ORIGINAL ARTICLE

Prognostic and Predictive Effect of *TP53* Mutations in Patients with Non–Small Cell Lung Cancer from Adjuvant Cisplatin–Based Therapy Randomized Trials: A LACE-Bio Pooled Analysis

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ABSTRACT

Introduction: Tumor protein p53 gene (*TP53*) mutations are common in stage I through III non–small cell lung cancer, but clinical trials have shown inconsistent results regarding their relationship to the effects of adjuvant therapy. The objective is to clarify their putative prognostic and predictive effects.

Methods: A pooled analysis of *TP53* mutations (exons 5–8) was conducted in four randomized trials (the International Adjuvant Lung Cancer Trial, J BRonchus 10, Cancer and Leukemia Group B-9633, and Adjuvant Navelbine International Trialist Association trial) of platinum-based adjuvant chemotherapy (ACT) versus observation (OBS). Hazard ratios (HRs) and 95% confidence intervals (CIs) of mutant

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versus wild-type (WT) *TP53* for overall survival (OS) and disease-free survival (DFS) were estimated using a multivariable Cox model stratified on trial and adjusted on sex, age, and clinicopathological variables. Predictive value was evaluated with an interaction between treatment and *TP53*.

Results: A total of 1209 patients (median follow-up 5.5 years) were included. There were 573 deaths (47%) and 653 DFS events (54%). Mutations (434 [36%]) had no prognostic effect (OBS $HR_{OS} = 0.99$, 95% CI: 0.77–1.28, $p = 0.95$; $HR_{DFS} = 0.99$, 95% CI: 0.78–1.25, $p = 0.92$) but were marginally predictive of benefit from ACT for OS (test for interaction: OS, $p = 0.06$; DFS, $p = 0.11$). Patients with WT *TP53* had a tendency toward better outcomes with ACT than did those in the OBS group ($HR_{OS} = 0.77$, 95% CI: 0.62–0.95, $p = 0.02$; $HR_{DFS} = 0.75$, 95% CI: 0.62–0.92, $p = 0.005$). In the ACT arm, a deleterious effect of mutant versus WT *TP53* was observed ($HR_{OS} = 1.40$, 95% CI: 1.10–1.78, $p = 0.006$; $HR_{DFS} = 1.31$, 95% CI: 1.04–1.64, $p = 0.02$).

Conclusions: *TP53* mutation had no prognostic effect but was marginally predictive for survival from ACT. In patients who received ACT, *TP53* mutation tended to be associated with shorter survival than wild-type *TP53*.

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Keywords: TP53 mutations; Lung cancer; Prognostic; Predictive

Introduction

Mutations in the tumor suppressor gene tumor protein p53 gene (*TP53*) are frequent in non–small cell lung cancer (NSCLC). Data from the Catalogue of Somatic Mutations in Cancer database indicate that *TP53* mutations are detected in approximately 39% of adenocarcinomas, 51% of squamous cell carcinomas, and 68% of large cell carcinomas, the main NSCLC subtypes.[1,2] Typically, mutations impair p53 suppressor function by hampering correct protein synthesis (nonsense, splicing, or frameshift mutations) or by altering the DNA-binding domain of the p53 protein (missense mutations).[3] More than 90% of missense mutations occur in exons 5 to 8 (residues 126–305, encoding the DNA-binding domain) and many of them lead to the accumulation of histochemically detectable mutant proteins.[4,5] *TP53* plays multiple roles in preventing and suppressing abnormal cell growth through cell cycle arrest, apoptosis, senescence, or control of metabolism and DNA repair.[6–9] However, data on its prognostic or predictive effect in NSCLC are limited and inconclusive. Kandioler et al. (2008) reported that *TP53* mutation was predictive of resistance to induction therapy (cisplatin/etoposide plus radiation) in a cohort of 35 patients with NSCLC from a prospective phase II trial.[6] However, in a study of 197 completely resected patients enrolled in a randomized trial of postoperative radiotherapy plus chemotherapy, Schiller et al. (2001) failed to identify a prognostic or predictive value.[10] Negative results were also reported in 397 patients from J BRonchus (JBR) 10, a randomized trial of patients with stage IB and II NSCLC assigned to treatment with cisplatin-based adjuvant chemotherapy (ACT) versus observation (OBS).[11] In the International Adjuvant Lung Cancer Trial (IALT), another randomized trial of ACT versus OBS in patients with stage I to III NSCLC, we reported that *TP53* mutation was neither prognostic nor predictive for overall survival after 8 years of follow-up, although it was noted that survival tended to be shorter among patients with mutations.[12] To clarify the putative prognostic and predictive value of *TP53* mutation for ACT, we performed a pooled analysis of four randomized trials of ACT versus OBS.

