The Clinical Value of Somatic *TP53* Gene Mutations in 1,794 Patients with Breast Cancer

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Abstract To investigate the clinical value of somatic *TP53* mutations in breast cancer, we assembled clinical and molecular data on 1,794 women with primary breast cancer with long-term follow-up and whose tumor has been screened for mutation in exons 5 to 8 of *TP53* by gene sequencing. *TP53* mutations were more frequent in tumors of ductal and medullar types, aggressive phenotype (high grade, large size, node positive cases, and low hormone receptor content) and in women <60 years old. *TP53* mutations within exons 5 to 8 conferred an elevated risk of breast cancer–specific death of 2.27 (relative risk >10 years; $P < 0.0001$) compared with patients with no such mutation. The prognostic value of *TP53* mutation was independent of tumor size, node status, and hormone receptor content, confirming and reconciling previous findings in smaller series. Moreover, an interaction between *TP53* mutation and progesterone receptor (PR) status was revealed, *TP53* mutation combined with the absence of progesterone receptor being associated with the worst prognosis. Whereas previous studies have emphasized the fact that missense mutations in the DNA-binding motifs have a worse prognosis than missense mutations outside these motifs, we show that non–missense mutations have prognostic value similar to missense mutations in DNA-binding motifs. Nonetheless, specific missense mutants (codon 179 and R248W) seem to be associated with an even worse prognosis. These results, obtained on the largest series analyzed thus far, show that *TP53* mutations identified by gene sequencing have an independent prognostic value in breast cancer and could have potential uses in clinical practice.

The tumor suppressor gene *TP53* plays a key role in many cellular pathways controlling cell proliferation, cell survival, and genomic integrity. It acts as a proliferation brake when cells experience stress conditions, such as DNA-damage, hypoxia, or oncogene activation. Disrupting *TP53* function promotes checkpoint defects, genomic instability, and inappropriate survival, leading to the uncontrolled proliferation of damaged cells. The proliferative advantage given by its inactivation, and the fact that it is ubiquitously expressed, explains why it is frequently found to be mutated in almost every type of cancer (1). It has been shown in various experimental *in vitro* systems, as well as in mouse models, that cell cycle arrest or apoptosis induced by radiotherapy and various chemotherapeutic drugs depends on an intact *TP53* pathway (2, 3). These results have raised the hypothesis that *TP53* could be a key player in defining tumor sensitivity to a broad range of anticancer treatments in patients with cancer. Moreover, the presence of a *TP53* mutation could be one of the underlying causes of drug resistance, the major cause of treatment failure and cancer death.

Several studies have investigated the predictive value of *TP53* mutation status for tumor response to treatment and patient outcome in various cancers. Different clinical and methodologic

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settings have been used and the results are often contradictory. A majority of studies have relied on immunohistochemistry to assess p53 alterations. This approach is, however, a poor surrogate for gene mutation detection because many mutations do not lead to protein accumulation, and because accumulation of wild-type p53 may also occur. Hence, the use of immunohistochemistry leads to an unacceptable number of misclassified cases and to a greater interstudy variability. By contrast, in studies that have screened *TP53* mutations by gene sequencing to precisely identify the mutation, the presence of a mutation has been correlated with a shorter survival or a poor response to treatment in several cancers (http://www-p53.iarc.fr/Somatic.html). Moreover, a number of studies have described specific types of mutation that were associated with a worse prognosis compared with other mutations. This is the case for mutations within the DNA-binding domain that have been repeatedly associated with poor prognosis in several types of cancer (4–7). These clinical results are substantiated by *in vitro* experimental evidence showing that different missense mutations have different functional consequences (see *TP53* Function Database, http://www-p53.iarc.fr/). Wild-type TP53 activities rely mainly on the capacity to transactivate specific target genes by binding to specific response elements. In human cancers, >1,800 different *TP53* missense mutations have been reported and functional assays have shown that mutant proteins show a great variability in their transactivation activities. Whereas hotspot missense mutations in the DNA-binding domain lead to a general loss of specific transactivation capacity, missense mutations outside the DNA-binding domain more often retain transcriptional activity on a variety of promoters (8, 9).

In breast cancer, more than 20 studies have analyzed the prognostic or predictive value of *TP53* mutation (10). In 18 of these studies, *TP53* mutation was clearly associated with poor prognosis, mutations at residues involved in DNA contacts being of worse prognosis in several of them. However, it is not clear from these studies whether *TP53* is a factor of prognosis that is independent of other clinicopathologic factors. Also, there is no clear consensus on the specific type of mutations carrying a worse prognosis because different classifications of mutations have been used and because the comparison of individual mutations was limited by a lack of statistical power.

Using a more powerful analysis in order to assess whether the identification of *TP53* mutation presents a real benefit over currently available factors of prognosis (such as tumor size, node status, and estrogen and progesterone receptor contents), we collected and pooled clinical and molecular data from 1,794 European patients with breast cancer who were followed-up for at least 10 years and whose tumors were screened for somatic *TP53* gene mutation.

