

Type of TP53 mutation and ERBB2 amplification affects survival in node-negative breast cancer

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Abstract Alterations of TP53 and ERBB2 have been shown to play important roles in the prognosis of breast cancer. The primary objective of this study is to characterize TP53 mutation types in node negative breast cancer and investigate their prognostic value, alone and in combination with ERBB2 amplification status. TP53 mutational status (exons 2–10) and ERBB2 amplification status were determined in tumor specimens from a prospective cohort of 543 women with node-negative breast cancer. During a median follow-up of 120 months, there were 111 disease recurrences, and 81 disease-related deaths (3 with cancer; 78 from cancer). Of 543 women, 133 (24.5%)

carried mutations in exons 4–9 of the TP53 gene. Seventy-one (53.4%) of these mutations were missense; whereas 62 (46.6%) were protein-truncating mutations. Women whose tumors had missense TP53 mutations were found to be at significantly higher risk of recurrence and death compared to those with wild type TP53, and they also tended to have worse prognosis compared to those with truncating mutations. Those with short truncated proteins tended to have good prognosis compared to those with long truncated proteins, but the risk of recurrence and death did not differ between those whose tumors exhibited conserved versus non-conserved mutations. Missense mutations, in combination with ERBB2 amplification, were observed in 4.6% of the tumors and dramatically affected the disease-specific survival (DSS) and disease-free survival (DFS) of the breast cancer patients. Our study suggests that the type of TP53 mutation, especially missense mutation, is a strong prognostic indicator for DFS and DSS in node-negative breast cancer, particularly in combination with ERBB2 amplification.

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Introduction

TP53 consists of three major functional domains; the NH2 terminus (amino acids 1–95) involved in the trans-activation, the central region (amino acids 102–292) involved in DNA binding and the COOH terminus (amino acids 300–393) engaged in oligomerization

and DNA damage recognition. Four of the five evolutionary conserved segments of TP53 lie in the central region of the protein [1, 2]. Mutations in the form of single base substitutions and small insertions and deletions have been shown to be the major mechanism of TP53 inactivation [3]. Mutations of TP53 can be categorized into two major classes as missense and protein truncating mutations. Missense mutations are known to be associated with increased stability of the TP53 protein which can be detected by immunohistochemical (IHC) analysis, where missense mutations result in significantly higher tumor tissue staining compared to tumors with protein truncating mutations and wild type [4]. TP53 proteins bearing missense mutations can have a dominant-negative effect with gain-of function properties [2, 5]. In contrast, tumor tissues with protein truncating mutations generally do not stain positively by IHC analysis, similar to tumors without TP53 mutations, suggesting lack of increased stability of the resulting truncated protein. Whether these truncated TP53 products are degraded immediately, or not, is not well understood.

Both TP53 and ERBB2 have been shown to play important roles in the molecular pathways controlling cell growth and death [6, 7]. Genetic alterations of TP53 and ERBB2 are among the most common abnormalities in breast tumors, and they have been extensively studied as prognostic indicators for breast cancer. Over-expression of ERBB2 and expression of mutant TP53 proteins (mutations in TP53) have been shown to have prognostic value individually; however the prognostic significance of their co-existence in the same tumor is still controversial [8–17].

In a 2004 report of disease-free and overall survival in a cohort of axillary node negative (ANN) patients [18], a significant association of TP53 mutation in univariate analysis was substantially accounted for by known prognostic factors (including ER status, tumor size and grade). In addition, however, the combination of TP53 mutation and ERBB2 amplification was determined to be a significant and independent prognostic factor for DFS in both univariate and multivariate analysis. Our purpose in this report is to further explain the previous TP53 mutation findings, as a main effect and as an interaction with ERBB2 amplification, in terms of mutation types. The primary objective of this study was to assess the association of TP53 missense and truncation mutations with risk of disease recurrence and disease-specific death in node-negative breast cancer patients. Secondary analyses also evaluated the prognostic value of short versus long truncation mutations and conserved versus non-conserved missense mutations, and assessed the consistency of

associations with mutation type across adjuvant treatment subgroups.

Materials and methods

Study population

In our prospective study, a consecutive series of women with node-negative breast cancer was enrolled from 8 Toronto hospitals from September 1987 until March 1993 as previously described [19]. We have continued to follow up all eligible women for recurrence and death. Characteristics of the patients are listed in Table 1. Disease-free survival (DFS) was taken as the time between diagnosis and the confirmation of non-breast recurrence. All patients were monitored for death whether or not they experienced disease recurrence. Disease-specific survival (DSS) was taken as the time between diagnosis and death from or with disease, regardless of recurrence events. Patient status on January 10, 2002 determined both DFS and DSS survival times and censoring status using clinical follow-up data. Follow-up data were monitored for an additional 6 months (up to July 10, 2002) to confirm patient status at the termination date. We observed 111 disease recurrences, 81 deaths (3 with cancer; 78 from cancer) and a median follow-up of 120 months among these patients. There were 51 deaths from other causes and these were treated as censored in DSS. Further details about patient eligibility, clinical follow-up, and determination of survival times can be found in our recent report [18]. Written consent was obtained from all the patients included in this study.