Materials and Methods

Clinical Trials and Tumor Samples

This study used the Lung Adjuvant Cisplatin Evaluation (LACE)-Bio Collaborative Group platform, which investigates biomarkers in biosamples of the LACE trials of cisplatin-based ACT[13] (IALT,[14,15] Adjuvant Navelbine International Trialist Association [ANITA],[16] and JBR 10[17,18]) and the Cancer and Leukemia Group B (now the Alliance for Clinical Trials in Oncology) trial 9633 (CALGB-9633), which used carboplatin-based ACT.[19] Samples were available for 1546 patients (44% of the randomized cases [see the flowchart in Supplementary Fig. 1]). Tumors were classified according to the 2004 World Health Organization classification[20] and the 2011 International Association for the Study of Lung Cancer classification.[21] The cisplatin-based adjuvant trials Italian Lung Project Italy and Big Lung Trial had no available tumor tissues and were not included in this pooled analysis.

TP53 *Mutation Detection*

Mutations were analyzed by direct sequencing of polymerase chain reaction products of DNA extracted from informative formalin-fixed paraffin-embedded sections as described in Supplementary Methods 1. Mutation data for IALT[12] and JBR 10[11] have been reported previously. *TP53* mutation analyses were performed at the International Agency for Research on Cancer (Lyon, France) for IALT, ANITA, and CALGB-9633, and at the Ontario Cancer Institute (Toronto, Canada) for JBR 10 (see Supplementary Methods 1 for the details). Only samples with confirmed results and sequence data including the entire coding sequence of exons 5 to 8 and flanking splice sites were used in the analyses. These samples were classified as wild type (WT) (no mutation detected

in exons 5 to 8) or mutant (MUT) (confirmed mutation in exons 5 to 8). Silent mutations and rare *TP53* polymorphisms were classified as WT (for a list of all mutations see Supplementary Table 1). To analyze the effect of different types and classes of *TP53* mutations, mutations in exons 5 to 8 were grouped into predefined classes according to their predicted effects on mutant p53 protein (missense or null mutation) and their position with respect to DNA binding motifs of the p53 protein (for the classification algorithm, see Supplementary Methods 2 and Supplementary Fig. 2; for the correspondence between classifications, see Supplementary Tables 2 and 3).

Immunohistochemical Staining for p53

Immunostaining for p53 was performed on serial sections from the same paraffin block as for detection of *TP53* mutations (Supplementary Methods 3).[9,22] A composite staining score (from 0 to 12) was obtained by multiplying the percentage of positively stained nuclei by the intensity of staining. Percentage was used as a categorical variable (0 = absent, 1 = [0–25], 2 = [25–50], 3 = [50–75], and 4 = [75–100]). Intensity was evaluated on a scale of 0 to 3 (0 = absent, 1 < 10%, 2 = [10%–50%], and 3 = [50%–100%]). A tumor was considered positive for p53 when the score was 4 or higher.

Statistical Analyses

Analyses were based on intention to treat using individual patient data on patients with NSCLC with confirmed *TP53* mutation (WT and MUT) results. End points were overall survival (OS) (primary end point) and disease-free survival (DFS), which was defined as time from randomization to death from any cause (OS), recurrence (DFS), or last follow-up for surviving patients. All variables associated with *TP53* at $p < 0.20$ in a univariate logistic model stratified by trial were included in a multivariable logistic regression model and considered confounders if related to *TP53* at $\alpha = 0.05$. Kaplan-Meier–based survival curves were compared using the log-rank test. A multivariable Cox model stratified by trial was the main analysis. Variables included treatment, sex, age (<55, 55–64, or >64 years), tumor stage (T1, T2, or T3/4), nodal stage (N0, N1, or N2), and histologic diagnosis (squamous cell carcinoma [SCC], adenocarcinoma [ADC], or other histologic diagnoses) (plus potential confounders) and were used to estimate prognostic and predictive effects (interaction between *TP53* and treatment). Heterogeneity among trials of these effects was also evaluated. Prognostic analyses were performed in the OBS arm. We also investigated the prognostic and predictive effect of *TP53* across three histologic diagnosis groups (SCC, ADC, and other) using second- and third-order interaction terms. Exploratory analyses were performed (when appropriate depending on the sample size) to assess the effect of different classes of *TP53* mutations (see Supplementary Methods 2). All p values were two sided and statistical significance was set at $p = 0.01$ within pooled analyses to limit the risk of a false-positive result. Statistical analyses were performed using SAS Software, version 9.3 (SAS Institute, Cary, NC).