Materials and Methods

Patients and selection criteria. Patients were from 10 hospitals in seven European countries (Norway, Finland, Iceland, France, Sweden, United Kingdom, and Germany). Breast cancer cases were selected from cohorts of patients included in previous studies related to *TP53* gene analysis that have been approved by local ethical committees. The largest cohorts have previously been described in detail in refs. (11–16), whereas clinical and molecular data from 600 patients have not been reported before. Updated follow-up information has been obtained on about half of the previously reported cases. Patients were considered eligible for the present study if they had a primary unilateral breast cancer and if their tumor has been screened for *TP53* mutation by gene sequencing. Patients in whom *TP53* mutation had not been confirmed were not considered eligible (84 patients were excluded). Three patients whose tumors carried more than one mutation were also excluded because they could not be grouped according to the type of mutation (mutations of different type in the same tumor). A total of 1,794 patients were eligible. Clinical data were recovered from hospital pathologic records and included, histopathologic subtype, histopathologic grade, nodal status, tumor size, estrogen receptor (ER) and progesterone receptor (PR) status, age at diagnosis, presence of a *TP53* mutation and characteristics of *TP53* mutation if present, time elapsed between surgery and death or last follow-up, and cause of death (breast cancer or other). ER and PR status were assessed in laboratory hospitals by standard biochemical assays in 90% of the cases, with a cutoff value of 10 fmol/mg of protein, and by immunohistochemistry in 10% of the cases (see details in Supplementary Table S1).

Tumor material and TP53 mutation screening. The samples analyzed were either biopsies or surgery specimens, either fresh-frozen or paraffin-embedded. Histopathology, grading, and hormone receptor contents were determined independently for each cohort at their respective institutions. *TP53* mutation screening was done on genomic DNA, except for 311 patients from the Swedish cohort, in which RNA was analyzed. Constant denaturing gel electrophoresis/denaturing gradient gel electrophoresis/temporal temperature gradient electrophoresis, single-strand conformational polymorphism, or denaturing high-pressure liquid chromatography prescreening methods were used to detect mutations and sequencing was done to precisely identify the mutation in all cohorts except for the Swedish cohort, in which no prescreening was done (direct sequencing of cDNA was applied). The entire coding sequence of *TP53* gene (exons 2-11) was screened in 651 tumors, whereas only exons 5 to 8 were analyzed in 1,124 samples, and exons 5 to 11 in 19 samples. Details of methods and PCR primers have been described previously (13–15, 17).

TP53 mutation classifications. Mutations in exons 5 to 8 (including introns) were classified according to their position, nature, and suspected effect on protein structure and activity (18, 19). The following groups were defined:

- Silent. Mutations in the coding sequence that do not change the amino acid sequence or mutation in introns, excluding splice sites and branch sites;
- Non-missense. Any mutation other than missense, including nonsense (introducing a stop codon), deletions and insertions (in-frame or producing a frame shift), and substitutions at splice sites.
- Missense. Mutation resulting in a single amino acid change. This category was further subdivided as:

  (a) Missense DNA-binding motifs. Missense mutations in DNA-binding motifs (DBM) formed by the L2 and L3 loops (codons 164-194 and 237-250, respectively) involved in DNA contacts in the minor groove, and by the LSH motif (codons 119-135 and codons 272-287) involved in DNA contacts in the major groove.

  (b) Missense non-DBM. Missense mutations outside the above-defined DBMs (L2/L3 and LSH).

  (c) Missense Zn. Missense mutations affecting residues involved in binding a zinc atom (codons 176, 179, 238, and 242).

  (d) Missense DNA. Missense mutations affecting residues involved in DNA contacts (codons 119, 120, 121, 239, 241, 243, 247, 248, 273, 275, 276, 27, 280, and 283).

- Codons. Codons most frequently affected by missense mutations in the present series (codons 163, 175, 179, 220, 245, 248, 249, 273, and 282).
- Mutants. Frequent substitutions at "hotspot" codons in the present series as well as in many other cancer types (R248Q, R248W, R175H, R273H, R273C, and G245S).

- Missense conserved/nonconserved. Missense mutation at an amino acid residue conserved (or not conserved) during evolution (conservation based on CLUSTALW alignment of 35 p53 protein sequences from vertebrate species).
- Functional classes. Results of yeast-based functional assays have been used to classify missense mutations according to their capacity to transactivate the promoters of several p53 target genes (9). The promoters used were the p53-binding elements of *WAF1*, *MDM2*, *BAX*, *GADD45*, *14-3-3σ*, *p53AIP1*, and *Noxa* genes. Functional groups were defined as follows: 1, active or partially active on all promoters; 2, inactive on one to two promoters; 3, inactive on three to five promoters; 4, inactive on six to seven promoters. For individual promoters, three groups were considered: 1, inactive; 2, partially active; 3, activity similar to wild-type.
- Structural classes. Results of a systematic computerized prediction model of the effects of *TP53* mutations on the structure of the core domain of the protein were used to classify *TP53* missense mutations into two classes: 1, mutations predicted to impair correct folding of the core domain (structurally explained); 2, mutations predicted to have no effect on the folding of the core domain (not structurally explained). Criteria taken into account include hydrogen bonding, structural clashes, mutation from glycine or to proline, direct contact with DNA or zinc binding (20).

Statistical analysis. Statistics were done using SPSS and Minitab software. To avoid a possible selection bias, patients with missing data for clinical prognostic variables were included by creating a category labeled as "missing" for each variable. Patient follow-ups were computed as the time interval between surgery date and the date of last follow-up, or as the time interval between the date of surgery and the date of death. To reduce heterogeneity among hospitals for duration of follow-up (three hospitals, accounting for <30% of the patients, had <10 years of maximum follow-up), follow-up was censored at 120 months (10 years − censoring time). Date of follow-up was set at 10 years after surgery for patients whose survival exceeded 10 years. Death from breast cancer within 10 years after surgery was considered as the primary outcome variable. Due to censoring, all breast cancer deaths after 10 years of follow-up were not taken into consideration. Patients who died from causes other than breast cancer were censored at the time of their death when in the 10-year follow-up period or at 10 years if survival exceeded 10 years.