Molecular analysis

Mutational analysis of exons 2–10 of the TP53 gene was carried out using single stranded conformation polymorphism (SSCP) and direct sequencing analysis. The amplicons studied also included intronic sequences in order to capture the splice site variants along with other type of mutations. The details of molecular analysis of TP53 and ERBB2 have been described previously [18–22].

Statistical analysis

Survival analytic techniques [23] were used to examine the long-term and short-term survival experiences among subgroups defined by type and location of TP53 mutation. In this study, our primary interest was to assess the biological effects of the types of TP53

Table 1 Patient and molecular marker characteristics according to TP53 mutation types

Characteristic	Mutation type									
	Wild		Truncation				Missense			
			Short		Long		Conserved		Non-conserved	
	<i>N</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
No. of patients, <i>n</i> = 543	410	75.5	14	2.6	48	8.8	30	5.5	41	7.6
No. of relapses, <i>n</i> = 111	79	71.2	0	0.0	10	9.0	8	7.2	14	12.6
No. of deaths, <i>n</i> = 132	89	67.4	2	1.5	14	10.6	10	7.6	17	12.9
From disease, <i>n</i> = 78	47	60.3	0	0.0	10	12.8	8	10.2	13	16.7
With disease, <i>n</i> = 3	3	100.0	0	0.0	0	0.0	0	0.0	0	0.0
Without Disease, <i>n</i> = 51	39	76.6	2	3.9	4	7.8	2	3.9	4	7.8
ERBB2 status										
Amplified, <i>n</i> = 108	66	61.1	6	5.6	11	10.2	13	12.0	12	11.1
Not amplified, <i>n</i> = 435	344	79.1	8	1.8	37	8.5	17	3.9	29	6.7
Adjuvant therapy received										
Untreated, <i>n</i> = 258	193	74.8	8	3.1	25	9.7	11	4.3	21	8.1
Hormone therapy only, <i>n</i> = 212	174	82.1	2	0.9	14	6.6	12	5.7	10	4.7
Any chemotherapy, <i>n</i> = 73	43	58.9	4	5.5	9	12.3	7	9.6	10	13.7

mutations and their joint associations with ERBB2 amplification. Therefore, our survival analyses examined mutation type alone and in combination with the amplification variable, without considering other known prognostic variables. The associations of TP53 mutation type with the risk of recurrence and death in node-negative breast cancer were examined in four different models. Model 1 compared missense mutation, protein-truncating mutation, and wild type. In Model 2, protein-truncating mutations were dichotomized into short protein-truncating type (amino acids less than or equal to 95), and long protein-truncating type (within amino acids 95–393), and compared with wild type. Model 3 similarly compared missense mutations with wild type according to location, conserved or non-conserved. In a fourth Model, the TP53 mutation categories (missense, truncation, and wild type) were evaluated in combination with ERBB2 status (amplified, not amplified) yielding six subgroups for comparison.

For each of the comparisons of interest, Kaplan–Meier (K–M) estimates of survival were calculated for DFS and DSS and plotted as a function of time to yield graphs of survival experience for the different subgroups, using R statistical software version 1.9.1 (<http://www.r-project.org/>). The differences in survival curves were evaluated by exact log-rank tests [24] as implemented in StatXact statistical software version 5.0.3 (Cytel Software Corporation, Cambridge, MA, USA). Univariate Cox proportional hazards models [25] were fitted separately for DFS and DSS; the relative survival between subgroups was summarized by the relative risk (RR) as estimated by the hazard ratio in the Cox model. Cox modeling was performed using SAS

statistical software, PROC PHREG, version 8.2 (SAS Inc., Cary, NC, USA).

Based on previous findings that the risk of recurrence depended on duration of follow-up [18], analyses for short-term effects were conducted by censoring follow-up at 36 months for DFS analysis and at 60 months for DSS analysis. In our 2004 DFS analysis, we had defined early recurrences to be those occurring in the first 36 months post-diagnosis, and found a significant decrease in the relative risk of recurrence for TP53 mutation and for ERBB2 amplification after this cut-off; in the present analysis 36 months corresponded to the median time to disease recurrence. For DSS, we defined early disease-specific mortality to be events occurring within 5 years of diagnosis, which is a conventional reporting period for overall survival. Formal tests for suspected time-dependent hazard ratios were conducted by adding an indicator term for interaction with follow-up time [26].

To reduce concerns arising from consideration of multiple hypotheses, we reduced the potential number of tests conducted by carrying out significance testing in a hierarchical fashion, as follows. Given that the previous study analysis had detected significant TP53 mutation associations [18], we first classified mutations according to truncation or missense type and tested for an overall association of TP53 mutations with DFS and DSS and for differences between truncation and missense mutation groups (model 1). For overall comparisons among subgroups, $p < 0.05$ was considered to be statistically significant. We did not consider short-term effects to be differential unless a test for time dependence was statistically significant ($p < 0.05$). Then, given that significant associations were detected

