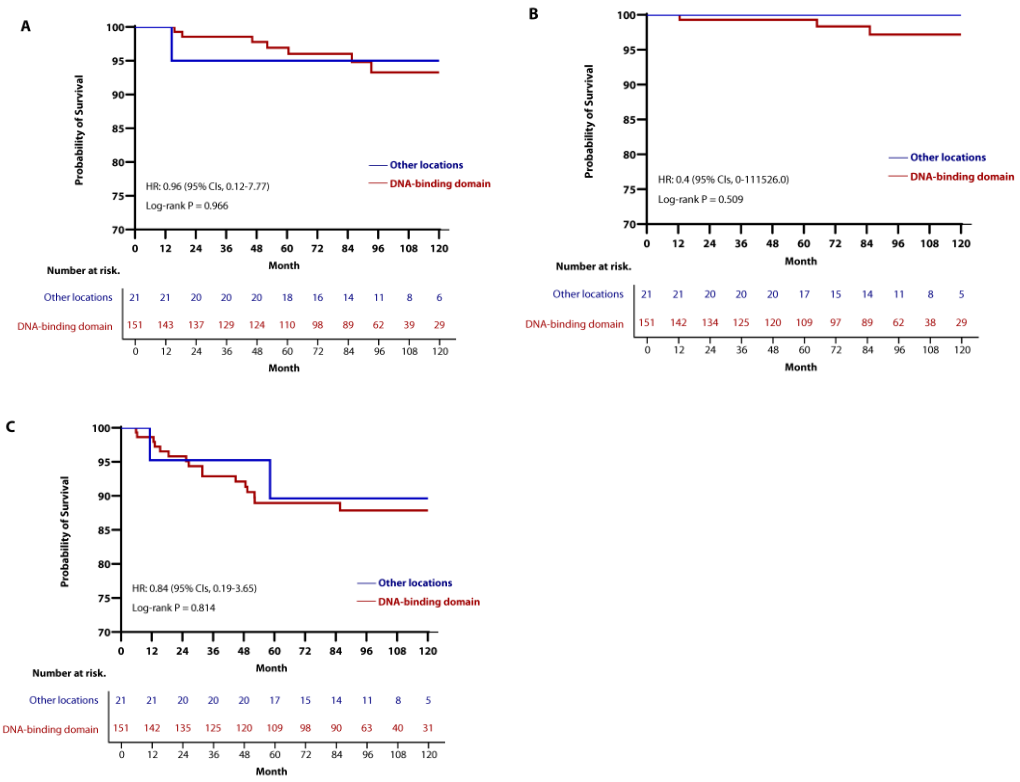
Supplementary Figure 1. Characteristics of *TP53* mutations in patients within the *TP53*-mutated group. More than half of the identified *TP53* mutations were missense mutations, with the majority occurring in the DNA-binding domain (DBD). Each circle represents a codon where a *TP53* mutation occurred, with mutation types distinguishing by color. The number of circles indicates the total number of mutations occurring within specific codons