Mortality rates were computed with a censoring at 10 years and using the cumulated number of person years (PY) in each category as a denominator. Kaplan-Meier survival curves and hazard rates estimated by a Cox proportional hazard model were computed to quantify the effect of *TP53* mutation on breast cancer–specific mortality after adjustment for known clinical cofactors (tumor size, node status, ER and PR contents, and age at diagnosis). Histopathologic subtypes and grading were also considered in a descriptive analysis but were not used as predictors of survival because of missing data and possible differences in the classification systems used by participating hospitals.

All Cox models were stratified by hospital using a different baseline hazard function for each hospital to adjust for differences between centers that may be attributable to differences in tumor classification methods, hormone receptor measurements, treatment regimen (surgery and/or chemotherapy), or disease severity (some centers may be more likely than others to admit patients with more severe disease). Patients were grouped according to the mutation present in their tumor as described above. Proportionality assumption for the Cox model was verified by looking at parallelism of survival curves in Kaplan-Meier analysis. To identify possible interactions with *TP53*, Kaplan Meier curves were also stratified according to the presence of a *TP53* mutation.

Because the detection of *TP53* mutation outside exons 5 to 8 was done in a limited number of cases, we focused on mutations occurring in exons 5 to 8 and two distinct analyses were done. In the first analysis, all patients were considered and patients with a mutation outside exons 5 to 8 were included in the "no-mutation" group. Multivariate analysis was restricted to patients who had complete data for the variables that remained associated in the final Cox model. Several sensitivity analyses were previewed to validate the results obtained in the final Cox model under the presence of missing data for some clinical cofactors or when using a different definition of the outcome measure:

(a) The estimated coefficients in the final Cox model were compared with those obtained when using the whole population, which included individuals with missing values for some cofactors who were included in the analyses and labeled as "unknown" or "missing information."

(b) Estimated coefficients in the final Cox model were compared with those obtained when death for breast cancer was replaced by death for all causes.

(c) The set of predictors and the estimated coefficients in the Cox model were compared with those obtained when censoring time was set at 5 years instead of 10 years.

In the second analysis, only patients with mutations in exons 5 to 8 were included to compare the prognostic value of specific *TP53* mutations. Mortality rates and Kaplan-Meier curves and log rank test were computed.

Results

Associations between TP53 mutations and clinicopathologic variables. Clinical and molecular data on primary breast cancer patients from 10 European hospitals were gathered to investigate the relationship between *TP53* mutation status and patient outcome (breast cancer survival) after adjustment for known clinical predictors. A total of 1,794 patients were eligible for the present study. All patients underwent surgery between 1987 and 1997 and were followed-up for a median period of 78 months (interquartile range, 46-121). The number of eligible patients by center varied from 41 to 389 with follow-up varying from 95 to 262 months. *TP53* gene was sequenced from exons 5 to 8 in 1,143 tumors and from exons 2 to 11 in 651 tumors. The clinical and molecular characteristics of patients and tumors are summarized in Table 1. The median age at surgery was 59 years (interquartile range, 48-70). The majority of cases were invasive ductal carcinomas of size <5 cm without node invasion and with positive ER and PR expression. A total of 308 samples had a single mutation within exons 5 to 8 (17%, 308 of 1,794), whereas 26 had a single mutation outside exons 5 to 8 (4%, 26 of 651).

The association of *TP53* mutation with clinical and molecular variables was investigated (Table 1). For this analysis, patients whose tumors carried a mutation outside exons 5 to 8 (26 of 651 patients analyzed for exons 2-11) were included in the no-mutation group because mutations in exons 2 to 4 and exons 9 to 11 should also be expected to occur in the 1,143 patients whose tumors were analyzed only for exons 5 to 8. *TP53* mutation was significantly associated with age at diagnosis ($P = 0.037$), histopathologic subtype ($P < 0.001$), size ($P < 0.001$), histopathologic grading ($P < 0.001$), node status ($P = 0.016$), and hormone receptor status (ER and PR, $P < 0.001$). *TP53* mutation was more prevalent in patients <60 years old, tumors of ductal and medullar histology, high grade, large size, in node-positive cases, and in tumors with low hormone receptor contents.

Prognostic value of TP53 gene status. In univariate analysis, known prognostic factors of survival such as tumor size, histopathologic subtype, grading, node status, and hormone

Table 1. Associations between *TP53* mutation within exons 5 to 8 and clinicopathologic variables

n = 1,794	*n*	*TP53* wild-type*	*TP53* mutation†	Mutated (%)	χ^2 test (*P*)
Age group (quartiles)					
<49	464	373	91	19.6	0.037
49-59	458	368	90	19.7	
60-70	456	386	70	15.4	
>70	416	359	57	13.7	
Tumor subtype					
ductal	1,162	942	220	18.9	<0.001
lobular	175	165	10	5.7	
medullar	18	11	7	38.9	
tubular	34	34	0	0	
mucinous	27	27	0	0	
other	71	57	14	19.7	
missing	307				
Tumor grade					
1	118	117	1	0.8	<0.001
2	458	397	61	13.3	
3	345	267	78	22.6	
missing	873				
Tumor size					
T1	547	488	59	10.8	<0.001
T2	918	737	181	19.7	
T3	252	201	51	20.2	
T4	20	13	7	35.0	
missing	57				
Nodal status					
N0	955	811	144	15.1	0.016
N ≥ 1	690	504	136	19.7	
missing	149				
ER status					
ER+	1,152	1,022	130	11.3	<0.001
ER−	503	349	154	30.6	
missing	139				
PR status					
PR+	1,107	963	144	13.0	<0.001
PR−	547	408	139	25.4	
missing	140				

*No *TP53* mutation within exons 5 to 8.
†Any *TP53* mutation within exons 5 to 8.

receptor status were all associated with patient survival (Table 2). The effect of tumor histopathologic subtype was mainly due to tubular and medullar types that were associated with lower and higher rates of death, respectively. Large tumor size, high histopathologic grade, presence of nodes, and absence of hormone receptors were associated with high mortality rates. Patients with a *TP53* mutation had a relative risk of breast cancer–specific death of ~2 over a period of 10 years following surgery compared with patients with no mutation (Table 2).