Results

Patients and Mutations

Conclusive results on *TP53* mutation in exons 5 to 8 and flanking splice sites were obtained for 1209 of the 1546 patients representative of the LACE-Bio cohort who were tested (Supplementary Fig. 1). Results for other exons were incomplete owing to the use of archived formalin-fixed paraffin-embedded materials and were not considered in this analysis. Of these 1209 patients, 75% were male, 68% were younger than 65 years old, and 84% were in stage I to II; 573 deaths (47%) and 653 events (54%) were observed with a median follow-up time of 5.5 years (range 35 days to 11 years) (Table 1). *TP53* mutations were identified in 434 of 1209 patients (36%) (for a list of all mutations, see Supplementary Table 1). *TP53* mutation was significantly correlated with stage, histologic diagnosis, and type of operation (Table 2). In multivariable analysis, only histologic diagnosis remained significant ($p < 0.001$). A total of 995 patients were evaluated for both *TP53* mutation and p53 immunohistochemical (IHC) analysis results. Despite an overall strong association ($p < 0.0001$), the results of IHC staining were positive for approximately 33% of tumors with WT *TP53* but negative for 39% of tumors with MUT TP53 (Table 3 and Supplementary Fig. 3).

Prognostic Effect

In the OBS group, patients with WT *TP53* did not have significantly different OS compared with patients with MUT *TP53* in the univariable (Fig. 1*A*, log-rank test $p = 0.35$) and multivariable analyses (hazard ratio $[HR]_{MUT\ vs.\ WT} = 0.99$, 95% confidence interval [CI]: 0.77–1.28, $p = 0.95$ [see Supplementary Table 4 for all cases]), with no heterogeneity among trials ($p = 0.90$). There was no prognostic effect in the three histologic diagnosis groups (interaction test $p = 0.28$ [Supplementary Table 4]). Similar results were observed for DFS (Supplementary Table 5). When *TP53* (WT, MUT) and IHC staining results (<4 versus ≥4) were combined, no significant difference was observed among the four groups ($p = 0.48$ [Fig. 1*B*]). After adjustment for covariates and IHC staining results, the difference remained nonsignificant ($HR_{MUT\ vs.\ WT} = 1.04$, 95% CI: 0.79–1.36, $p = 0.80$).