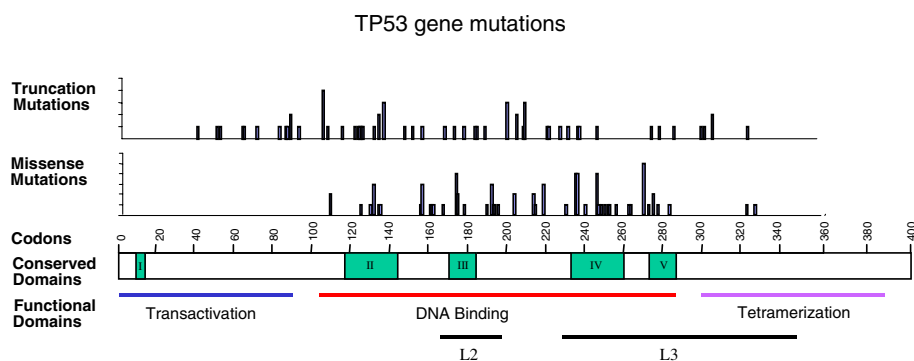
for truncation or missense mutations alone, we further examined each of these by subtype (models 2 and 3), similarly requiring significant heterogeneity tests for conclusions of differential associations with outcome. Similar analyses by mutation type were conducted to determine whether the synergy of TP53 mutation and ERBB2 amplification could be further explained by mutation type (model 4).

To assess whether the mutation group differences detected in Model 1 for the entire group persisted independently of any adjuvant treatment received, subgroup analyses were conducted separately for those treated and untreated, and for those who received any chemotherapy versus hormone therapy alone. Overall likelihood ratio (LR) tests for variation by treatment received were implemented by joint tests of interactions for mutation-type by treatment-subgroup terms. Given no prior evidence in our dataset for differences in ERBB2 association by treatment subgroup, we did not consider examination of 3-way interactions of mutation type by ERBB2 amplification by treatment to be well justified, especially with the small size of the resulting subgroups.

Results

We carried out somatic mutational analysis of exons 2–10 of TP53 in 543 women with node-negative breast cancer. Of 543 women, 133 (24.5%) carried mutations in exons 4–9; 71 (53.4%) of these mutations were missense whereas 62 (46.6%) were protein-truncating mutations. Among 71 missense mutations, 30 (42.3%) were located outside (non-conserved) and 41 (57.7%) were located inside (conserved) the five evolutionary conserved segments of TP53. The distribution of missense and protein-truncating mutations along the coding region of TP53 are presented in Fig. 1. A *supplementary table* for the type of TP53 mutations is presented online (www.ozceliklab.com/TP53 table).

Fig. 1 Distribution of missense and nonsense mutations along the coding region of TP53



We also studied the relationship between TP53 mutation type (missense vs. truncation) and ERBB2 status. ERBB2 was amplified in 19.9% of the specimens in this study. TP53 mutation types (short truncation, long truncation, non-conserved, and conserved) were more frequent in the specimens with ERBB2 amplification than those without as shown in Table 1 ($p = 0.0003$). Although tumors with TP53 mutations were larger, of higher grade, were less likely to be hormone receptor positive compared to wild type, there were no differences between tumors with truncation versus missense mutations for these factors (data not shown). Compared to wild type, tumors with TP53 mutations were also less likely to have received adjuvant hormone therapy (Table 1).

The overall log-rank tests for survival rate differences among the three mutation groups: missense, truncating, and wild type (Model 1), were statistically significant for both DFS (Table 2, Fig. 2a), and DSS (Table 3, Fig. 2b), with particularly strong results for the latter. Comparisons among mutation groups within adjuvant treated and untreated subgroups, as well as within hormone only and chemotherapy treatment subgroups, did not detect significant variation in the mutation group differences among these subgroups (Supplementary Table 2). While the overall LR tests for subgroup variation were non-significant for both DFS and DSS, subgroup analysis including women who received hormone therapy (without any chemotherapy) indicated most clearly that women carrying a missense mutation had worse DFS and DSS compared to wild type (RR = 3.05 and 4.72 respectively, Supplementary Table 2).

Further pair-wise analyses of the mutation groups in Model 1 found that the survival of the missense group was significantly worse than the wild type group for both long- and short-term follow-up. The relative risks were significantly higher in the short-term, compared to full follow-up, decreasing from 3.35 to 1.86 for DFS (Table 2) and from 4.52 to 2.81 for DSS (Table 3). In the comparisons of the truncation group to the wild

Table 2 Results of DFS analysis by log-rank and univariate Cox proportional hazards models ($n = 543$ with 111 recurrences) for the entire follow-up period (median time, 120 months) and short follow-up period (36 months)