In multivariate analysis including *TP53* mutation, tumor size, node status and ER/PR status, an interaction between *TP53* mutation and PR status was revealed (Table 3). Whereas the lack of PR expression increased the relative risk of breast cancer death by 2-fold in patients without *TP53* mutation, PR status did not affect the survival of patients with a *TP53* mutation in exons 5 to 8 (Table 3; Fig. 1A). These results were confirmed after multiple adjustments for tumor size and node status. By contrast, ER status, even when forced in the final multivariate model, was not significantly associated with outcome, either as the main effect or as a factor interacting with *TP53* mutation status.

A sensitivity analysis was carried out (Table 3, sensitivity analysis) on the whole data set by including patients with missing information on nodal status or tumor size (labeled as "missing" in Table 3). This analysis confirmed the result above, as did an analysis made using a more extended definition of the outcome (overall survival – data not shown), or using 5 years as censoring time (Table 3).

Because data on tumor grade was lacking for too many cases to be included in the multivariate analysis, a separate analysis

on a subset of patients with information on grading was carried out. Patients predicted to have a favorable outcome were selected (tumor grade <3, tumor size <5 cm, no node invasion, positive ER or PR receptor). Ten-year survival analysis of these patients stratified by *TP53* mutation status showed that patients with a *TP53* mutation had a large and significant reduction in survival compared with patients without mutation (close to 60% at 10 years; Fig. 1B).

Table 2. Univariate analysis of breast-specific mortality rates >10 years, according to clinical and molecular characteristics of patients with breast cancer and samples (n = 1,794)

n = 1,794	n (%)	Death (n)	Ten-year mortality rate (/1,000)	Relative risk	Log rank test (P)
Age group (quartiles)					
<49	464 (25.86)	121	40.15	1.00	0.1178
49-59	458 (25.53)	102	33.15	0.83	
60-70	456 (25.42)	98	31.99	0.80	
>70	416 (23.19)	96	42.75	1.06	
Tumor subtype					
ductal	1,162 (64.77)	271	37.26	1.00	0.0302
lobular	175 (9.75)	40	37.67	1.01	
medullar	18 (1.00)	6	49.11	1.32	
tubular	34 (1.90)	0	0.00	0.00	
mucinous	27 (1.51)	4	25.65	0.69	
other	71 (3.96)	14	27.89	0.75	
missing	307 (17.11)				
Tumor grade					
1	118 (6.58)	11	12.87	1.00	<0.0001
2	458 (25.53)	83	30.90	2.40	
3	345 (19.23)	98	52.52	4.08	
missing	873 (48.66)				
Tumor size					
T1	547 (30.49)	70	17.39	1.00	<0.0001
T2	918 (51.17)	211	37.63	2.16	
T3	252 (14.05)	115	85.85	4.94	
T4	20 (1.11)	14	191.78	11.03	
missing	57 (3.18)				
Nodal status					
N0	955 (53.23)	134	19.99	1.00	
N ≥ 1	690 (38.46)	243	62.12	3.11	<0.0001
missing	149 (8.31)				
ER status					
ER+	1,152 (64.21)	234	31.11	1.00	
ER−	503 (28.04)	148	49.35	1.59	<0.0001
missing	139 (7.75)				
PR status					
PR+	1,107 (61.71)	207	28.47	1.00	
PR−	547 (30.49)	174	53.95	1.90	<0.0001
missing	140 (7.80)				
TP53 mutation*					
none	1,460 (81.38)	292	30.53	1.00	<0.0001
outside exons 5-8	26 (1.45)	8	55.56	1.82	
exons 5-8	308 (17.17)	117	69.18	2.27	
TP53 mutation in exons 5-8 and PR status					
wild-type† PR+	963 (53.7)	155	23.87	1.00	<0.0001
wild-type† PR−	408 (22.7)	119	48.51	2.03	
mutant PR+	144 (8.0)	52	66.87	2.80	
mutant PR−	139 (7.7)	55	71.23	2.98	
missing	140 (7.8)				

NOTE: Relative risks and log rank test P values are shown for each clinical and molecular category.
*TP53 gene has been analyzed between exons 5 to 8 in 1143 tumors whereas the whole coding sequence has been analyzed in 651 cases.
†The 26 TP53 mutations found outside exons 5 to 8 are included in the wild-type group (see text).

Table 3. Multivariate Cox proportional hazards models and sensitivity analysis of 10-year mortality rates

	Complete case analysis	Sensitivity analysis	
	Cox model, 10 years (n = 1,470)	Cox model, 10 years including missing (n = 1,750)*	Cox model, 5 years (n = 1,470)
	HR† (95% CI)	HR† (95% CI)	HR† (95% CI)
Tumor size			
T1	1	1	1
T2	1.78 (1.32-2.42)	1.83 (1.38-2.43)	2.09 (1.40-3.11)
T3	3.24 (2.24-4.69)	3.37 (2.41-4.70)	3.48 (2.18-5.55)
T4	8.47 (4.15-17.29)	5.65 (3.02-10.54)	9.54 (4.37-20.84)
missing		0.68 (0.29-1.56)	
Nodal status			
N0	1	1	1
N ≥ 1	2.53 (2.00-3.21)	2.56 (2.04-3.20)	3.00 (2.34-4.02)
missing		2.52 (1.70-3.73)	
TP53 mutation in exons 5-8 and PR status			
wild-type‡ PR+	1	1	1
wild-type‡ PR−	1.83 (1.41-2.39)	1.80 (1.40-2.30)	2.06 (1.50-2.83)
mutant PR+	2.40 (1.70-3.38)	2.48 (1.79-3.44)	3.00 (2.03-4.44)
mutant PR−	2.63 (1.89-3.66)	2.58 (1.87-3.55)	2.67 (1.78-3.99)
missing		1.14 (0.77-1.67)	

*The total does not add to 1,794 as the stratified Cox model drops some observations from the analysis.
†Hazard rate ratio.
‡The 26 TP53 mutations found outside exons 5 to 8 are included in the wild-type group (see text).