Table 1. Patients' Characteristics by Trial and Overall

	ANITA		IALT		JBR 10		CALGB		All	
	N = 105	%	N = 524	%	N = 397	%	N = 183	%	N = 1209	%
Age, y										
<55	31	29.5	149	28.4	108	27.2	51	27.9	339	28.0
55-64	40	38.1	227	43.3	165	41.6	57	31.1	489	40.4
≥65	34	32.4	148	28.2	124	31.2	75	41.0	381	31.5
Sex										
Male	97	92.4	431	82.3	259	65.2	125	68.3	912	75.4
Female	8	7.6	93	17.7	138	34.8	58	31.7	297	24.6
T stage[a]										
1	4	3.8	80	15.3	50	12.6	1	0.5	135	11.2
2	90	85.7	317	60.5	347	87.4	178	97.3	932	77.1
3/4	11	10.5	127	24.2	0	–	1	0.5	139	11.5
Unknown	0	–	0	–	0	–	3	1.6	3	0.2
N stage[a]										
0	52	49.5	231	44.1	187	47.1	177	96.7	647	53.5
1	25	23.8	158	30.2	210	52.9	1	0.5	394	32.6
2	28	26.7	135	25.8	0	–	0	–	163	13.5
Unknown	0	–	0	–	0	–	5	2.7	5	0.4
Stage[a]										
Stage I	48	45.7	172	32.8	187	47.1	177	96.7	584	48.3
Stage II	25	23.8	187	35.7	210	52.9	3	1.6	425	35.2
Stage III	32	30.5	165	31.5	0	–	0	–	197	16.3
Unknown	0	–	0	–	0	–	3	1.6	3	0.2
WHO performance status										
0	52	49.5	281	53.6	192	48.4	112	61.2	637	52.7
1/2	52	49.5	243	46.4	204	51.4	68	37.2	567	46.9
Unknown	1	1.0	0	–	1	0.3	3	1.6	5	0.4
Histologic diagnosis										
Squamous cell carcinoma	55	52.4	282	53.8	132	33.2	54	29.5	523	43.3
Adenocarcinoma	30	28.6	182	34.7	193	48.6	102	55.7	507	41.9
Other[b]	20	19.0	60	11.5	72	18.1	27	14.8	179	14.8
Type of surgery										
Pneumonectomy	70	66.7	315	60.1	307	77.3	163	89.1	855	70.7
Other	35	33.3	209	39.9	90	22.7	18	9.8	352	29.1
Unknown	0	–	0	–	0	–	2	1.1	2	0.2
Radiotherapy										
No	69	65.7	386	73.7	397	100	183	100	1035	85.6
Yes	36	34.3	138	26.3	0	–	0	–	174	14.4
Follow-up (range), y	6.0 (3.6-9.1)		4.7 (0.7-7.4)		5.4 (0.4-9.3)		7.5 (0.1-11.3)		5.5 (0.1-11.3)	
No. deaths (OS)	54	51.4	272	51.9	162	40.8	85	46.5	573	47.4
No. events (DFS)	62	59.1	309	59.0	191	48.1	91	49.7	653	54.0

[a]Stages were defined as described in Greene et al.[23]
[b]Other subtype includes large cell, adenosquamous, sarcomatoid, basaloid, and unclassifiable NSCLC.
ANITA, Adjuvant Navelbine International Trialist Association; IALT, International Adjuvant Lung Trial; JBR, J BRonchus; CALGB, Cancer and Leukemia Group B; T, tumor, N, node; WHO, World Health Organization; OS, overall survival; DFS, disease-free survival.

Predictive Effect

The HR for OS when the ACT and OBS arms were compared was 0.77 (95% CI: 0.62–0.95, $p = 0.02$) in patients with WT *TP53* tumors versus 1.07 (95% CI: 0.82–1.39, $p = 0.63$) in patients with MUT *TP53* tumors, with only a marginal difference in treatment effect between WT *TP53* and MUT *TP53* (interaction $p = 0.06$) (Table 4 [all cases] and Supplementary Fig. 4). The interaction term between treatment and *TP53* was not heterogeneous across trials ($p = 0.96$, Fig. 2). No predictive effect of *TP53* was observed in any histologic diagnosis subgroup (interaction $p = 0.17$ for SCC, $p = 0.23$ for ADC, and $p = 0.57$ for other NSCLC [Table 4]). In the ACT arm, patients with *TP53* mutation had a significantly poorer outcome compared with that of patients with WT *TP53* (HR = 1.40, 95% CI: 1.10–1.78, $p = 0.006$, heterogeneity among trials $p = 0.96$ [Fig. 2]). This effect was marked in SCC, for which the

Table 2. Correlation between Patient/Tumor Characteristics and *TP53* Mutation

	WT		MUT			Total	
	n = 775	%	n = 434	%	*p* Value (Trend Test)	N = 1209	%
Age,					0.07 (0.11)		
<55	214	63	125	37		339	28
55-64	299	61	190	39		489	40
≥65	262	69	119	31		381	32
Sex					0.05		
Male	567	62	345	38		912	75
Female	208	70	89	30		297	25
T stage[a]					0.002 (<0.001)		
1	97	72	38	28		135	11
2	610	65	322	35		932	77
3/4	66	47	73	53		139	11
Unknown[b]	2	67	1	33		3	<1
N stage[a]					0.14 (0.05)		
0	428	66	219	34		647	54
1	253	64	141	36		394	33
2	91	56	72	44		163	13
Unknown[b]	3	60	2	40		5	<1
Stage[a]					0.01 (0.003)		
Stage I	396	68	188	32		584	48
Stage II	272	64	153	36		425	35
Stage III	105	53	92	47		197	16
Unknown[b]	2	67	1	33		3	<1
WHO performance status					0.34		
0	414	65	223	35		637	53
1/2	358	63	209	37		567	47
Unknown[b]	3	60	2	40		5	<1
Histologic diagnosis					<0.0001		
Squamous cell carcinoma	307	59	216	41		523	43
Adenocarcinoma	364	72	143	28		507	42
Other[c]	104	58	75	42		179	15
Type of surgery					0.002		
Pneumonectomy	198	56	154	44		352	29
Other	575	67	280	33		855	71
Unknown[b]	2	100	0	0		2	<1
Radiotherapy					0.69		
No	672	60	363	40		1035	86
Yes	103	59	71	41		174	14