Comparison	Full follow-up (median of 120 months)		Short follow-up (36 months)		Time-dependence <i>p</i> -value (asymptotic)
	<i>p</i> -value (exact)	RR (CI) (asymptotic)	<i>p</i> -value (exact)	RR (CI) (asymptotic)	
<i>Model 1</i>					
Missense vs. Truncation vs. Wild type ($n = 543$, 2 df)	0.03		0.005		
Missense vs. Wild	0.01	1.86 (1.16–2.98)	0.002	3.35 (1.82–6.16)	0.01
Truncation vs. Wild	0.70	0.88 (0.45–1.69)	0.15	2.14 (1.01–4.51)	0.01
Missense vs. Truncation	0.06	2.11 (1.00–4.47)	0.32	1.57 (0.69–3.55)	0.23
<i>Model 2</i>					
Long vs. Short trunc vs. Wild type ($n = 472$, 2 df)	0.14		0.03		
Long Trunc vs. Wild	0.61	1.20 (0.62–2.31)	0.02	2.84 (1.35–6.00)	0.01
Short Trunc vs. Wild	0.05	NA	0.38	NA	NA
Long vs. Short Trunc	0.07	NA	0.10	NA	NA
<i>Model 3</i>					
Conserved vs. Non-conserved vs. Wild type ($n = 481$, 2 df)	0.04		0.007		
Conserv vs. Wild	0.01	2.02 (1.14–3.56)	0.04	3.15 (1.49–6.66)	0.14
Non-conserved vs. Wild	0.22	1.63 (0.79–3.37)	0.008	3.64 (1.59–8.30)	0.03
Conserv vs. Non-conserved	0.64	1.24 (0.52–2.95)	0.58	0.87 (0.32–2.33)	0.23
<i>Model 4</i>					
Missense vs. Trunc vs. Wild by Amplification ($n = 543$, 5 df)	0.002		0.001		
Amp/Missense vs. Amp/Wild	0.0004	4.72 (2.07–10.8)	0.03	4.57 (1.62–12.8)	NA
Amp/Trunc vs. Amp/Wild	0.31	1.76 (0.55–5.60)	0.22	2.90 (0.82–10.3)	NA
Amp/Missense vs. Amp/Trunc	0.10	2.69 (0.88–8.25)	0.60	1.58 (0.49–5.12)	NA
Amp/Missense vs. No Amp/Wild	0.0002	3.69 (2.04–6.67)	0.0002	6.52 (3.02–14.1)	0.07
Amp/Trunc vs. No Amp/Wild	0.55	1.37 (0.50–3.77)	0.001	4.14 (1.43–11.9)	NA

Exact *p*-values were obtained from log-rank tests. RR and CI were obtained from the Cox proportional hazards model. Time dependence *p*-values in the Cox model were used to assess whether the RR in the first 36 months was different from RR after 36 months

NA (Not Available) asymptotic *p*-values and CIs are due to no events in one of the comparison groups

type group, the patterns of non-proportional hazards were extreme (Fig. 2a, b) with the relative risks decreasing from 2.14 to 0.88 for DFS (Table 2) and from 3.65 to 1.43 for DSS (Table 3). For the short-term follow-up, the truncation and missense groups had very similar survival for both DFS and DSS, but after the initial periods, the cumulative survival rate continued to decrease for the missense group, whereas for the truncation group it leveled off (Fig. 2a, b). As a result, in the full follow-up analysis the truncation group tended to have better DFS and DSS than the missense group with no significant differences between the truncation and the wild type groups (Tables 2 and 3).

Examination of the truncation group according to short versus long truncations provided some further insight into the time-dependent trends (Model 2, Fig. 3a, b). In the longer-term, the short truncation group had better DFS and DSS than the wild type group, while the long truncation group did worse than

the wild type group in the short-term. This yielded weak but consistent evidence from the exact log-rank tests of differences between the long and short truncation groups (Tables 2 and 3). Thus, we found statistically significant relative risks of disease recurrence and disease-specific death associated with the presence of a long truncation type compared to wild type, particularly in the early follow-up periods.

The overall log-rank tests for survival rate differences among the three groups: conserved, non-conserved, and wild type (Model 3), were statistically significant for both DFS (Table 2, Fig. 4a), and DSS (Table 3, Fig. 4b). Further pair-wise analyses of groups in this model found that the survival rates of the non-conserved and conserved groups did not differ significantly for either long or short-term follow-up. The significance of the overall log-rank test thus reflects the combined association of the missense mutations.