Prognostic values of specific TP53 mutations. Mutations within exons 5 to 8 were classified in different groups according to the effect or position of the mutation in the primary or tertiary sequence of the protein (see Materials and Methods). Kaplan-Meier survival analysis of patients grouped according to the type of *TP53* mutation found in their tumor showed that non–missense mutations (any mutation other than missense) and missense mutations in the DNA-bindings motifs (DBM) were associated with a strong reduction in survival compared with patients without mutations, whereas missense mutations outside the DNA-bindings motifs (non-DBM) were associated with an intermediate reduction of survival (Fig. 2A). The 10-year mortality rates for non-DBM and DBM mutations were, respectively, 43.92 and 73.42 (per 1,000 persons, P = 0.0897; Table 4). If the non-DBL missense mutations were grouped with silent mutations (associated with similar mortality rate) and used as a reference group, the relative risk associated with DBM mutations was 1.8 (1.03-3.18) and the one of non–missense mutations was 2.9 (1.44-5.38). These results remained valid after adjustment for tumor size, node status, and hormone receptor status. Analysis of mortality rates for the most frequent (hotspots) missense mutations in this series identified mutations with higher or lower mortality rates compared with other missense mutations (Table 4). Figure 2B shows that missense mutations at codon 179 and the R248W mutation were associated with reduced survival, whereas the G245S and Y220C mutations were associated with better survival compared with any other missense mutation. Of note, other mutation hotspots, which are general hotspots for all breast cancers (R175H, R248Q, R273H/C, codons 163, 249, and 282), were associated with mortality rates similar to those of non–hotspot missense mutations (these mutations were included in the "other" category in Fig. 2).

Several groupings of missense mutations were done based on functional, structural, or conservation properties of mutant proteins (Table 4). First, mutations were classified as conserved or nonconserved if the affected residue is conserved or not in vertebrate species. Mutations were also classified according to their capacity to transactivate p53-responsive promoters in yeast-based assays (9). Groups were made according to mutant protein activity on a single promoter or to its global activity on seven different promoters. Finally, mutations were classified according to the predicted structural effects of amino acid substitution (20). Two categories were made, one for mutations predicted to affect protein folding or protein-DNA interaction, and another for all other mutations. None of these classifications detected significant differences in patient survival (Table 4).

Discussion

In this study, which is the largest to date, on the prognostic value of *TP53* gene mutation in breast cancer, we show that *TP53* gene mutation is an independent factor of prognosis for breast cancer survival after adjustment for tumor size, node status, and hormone receptor status (ER and PR). The relative risk of dying of breast cancer within 10 years following surgery for patients with a *TP53* mutation within exons 5 to 8 in their tumor was between 2 and 3 compared with patients without any mutation. In a subset of patients with available tumor grade information and favorable outcome (low grade, limited

size, no nodes, and the presence of hormone receptors), the presence of a *TP53* mutation was associated with a reduction in survival close to 60% at 10 years. This result shows the value of *TP53* mutation as an additional prognostic indicator in this group of patients. It is of note that our analysis was focused on mutations occurring within exons 5 to 8. These exons contain >90% of the mutations reported in breast cancer (19). Thus, most published studies have analyzed only these exons, as it is the case for 1,143 of the 1,794 tumors included in the present study. Analysis of the whole coding sequence (and splice junctions) in 651 cases revealed the presence of 26 mutations outside exons 5 to 8 (4%). These 26 mutations were associated with a relative risk of death close to 2 compared with wild-type cases. Thus, among the 835 mutation-negative breast cancers analyzed for exons 5 to 8 only, about 40 cases may contain a *TP53* mutation. Thus, the actual risk associated with *TP53* mutation may be greater than that estimated here. In clinical practice, it is therefore recommended to conduct mutation analysis on all coding exons and splice junctions.

There was a linear relationship between the size of the tumor and the frequency of *TP53* mutations, and a strong association