[a]Stages were defined as described in Greene et al.[23]
[b]Unknown category was excluded from the logistic regression stratified by trial.
[c]Other subtype includes large cell, adenosquamous, sarcomatoid, basaloid, and unclassifiable NSCLC.
TP53, tumor protein p53 gene; WT, wild type; MUT, mutation; T, tumor; N, node; WHO, World Health Organization.

HR comparing MUT *TP53* to WT *TP53* in the ACT arm was 1.65 (95% CI: 1.15–2.38, $p = 0.007$). Such an effect was not seen in the OBS arm (HR = 1.16, 95% CI: 0.81–1.66, $p = 0.43$), suggesting a tendency toward a detrimental effect of MUT *TP53* in relation to ACT in SCC. For DFS, the HR comparing ACT to OBS was 0.75

Table 3. Association of *TP53* Mutation and Results of Immunohistochemical Staining for p53

	IHC Staining Negative		IHC Staining Positive		
TP53 Mutation	n	%	n	%	Total
Wild-type	411	67	206	33	617
Mutant	148	39	230	61	378
Total	559	100	436	100	995

Note: IHC status unavailable for 214 patients. Chi-square test: $p < 0.0001$.
TP53, tumor protein p53 gene; IHC, immunohistochemical.

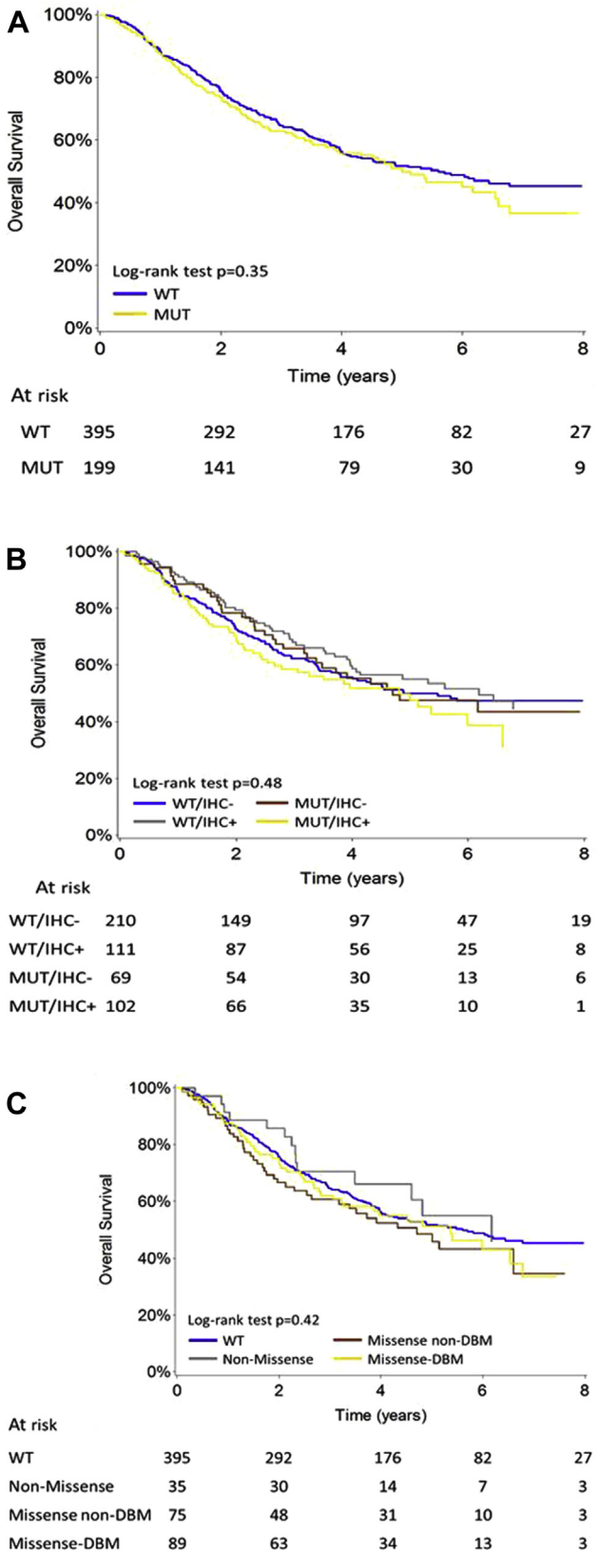

Figure 1. Unadjusted Kaplan-Meier curves for effect of *TP53* mutation (wild type [WT] and mutant [MUT]) on overall survival in the control arm. (*A*) Effect of *TP53* mutation (WT versus MUT). (*B*) Combination between *TP53* mutation (WT, MUT) and immunohistochemical (IHC) staining for p53 (positive/negative). (*C*) Effect of *TP53* mutation according to three structure-effect groups. Nonmissense, mutations predicting null p53 protein; Missense DBM, substitutions at residues in the DNA-binding motifs of the protein (including loops and helix directly contacting target DNA); Missense non-DBM, substitutions at residues of the core domain of p53 located in β strands and intervening loops that are not directly contacting target DNA. Reprinted with permission from Ma et al.[12]