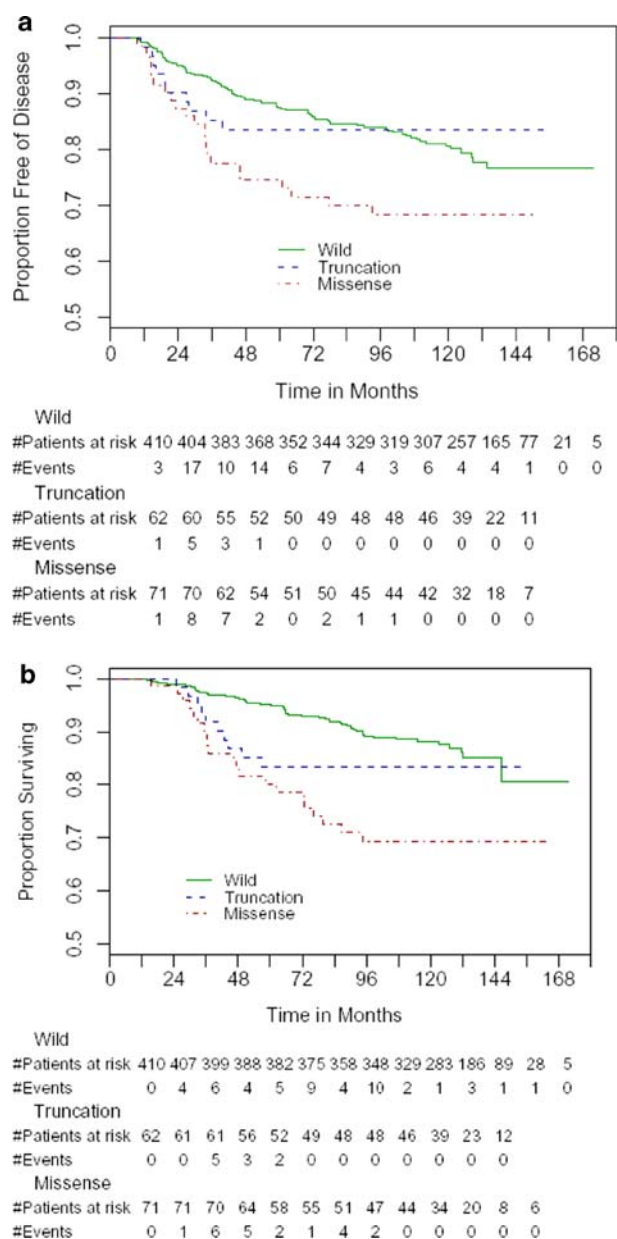


Fig. 2 Model 1: Kaplan–Meier DFS (**a**) and DSS (**b**) curves stratified by type of TP53 mutation (missense, truncating, and wild type)

Kaplan–Meier curves for subgroups corresponding to the combination of TP53 mutation type and ERBB2 amplification are shown in Fig. 5. The overall log-rank test for survival rate differences among the subgroups (Model 4) was highly statistically significant for both DFS (Table 2, Fig. 5a) and DSS (Table 3, Fig. 5b). Pair-wise analysis of groups in this model found that the survival of the women with missense TP53 and ERBB2 amplification was significantly worse than the wild type group for both long- and short-term follow-up. The relative risks

tended to be higher in the short-term, compared to full follow-up, decreasing from 6.52 to 3.69 for DFS ($p = 0.07$, Table 2) and from 10.52 to 4.82 for DSS ($p = 0.02$, Table 3). In the short-term follow-up period, women with truncation TP53 mutations and ERBB2 amplification had relatively worse survival compared to those with wild type TP53 and no ERBB2 amplification for both DFS and DSS, with relative risks of 4.14 and 6.70, respectively. Even taking into account that 10 pair-wise tests were conducted for each of DFS and DSS (5 for full follow-up and 5 for short-term follow-up), a crude Bonferroni adjustment nevertheless yields a significant p -value at the 5% level.

Discussion

Survival comparisons by TP53 mutation type

We found that 24.5% of women with node-negative breast cancer in our cohort carried TP53 mutations. The higher fraction of protein truncating mutations (46.6%) detected in our cohort differs from the majority of the studies focusing on exons 5–8 of the TP53 gene [3, 27]. The fraction of protein truncating mutations outside the predominantly studied region (exons 5–8) was 25%, whereas the fraction of missense mutations was 5%. Thus, 30% of TP53 mutations would have been missed in studies focusing only to exons 5–8 of the TP53 gene. Other studies have also shown that a considerable fraction of protein truncating mutations occurs outside the evolutionary conserved region of TP53 [3, 28].

Several studies including ours have shown that breast cancer patients whose tumors have TP53 mutations have a worse DFS and DSS than those whose tumors do not contain mutations [18, 29, 30]. In this study, long-term follow-up (120 months) has shown that women carrying missense TP53 mutations, compared to women with wild type TP53, tended to have worse DFS and DSS. Women carrying truncating mutations, compared to woman with wild type TP53, did not have a statistically significant difference in DFS and DSS when the entire follow-up period was examined. A comparison of IHC and mutation type in a subset of the sample ($n = 227$) indicated that missense mutations were predominantly IHC positive (92.9%), and truncation mutations were predominantly IHC negative (88.5%) (unpublished data). We found no evidence that the nature of the associations with mutation type varied by adjuvant treatment received, and in the hormone-

Table 3 Results of DSS analysis by log-rank and univariate Cox proportional hazards models ($N = 543$ with 81 deaths—3 with cancer, and 78 from cancer) for the entire follow-up period (median time, 120 months) and short follow-up period (60 months)