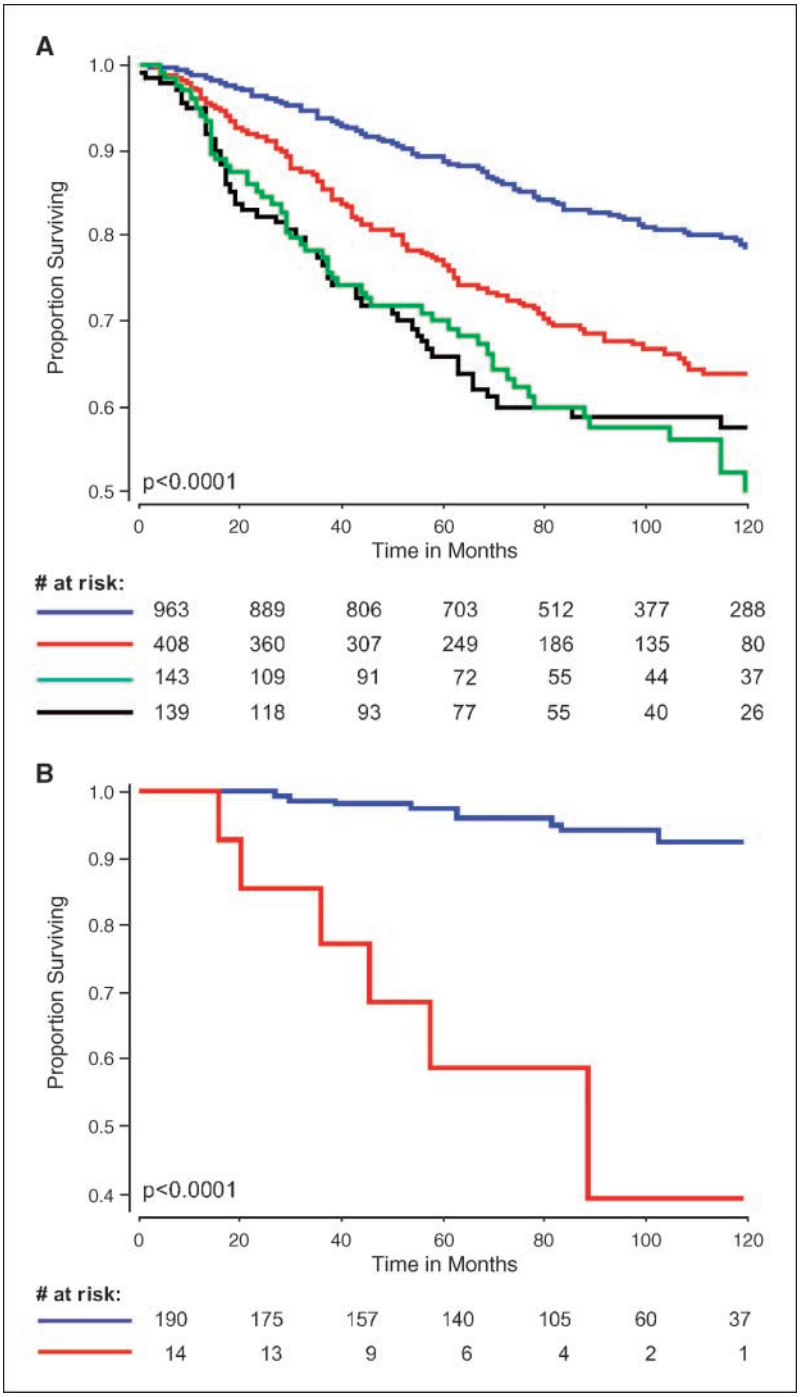

Fig. 1. Kaplan-Meier survival curves of patients with breast cancer stratified by *TP53* gene mutation status. *A,* entire cohort of 1,794 patients. Survival of patients without a mutation within exons 5 to 8 and with a positive PR status (*blue line*), without a mutation within exons 5 to 8 and with a negative PR status (*red line*), with a mutation within exons 5 to 8 and with a positive PR status (*green line*), with a mutation within exons 5 to 8 and with a negative PR status (*black line*). *B,* subset of 204 patients with favorable outcome (tumor grade <3, tumor size <5 cm, node negative and ER or PR positive cases). Survival of patients without a mutation within exons 5 to 8 (*blue line*), compared with patients with a mutation within exons 5 to 8 (*red line*).