(95% CI: 0.62–0.92, $p = 0.005$) for patients with WT *TP53* and 0.98 (95% CI: 0.76–1.26, $p = 0.86$) for patients with MUT *TP53* (interaction $p = 0.11$) (Table 5 and Supplementary Figs. 5 and 6). Predictive effect for DFS was similar to that for OS (HR = 1.56, 95% CI: 1.11–2.20, $p = 0.01$ in the SCC ACT arm [Table 5]). Overall, there was no heterogeneity of *TP53* effect among histologic diagnosis groups in the ACT arm ($p = 0.50$ for OS and 0.20 for DFS).

Mutation Classifications

Depending on their impact on protein structure, mutations in *TP53* may exert biological effects ranging from partial or complete loss of function to gain of function (GOF) effects.[4,24] To determine whether prognostic or predictive effects vary with mutation effect, *TP53* were subdivided into four classes according to their nature, position, and suspected effect on p53 protein function (structure-effect classification): WT, nonmissense, missense non–DNA-binding motifs (non-DBMs) and missense DBMs (classification algorithm in Supplementary Methods 2). Supplementary Table 2 shows the correspondence between this classification and two other classifications, based on the scale-invariant feature transform algorithm or on the transcriptional activity of mutants measured in an in vitro assay (transcription class).

The three structure-effect classes had no prognostic effect on OS or DFS in univariate analysis (OS: log-rank test $p = 0.42$, [see Fig. 1*C*]) or after adjustment (OS: $p = 0.72$; DFS: $p = 0.83$) (Supplementary Table 6). Altogether, the classification was not predictive for OS benefit from ACT (interaction $p = 0.20$ [Supplementary Table 7]) or DFS (interaction $p = 0.34$ [Supplementary Table 8]). However, a statistically significant effect of *TP53* mutation class was observed in the ACT arm ($p = 0.01$) for OS. Compared with WT *TP53*, a worse outcome was observed for the missense non-DBM class (HR = 1.68, 95% CI: 1.20–2.35, $p = 0.002$) and a marginal effect was observed for the nonmissense class (HR = 1.49, 95% CI: 1.01–2.19, $p = 0.05$). For DFS, a marginally significant effect was observed ($p = 0.05$) in the ACT arm, with a significant effect between