Comparison	Full follow-up (median of 120 months)		Short follow-up (60 months)		Time-dependence
	<i>p</i> -value (exact)	RR (CI) (asymptotic)	<i>p</i> -value (exact)	RR (CI) (asymptotic)	
<i>Model 1</i>					
Missense vs. Truncation vs. Wild type ($n = 543$, 2 df)	0.0006		0.0005		
Missense vs. Wild	0.0001	2.81 (1.68–4.68)	0.001	4.52 (2.27–9.02)	0.07
Truncation vs. Wild	0.31	1.43 (0.73–2.83)	0.005	3.65 (1.70–7.85)	NA
Missense vs. Truncation	0.09	1.96 (0.92–4.16)	0.79	1.24 (0.55–2.79)	NA
<i>Model 2</i>					
Long vs. Short Trunc vs. Wild type ($n = 472$, 2 df)	0.05		0.001		
Long Trunc vs. Wild	0.06	1.97 (0.99–3.88)	0.0005	4.86 (2.26–10.5)	NA
Short Trunc vs. Wild	0.11	NA	0.39	NA	NA
Long Trunc vs. Short	0.07	NA	0.05	NA	NA
<i>Model 3</i>					
Conserv. vs. Non-conserved vs. Wild type ($n = 481$, 2 df)	0.0006		0.005		
Conserved vs. Wild	0.0007	3.01 (1.63–5.54)	0.01	3.82 (1.61–9.10)	0.52
Non-conserved vs. Wild	0.01	2.53 (1.20–5.35)	0.01	5.53 (2.33–13.2)	0.03
Conserv vs. Non-conserved	0.75	1.19 (0.49–2.86)	0.79	0.69 (0.24–1.97)	0.11
<i>Model 4</i>					
Missense vs. Trunc vs. Wild by amplification $n = 543$, 5 df)	0.0004		0.0002		
Amp/Missense vs. Amp/Wild	0.0006	5.43 (2.10–14.0)	0.03	5.29 (1.77–15.8)	0.84
Amp/Trunc vs. Amp/Wild	0.15	2.51 (0.74–8.59)	0.13	3.37 (0.91–12.6)	NA
Amp/Missense vs. Amp/Trunc	0.26	2.16 (0.69–6.80)	0.67	1.57 (0.48–5.09)	NA
Amp/Missense vs. No Amp/Wild	0.0001	4.82 (2.48–9.35)	0.0001	10.50 (4.54–24.3)	0.02
Amp/Trunc vs. No Amp/Wild	0.15	2.23 (0.80–6.21)	0.005	6.70 (2.21–20.4)	NA

Exact *p*-values were obtained from log-rank tests. RR and CI were obtained from the Cox proportional hazards model. Time dependence *p*-values in the Cox model were used to assess whether RR in the first 60 months was different from RR after 60 months. NA (Not Available) asymptotic *p*-values and CIs are due to no events in one of the comparison groups.

treated subgroup ($n = 212$) in particular, women carrying missense mutations had significantly worse DFS and DSS compared to wild type. These results may indicate that missense mutations are overall more deleterious than the truncating mutations with regard to the DFS and DSS of the node-negative breast cancer patients. The more deleterious phenotype associated with missense TP53 mutations may be due to the dominant-negative effect and gain-of-function associated with such mutations [2, 5].

We also investigated whether the size of the truncated protein has a role in the DFS and DSS of the patients. There were no recurrences in the small subgroup of women carrying short proteins (less than 92 amino acids). Remarkably, the survival of these women tended to be better than those with wild type TP53, as well as those with protein truncating mutations outside this region (within amino acids 93–393). Shorter proteins are unstable and more likely to be degraded; however, longer proteins containing partial or complete sequences of DNA binding and

tetramerization domains may be more stable. Thus, longer truncated proteins may exert gain-of-function properties similar to missense mutations. This observation needs to be studied further and replicated with a larger sample.

We also studied the effect of sub-classes of missense mutations (conserved and non-conserved). Analysis of patients carrying missense mutations located in and outside the evolutionary conserved regions of TP53 detected no statistically significant differences in terms of DFS or DSS. These results suggest that conservation status of missense mutations does not influence the DFS and DSS of node-negative patients.

Time-effect and survival comparisons by TP53 mutation type

In order to better understand changes in the relationship of TP53 mutations to survival over prolonged follow-up, we considered shorter follow up-periods

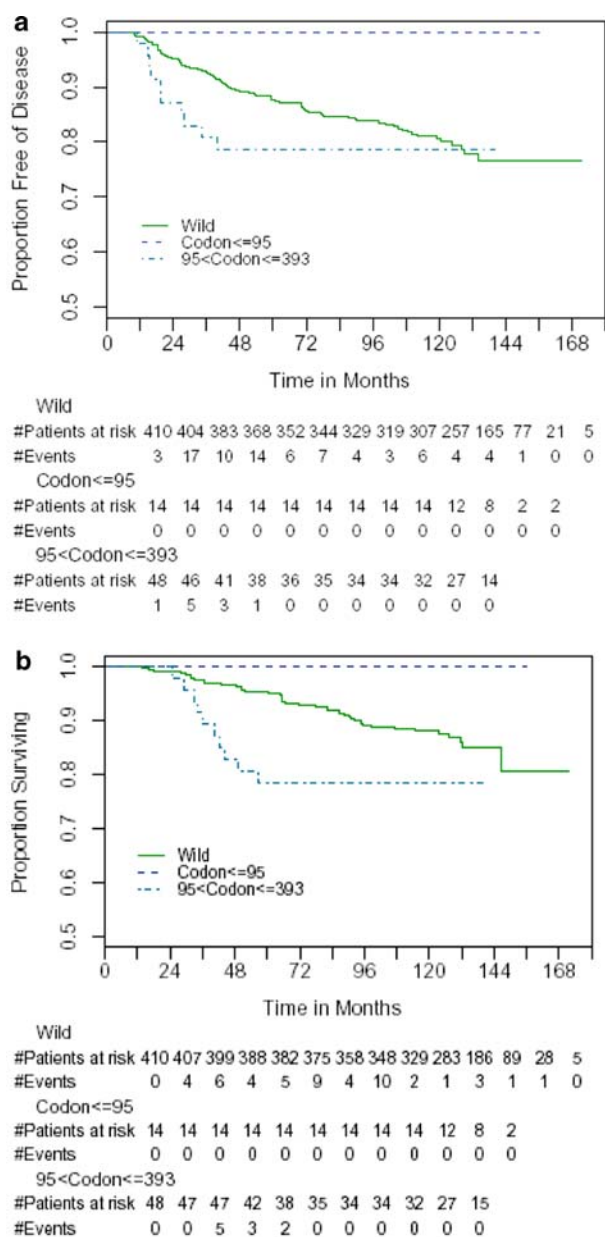


Fig. 3 Model 2: Kaplan–Meier DFS (**a**) and DSS (**b**) curves stratified by type of TP53 mutation on the basis of their type and subclasses of protein truncations by the size of the resulting protein