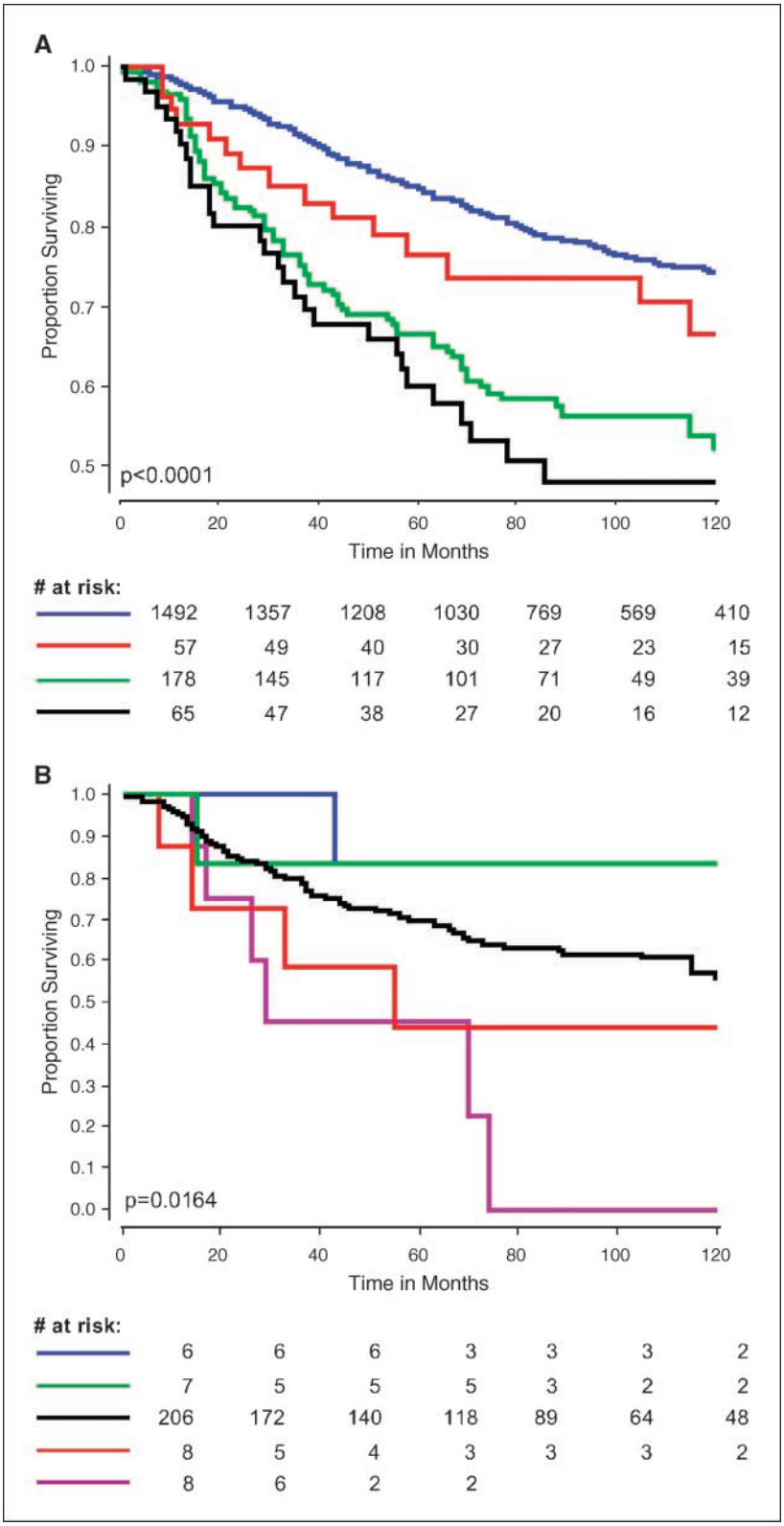

Fig. 2. Kaplan-Meier survival curves of patients with breast cancer stratified by the type of *TP53* gene mutation found in their tumor. *A*, survival of patients without mutation or with a silent mutation within exons 5 to 8 (*blue line*), with a missense mutation within exons 5 to 8 but outside the DBMs (*red line*), with a missense mutation in the DBMs (*green line*), with a mutation other than missense within exons 5 to 8 (*black line*). *B*, survival of patients with the Y220C mutation (*blue line*), the G245S mutation (*green line*), the R248W mutation (*red line*), with any missense mutation at codon 179 (*purple line*), or any other missense mutations (*black line*).

between the presence of a *TP53* mutation and high grade, positive node status, and loss of hormone receptors (ER and PR), which is in agreement with previous reports (http://www-p53.iarc.fr/Somatic.html). *TP53* mutations have also been found to be associated with increased global genomic instability (21–23) and with markers of increased cell proliferation such as high mitotic frequency, high expression of Ki-67, and high cyclin E expression (24, 25). These results show that *TP53* mutations are generally associated with an advanced and aggressive tumor phenotype.

TP53 mutations were significantly more frequent in young women and in medullar carcinoma as reported by others (25–27). Both early age at onset and medullar subtype may be indicative of inherited cancer due to BRCA1 germ line mutations (28, 29). BRCA1 breast cancers may represent up to 5% of the cases in a breast cancer series and these cancers usually present a high frequency of *TP53* somatic mutations (19). Because family history is not well documented in our series and <5% of the patients have been tested for BRCA1, we cannot exclude that some BRCA1 cases may contribute to the observed mutation frequency. To rule out any confounder effect of possible BRCA1 cases in subsequent analyses, results were systematically verified on subsets excluding patients <40 years and medullar cases.

The prognostic value of *TP53* mutation has been shown to be independent of tumor size, node status, or ER in a number of reports (13, 15, 25, 30, 31). The present study confirms these observations. In addition, we found an interaction between *TP53* mutation and PR content that has not been previously reported. *TP53* mutation combined with low PR had a very bad prognosis independently of tumor size, node status, and ER status. PR status, which reflects estrogen pathway integrity, has been shown to be more relevant than ER status for tumor response to tamoxifen and prediction of patient survival (32–34). In two independent retrospective series of patients (14, 35), one of them being included in our series (35), *TP53* mutation has been shown to affect tumor response to tamoxifen. These results suggest that *TP53* pathway may play a role in the response to antihormone therapy. However, the lack of information on treatment for a significant number of patients prevented us from exploring this hypothesis. Further studies are thus required to explore the possible interplay between *TP53* and ER pathways and the consequences on tumor development and behavior.

When comparing the prognostic value of different types of mutations, we found that the more severe mutations were non–missense mutations followed by missense mutations in the DBMs (L2/L3 and LSH), among which mutations at codon

Table 4. Univariate analysis of ten-years mortality rates and log rank test *P* values of breast cancer cases with specific *TP53* mutations within exons 5 to 8 ($n = 306$)

	n	Deaths (*n*)	Ten-year mortality rate (/1,000)	Log rank test (*P*)
Silent	6	2	43.17	0.3605
Non-missense	65	28	88.05	
Missense*	235	87	65.80	
Conserved	199	78	71.64	0.0944
Nonconserved	36	9	38.57	
Structurally explained†	151	57	70.44	0.3421
Not structurally explained	79	27	54.36	
Function 1	72	31	78.17	0.5165
Function 2	44	12	45.20	
Function 3	7	3	80.18	
Function 4	112	41	65.85	
DBM	178	72	73.42	0.0897
Non-DBM	57	15	43.92	
Zn	17	7	86.15	0.3325
DNA	61	27	83.38	
Non-Zn/DNA	157	53	57.79	
163	6	2	68.97	0.441
175	19	7	68.29	
179	8	6	254.42	
220	7	2	45.63	
245	9	2	37.68	
248	26	12	78.65	
249	7	3	71.29	
273	20	7	68.46	
282	7	3	71.01	
R175H	18	7	69.65	0.805
R248Q	18	8	69.06	
R248W	8	4	108.84	
R273H	9	3	67.29	
R273C	8	3	64.86	
G245S	7	1	24.49	

NOTE: Two patients were excluded because their *TP53* mutations were not precisely identified.
*Missense mutations were grouped as described in details in Materials and Methods. Survival analyses for the different subcategories are shown in the subsequent rows.
†Structural information was not available for five missense mutations.