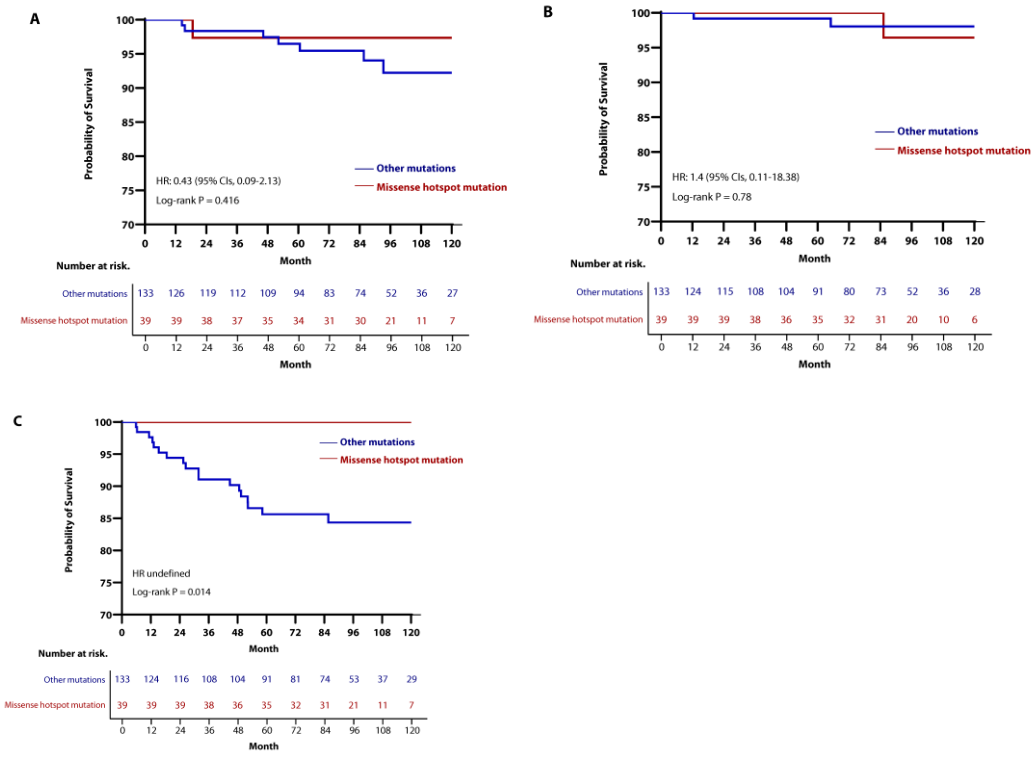
Supplementary Figure 2. Kaplan-Meier curve for (A) LRFS, (B) RRFS, and (C) DMFS in patients stratified by *TP53* mutation. Stratified log-rank test and Cox regression analysis presented that there were no significant differences between two groups. (The 5-year LRFS rate: 96.7% (95% CIs, 92.3-98.6) in *TP53* mutation group vs. 97.9% (95% CIs, 95.9-98.9) in *TP53* wild-type group, the 10-year LRFS rate: 93.6% (95% CIs, 87.2-96.8) in *TP53* mutation group vs. 96.9% (95% CIs, 94.6-98.3) in *TP53* wild-type group (HR, 1.82; 95% CIs, 0.76-4.38; $p = 0.173$)), (The 5-year RRFS rate: 99.4% (95% CIs, 95.7-99.9) in *TP53* mutation group vs. 99.3% (95% CIs, 97.9-99.8) in *TP53* wild-type group, the 10-year RRFS rate: 97.5% (95% CIs, 92.4-99.2) in *TP53* mutation group vs. 97.3% (95% CIs, 94.1-98.8) in *TP53* wild-type group (HR, 1.06; 95% CIs, 0.29-3.86; $p = 0.936$)), (The 5-year DMFS rate: 89.8% (95% CIs, 83.8-93.6) in *TP53* mutation group vs. 95.3% (95% CIs, 92.8-97.0) in *TP53* wild-type group, the 10-year DMFS rate: 88.1% (95% CIs, 91.7-92.4) in *TP53* mutation group vs. 91.0% (95% CIs, 87.3-93.6) in *TP53* wild-type group (HR, 1.54; 95% CIs, 0.87-2.71; $p = 0.135$))



Supplementary Figure 3. Kaplan-Meier curve for (A) LRFS, (B) RRFS, and (C) DMFS in patients with *TP53* mutation, stratified by types of mutation. Stratified log-rank test and Cox regression analysis presented that there were no significant differences between two groups. (The 5-year LRFS rate: 97.7% (95% CIs, 90.9-99.4) in missense mutation group vs. 95.4% (95% CIs, 86.5-98.5) in other mutations group, the 10-year LRFS rate: 97.7% (95% CIs, 90.9-99.4) in missense mutation group vs. 88.1% (95% CIs, 74.5-94.7) in other mutations group (HR, 0.24; 95% CIs, 0.05-1.19; $p = 0.06$)), (The 5-year RRFS rate: 98.5% (95% CIs, 90.0-99.8) in missense mutation group vs. 100% in other mutations group, the 10-year RRFS rate: 98.5% (95% CIs, 90.0-99.8) in missense mutation group vs. 96.9% (95% CIs, 88.0-99.2) in other mutations group (HR, 1.43; 95% CIs, 0.13-15.72; $p = 0.771$)), (The 5-year DMFS rate: 88.0% (95% CIs, 77.4-93.8) in missense mutation group vs. 89.9% (95% CIs, 81.4-94.6) in other mutations group, the 10-year DMFS rate: 88.0% (95% CIs, 77.4-93.8) in missense mutation group vs. 88.2% (95% CIs, 78.9-93.5) in other mutations group (HR, 1.13; 95% CIs, 0.45-2.87; $p = 0.793$)).