(36 months for DFS and 60 months for DSS) in our analysis. As discussed above, protein truncation mutations did not appear to be associated with DFS and DSS in long-term follow up (median 120 months), but did in short-term follow-up, particularly for DSS. These results may indicate functional differences among the truncating mutations, some of which may have a magnitude of association similar to that of missense mutations. Interestingly, compared to wild type, we found longer truncated proteins (between the

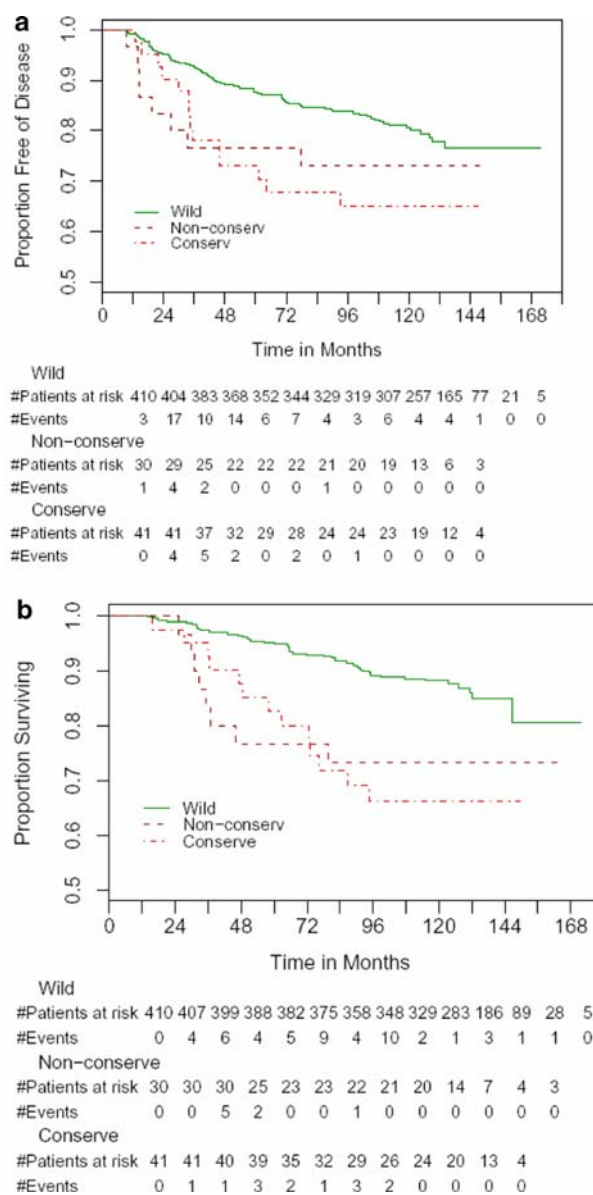


Fig. 4 Model 3: Kaplan–Meier DFS (**a**) and DSS (**b**) curves stratified by type of TP53 mutation on the basis of their type and location within the evolutionary conserved sequences

amino acids of 95–393) to be associated with DFS of breast cancer patients within 36 months of diagnosis, whereas this was not statistically significant for the long-term follow up. As indicated above, the potential gain-of-function properties of long truncated proteins may be responsible for these differences.

Several studies in the literature have also reported on the relationship between TP53 mutations and the DFS and DSS of breast cancer patients. However; unlike ours, none of these studies investigated the effect of missense versus truncating mutations [31–33]. Their mutation groups contained both missense and