179 and the R248W mutant were associated with the highest mortality rates. Grouping of missense mutations according to their loss of transcriptional activities measured by systematic yeast-based assays, or to their predicted effect on protein structure, did not correlate with patient outcome. Although these structural and functional analyses of p53 mutations are the most extensive that are currently available, they may not give an accurate assessment of the changes induced by mutation that really have an effect on clinical outcome. Indeed, other variables such as protein-protein interactions, transcriptional repression, and transactivation of other genes, not taken into account here, play an important role in the antiproliferative activity of p53 and in the activities of mutant proteins (36, 37). Mutations affecting the L2/L3 motif involved in specific DNA-binding and zinc coordination have been repeatedly described as "bad" mutations in breast cancer based on their association with poor tumor response to treatment and short patient survival (4, 26, 30, 38–41). Functional assessments of some of these mutations in human cells have shown not only loss of transcriptional activity and defects in the capacity to induce cell cycle arrest or apoptosis, but also gain of function properties and/or dominant-negative effects, resulting in growth-promoting activities and resistance to drug-induced apoptosis (http://www-p53.iarc.fr/p53MUTfunction.html). How these properties specifically affect tumor response to treatment and patient outcome is still under debate. Because deletions/insertions mutations are expected to result in a null phenotype (truncated and unfolded proteins), our results suggest that loss of transcriptional activity is the main determinant of the poor prognostic value of *TP53* mutations in breast cancer. Moreover, loss of transcriptional activity may be sufficient to promote breast tumor development, as suggested by studies on germ line mutations. First, non–missense mutations and missense mutations in the DBMs were associated with an earlier age at onset of breast tumors compared with missense mutations outside these motifs (32 versus 42 years; ref. 42). Second, *TP53* null mammary epithelium isolated from *TP53* null mice and transplanted into cleared mammary fat pads of *TP53* wild-type mice showed that the absence of *TP53* is sufficient to cause the development of primary tumors (43). If loss of transcriptional activity seems to be the main determinant of breast tumor development and behavior, it cannot be excluded that some specific mutants, such as codon 179 and R248W might exert dominant-negative effects and gain of function activities responsible for their very bad prognostic value.

Some limitations, mainly due to the multicenter structure of the study, have to be acknowledged. First, intercenter heterogeneity could be mostly controlled through stratification (44), as mentioned in Materials and Methods. In addition, differences of follow-up between hospitals were homogenized by censoring of follow-up. Second, *TP53* mutation detection was done in five different laboratories with four different prescreening methods that may differ in their sensitivities. Thus, hazard risk estimates for *TP53* mutation may have been underestimated. Third, the presence of missing values in known predictors of survival prevented the inclusion of some variables in multivariate analysis. It was particularly true for histopathologic grade and subtype with, respectively, 48% and 17% of missing values. However, histopathologic subtype and grade may reflect the presence of molecular alterations including *TP53* mutation and hormone receptor expression and thus may be collinear with *TP53* when entered in the multivariate model. The sensitivity analyses confirmed results when missing values for variables such as tumor size and nodal status were integrated in the analysis, and when censoring was made at the 5-year follow-up, or when using a more extended definition survival (overall mortality). These sensitivity analyses show the stability of the results. Nonetheless, the lack of information on family history, adjuvant treatments, and other markers such as *ERBB2* amplification, prevented us from investigating the influence of these variables on our results. In a recent study, Bull et al. have found that *TP53* gene mutations were more frequent than *ERBB2* amplification in women with node-negative breast cancer and that *TP53* mutation may be beneficial in identifying women at higher risk of disease recurrence and death when their tumor has *ERBB2* amplification (45). In two other studies, the use of expression microarrays has shown that *TP53* gene mutation is highly associated with groups of patients with similar gene expression profiles (46), and that tumor classification based on these profiles was a stronger predictor of outcome than any of the classical clinicopathologic markers, *TP53* mutation status being equally significant.[14] These results strongly support the fact that *TP53* mutations have a prognostic value in various groups of patients. However, further studies will be required to precisely identify which groups of patients would benefit or not from *TP53* mutation screening.

In the early 1990s, the rapid accumulation of data on *TP53* mutations in human cancer raised high expectations for clinical exploitation. However, most studies relied on immunohistochemistry to assess *TP53* status, a method prone to misclassification, as many *TP53* mutations do not correlate with protein accumulation. In the present study, the strongest association with poor survival was found for non–missense mutations, predicted to generate a negative immunostaining. It is thus highly recommended to perform gene sequencing to precisely identify the mutation. Several common polymorphisms in *TP53* (in exons 3, 4, and 6) may also deserve further investigation. Although a recent study argues against a role for these polymorphisms in breast cancer susceptibility (47), there is experimental evidence that codon 72 polymorphism (arginine to proline) may influence wild-type p53 activity in response to cytotoxic drugs (48). Mutation detection in our series was done by PCR-based prescreening methods followed by DNA sequencing, which are the gold standards for gene mutation identification, but they are labor-intensive, and thus, are not suitable for clinical practice. New techniques have been developed recently that may be more easily implemented for routine use, such as microarray-based methods (Affymetrix, arrayed primer extension assay; ref. 49). As these techniques will soon be available at an effective cost, *TP53* gene mutation may become an important marker for patient management.

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[14] A. Langerød and A-L. Børresen-Dale, unpublished data.

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