Supplementary Figure 4. Kaplan-Meier curve for (A) LRFS, (B) RRFS, and (C) DMFS in patients with *TP53* mutation, stratified by locations of mutation. Stratified log-rank test and Cox regression analysis presented that there were no significant differences between two groups. (The 5-year LRFS rate: 96.9% (95% CIs, 92.0-98.8) in DBD group vs. 95.0% (95% CIs, 69.5-99.3) in other locations group, the 10-year LRFS rate: 93.3% (95% CIs, 86.0-96.9) in DBD group vs. 95.0% (95% CIs, 69.5-99.3) in other locations group (HR, 0.96; 95% CIs, 0.12-7.77; $p = 0.966$)), (The 5-year RRFS rate: 99.3% (95% CIs, 95.1-99.9) in DBD group vs. 100% in other locations group, the 10-year RRFS rate: 97.2% (95% CIs, 91.4-99.1) in DBD group vs. 100% in other locations group (HR, 0.4; 95% CIs, 0-111526.0; $p = 0.509$)), (The 5-year DMFS rate: 89.0% (95% CIs, 82.3-93.2) in DBD group vs. 89.6% (95% CIs, 64.3-97.3) in other locations group, the 10-year DMFS rate: 87.9% (95% CIs, 80.8-92.4) in DBD group vs. 89.6% (95% CIs, 64.3-97.3) in other locations group (HR, 0.84; 95% CIs, 0.19-3.65; $p = 0.814$))



Supplementary Figure 5. Kaplan-Meier curve for (A) LRFS, (B) RRFS, and (C) DMFS in patients with *TP53* mutation, stratified by the presence or absence of missense hotspot mutation. Stratified log-rank test and Cox regression analysis presented that there were no significant differences between two groups. (The 5-year LRFS rate: 97.4% (95% CIs, 82.8-99.6) in missense hotspot mutation group vs. 96.5% (95% CIs, 90.9-98.7) in other mutations group, the 10-year LRFS rate: 97.4% (95% CIs, 82.8-99.6) in missense hotspot mutation group vs. 92.2% (95% CIs, 83.8-96.4) in other mutations group (HR, 0.43; 95% CIs, 0.09-2.13; $p = 0.416$)), (The 5-year RRFS rate: 100% in missense hotspot mutation group vs. 99.2 (95% CIs, 94.4-99.9) in other mutations group, the 10-year RRFS rate: 96.4% (95% CIs, 77.2-99.5) in missense hotspot mutation group vs. 98.0% (95% CIs, 92.3-99.5) in other mutations group (HR, 1.40; 95% CIs, 0.11-18.38; $p = 0.78$)), (The 5-year DMFS rate: 100% in missense hotspot mutation group vs. 85.6% (95% CIs, 77.9-90.8) in other mutations group, the 10-year DMFS rate: 100% in missense hotspot mutation group vs. 84.4% (95% CIs, 76.2-89.9) in other mutations group (undefined HR))

Abstract in Korean

유방암에 있어서 *TP53* 돌연변이의 임상적 관련성과 특징들에 대한 장기간 추적 자료 분석

TP53 돌연변이는 인간에게 발생하는 암들 중에서 가장 흔하게 발견되는 대표적인 유전자 돌연변이들 중 하나이다. 일반적으로 *TP53* 돌연변이가 있으면 예후가 불량하다고 알려져 있으나 일부의 경우에는 약간 다른 결론을 보여주었다. 그러나, 이들 연구는 대부분 작은 환자 대상군들, 일부 하위 형태의 유방암과 치료법 및 다양한 *TP53* 돌연변이 검사법을 사용하였다. 그러므로 우리는 모든 하위 형태와 통일된 치료법 및 검사법들을 포함하여 650명의 대규모 유방암 환자에 있어서 *TP53* 돌연변이의 임상적 관련성에 대한 확인을 하고자 하였다. 후향적으로 자료를 수집하여 분석하였고, 기존 연구들과는 다르게 *TP53* 돌연변이의 '타입과 위치'에 따른 임상적 차이를 비교 분석하고자 하였다. 650명 유방암 환자들 중 *TP53* 돌연변이가 관찰된 환자들은 172명(26.5%)였고, 이들 중 34명(19.8%)가 과오 호발부위 돌연변이(missense hotspot mutation)였다. *TP53* 돌연변이를 가진 환자들은 10년 무재발 생존율(83.5% vs. 86.6%, HR, 1.67; 95% CIs, 1.06−2.64; p = 0.026)과 10년 전체 생존율((88.1% vs. 91.0%, HR, 3.02; 95% CIs, 1.43−6.70, p = 0.003) 모두 각각 안 좋은 예후를 보였다. 하지만, *TP53* 돌연변이군 내에서의 돌연변이의 타입과 위치에 따른 하위 형태 분석에 있어서는 대부분 유의한 차이를 보이지는 않았다. 향후 과제로는 전향적 연구, 차세대염기서열분석(NGS; Next-Generation Sequencing)를 사용한 돌연변이 분석, 퍼제타 등 최신의 치료제 사용이 포함된 치료법 등을 통해서 좀 더 정밀한 연구가 필요하겠다.

핵심되는 말 : *TP*53 돌연변이, 과오 호발부위 돌연변이, 무재발 생존율, 전체 생존율