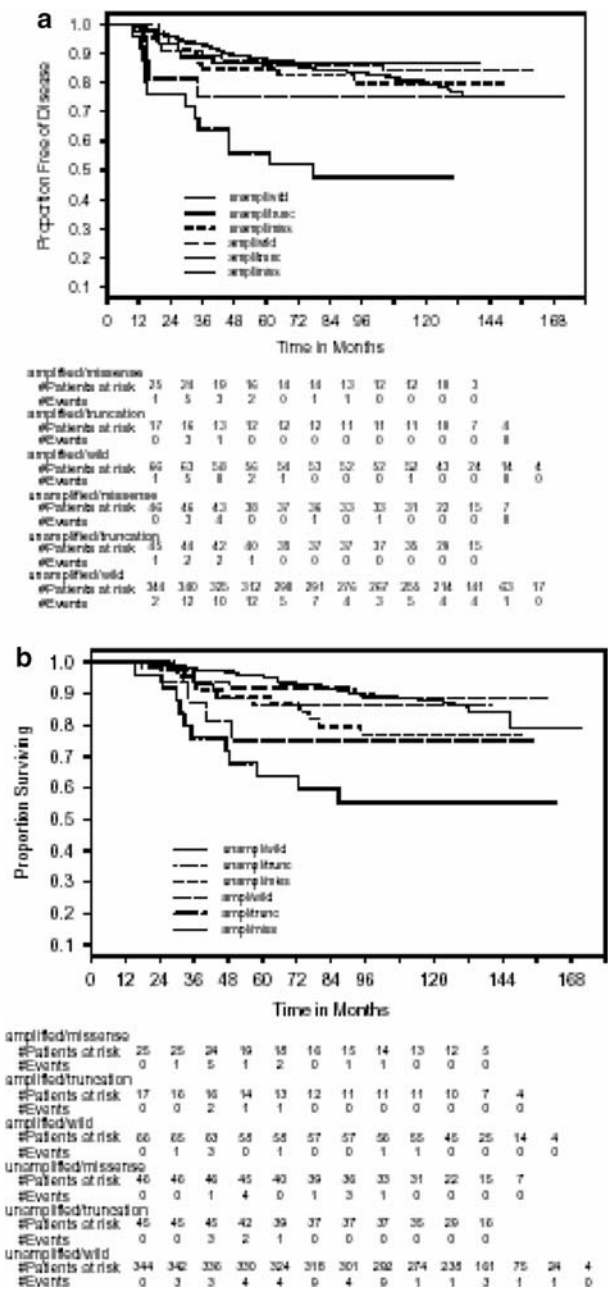


Fig. 5 Model 4: Kaplan–Meier DFS (a) and DSS (b) curves stratified by type of TP53 mutation and ERBB2 status

truncating mutations, although the ratio of missense to truncation mutations was higher compared to ours. Within 115 months follow-up (which is comparable to 120 months of follow-up period in our study), Berns et al. [31] showed that mutations, independent of their conservation status, are associated with DFS of breast cancer patients. This is similar to our finding although only 36% of the patients in their study were node-negative. Saitoh et al. [34] also investigated the effect of missense versus truncating mutations for a follow-up

period of 19 months. Thus, with a similar follow-up period to ours of 36 months, they found the DFS of patients with missense and truncating mutations to be similar and statistically different than the wild type.

Survival comparisons by TP53 mutation type and ERBB2 amplification status

We recently reported that TP53 mutation was not an independent prognostic factor in node-negative breast cancer, except in the presence of ERBB-2 amplification [18]. In the present study we have evaluated the joint prognostic value of various types of TP53 mutation in combination with ERBB2 amplification for DSS and DFS in node-negative women. In both short- and long-term follow-up, we found that women bearing the combination of missense TP53 mutations and ERBB2 amplification had significantly worse DFS and DSS than women with wild type TP53 with or without ERBB2 amplification. Compared to women with wild type TP53 and no amplification, the combination of truncating mutations and ERBB2 amplification was associated with a statistically significantly higher risk only in short-term follow up for DFS and DSS.

The co-expression of TP53 protein and ERBB2 has been extensively studied as prognostic indicator for breast cancer mainly using IHC based methods [8–17]. However, discrepancies among the study results exist, probably due to the qualitative and quantitative differences in the interpretation of IHC results. Our work is novel, in that, type of TP53 mutations rather than overall protein accumulation is studied in concert with ERBB2 amplification. Additional analysis of a large number of tumors carrying TP53 mutations and ERBB2 amplification simultaneously would allow definitive clarification of the prognostic value of these indicators.

Conclusion

In conclusion, with a median follow-up of 120 months, we found that missense mutations in TP53 were significantly associated with worse DFS and DSS of breast cancer patients, compared to wild type. We found no evidence that this association varied by adjuvant treatment received. In contrast, truncation mutations were not associated with either of DFS and DSS over the full follow-up period, and there was suggestive evidence in the entire cohort that missense mutations were more deleterious than truncating mutations. There was however, also suggestive evidence that longer truncating mutations were associated with

worse DSS compared to wild type and to shorter truncating mutations. Other mutational characteristics (conserved and non-conserved missense mutations) were not significantly associated with differences in DFS or DSS during this follow-up.

Interestingly, when short-term follow up periods were considered (36 for DFS and 60 for DSS), we were able to identify a subset of women with longer truncating mutations that had similar survival characteristics to those with the missense mutations. For DSS particularly within the follow-up period of 60 months, longer truncating mutations were associated with worse survival compared to shorter ones. This study strongly suggests that the type and location of TP53 mutations have differential influences on the DFS and DSS of node-negative breast cancer patients. We also found that women who carried a missense mutation along with ERBB2 amplification had the worst DSS and DFS. This combination represents a potentially excellent indicator of node-negative women with poor prognosis.

Although results based on relatively small sample sizes and multiple testing must be interpreted cautiously, and deserve replication in larger samples, the associations detected are highly statistically significant and are unlikely to have occurred by chance alone. The results of our study thus may provide insight into the clinical usefulness of different types of TP53 mutations, and particularly in combination with ERBB2 amplification, ultimately improve decision making for the treatment and the clinical management of breast cancer patients.

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