Table 4. Predictive Effect of *TP53* Mutation on Overall Survival

	Adjuvant Chemotherapy (Patient Deaths)	Observation (Patient Deaths)	HR (Adjuvant Chemotherapy vs. Observation) (95% CI)	Test for Interaction *TP53**Treatment
All cases				
WT n = 772	153 of 377	190 of 395	0.77 (0.62-0.95) $p = 0.02$	
MUT n = 432	127 of 233	100 of 199	1.07 (0.82-1.39) $p = 0.63$	
HR (MUT vs. WT) (95% CI)	1.40 (1.10-1.78) $p = 0.006$	1.01 (0.79-1.29) $p = 0.95$		$p = 0.06$
Squamous cell carcinoma				
WT n = 307	55 of 154	70 of 153	0.73 (0.51-1.04) $p = 0.08$	
MUT n = 215	65 of 117	53 of 98	1.04 (0.72-1.50) $p = 0.84$	
HR (MUT vs. WT) (95% CI)	1.65 (1.15-2.38) $p = 0.007$	1.16 (0.81-1.66) $p = 0.43$		$p = 0.17$
Adenocarcinoma				
WT n = 361	74 of 171	93 of 190	0.79 (0.58-1.07) $p = 0.13$	
MUT n = 143	34 of 71	30 of 72	1.13 (0.69-1.85) $p = 0.64$	
HR (MUT vs. WT) (95% CI)	1.23 (0.81-1.85) $p = 0.33$	0.86 (0.57-1.30) $p = 0.47$		$p = 0.23$
Other NSCLC histologic diagnoses				
WT n = 104	24 of 52	27 of 52	0.82 (0.47-1.42) $p = 0.47$	
MUT n = 74	28 of 45	17 of 29	1.04 (0.56-1.91) $p = 0.91$	
HR (MUT vs. WT) (95% CI)	1.24 (0.71-2.16) $p = 0.45$	0.97 (0.53-1.80) $p = 0.93$		$p = 0.57$
Heterogeneity test[a]	$p = 0.50$	$p = 0.56$		
Interaction test[b]				$p = 0.97$

[a]Test for equality of the three hazard ratios (MUT vs. WT) between histologic diagnosis subtypes in the adjuvant chemotherapy and observation arms, separately.
[b]*p* Value of the third-order interaction (histologic diagnosis**TP53**treatment) (i.e., equality of TP53*treatment interaction between histologic subtypes). The *p* value of the nullity of the three interaction terms was 0.30. Five patients were excluded owing to missing covariates.
TP53, tumor protein p53 gene; HR, hazard ratio of death; CI, confidence interval; WT, wild-type; MUT, mutation; HR (MUT vs. WT), hazard ratio for mutant versus wild-type; NSCLC, non-small cell lung cancer.

missense non-DBM and WT (HR = 1.53, 95% CI: 1.11–2.11, $p = 0.01$) (Supplementary Table 8).

Discussion and Conclusion

This LACE-Bio study of four randomized trials is the largest analysis to date of the value of *TP53* mutation for prognosis and prediction of benefit from ACT in completely resected NSCLC. The results on a total of 1209 patients show that mutation in the coding sequence or splicing junctions of exons 5 to 8 had no significant prognostic effect on OS and DFS in the OBS arm. A marginal predictive effect for benefit of ACT was observed for OS (interaction *TP53**treatment: $p = 0.06$). Specifically, patients with WT *TP53* had a tendency toward better outcomes with ACT than OBS (OS: $p = 0.02$; DFS: $p = 0.005$). This effect was not seen in patients with MUT *TP53* (OS: $p = 0.63$; DFS: $p = 0.86$). In addition, in the ACT arm, a deleterious effect of MUT *TP53* was observed for both OS ($p = 0.006$) and DFS ($p = 0.02$). This effect was significant only in SCC (OS: $p = 0.007$; DFS: $p = 0.01$) and not in other histologic diagnosis subtypes, and it was restricted to a specific class of mutations predicted to disrupt the structure of the DNA-binding domain of the p53 protein (missense non-DBM mutations; OS: $p = 0.002$; DFS: $p = 0.01$). Thus, despite no statistically significant interaction between *TP53* and treatment, at least some forms of *TP53* mutations appeared to be associated with worse outcome from ACT in patients with SCC. Although the regimens differ across trials (vinorelbine in ANITA; vindesine (n = 42), vinblastine (n = 51), vinorelbine (n = 160), and etoposide (n = 271) in IALT; and vinorelbine in JBR 10 and paclitaxel in CALGB), all prognostic and predictive results were homogeneous across trials. In addition, some patients received radiotherapy (ANITA and IALT trials), but sensitivity

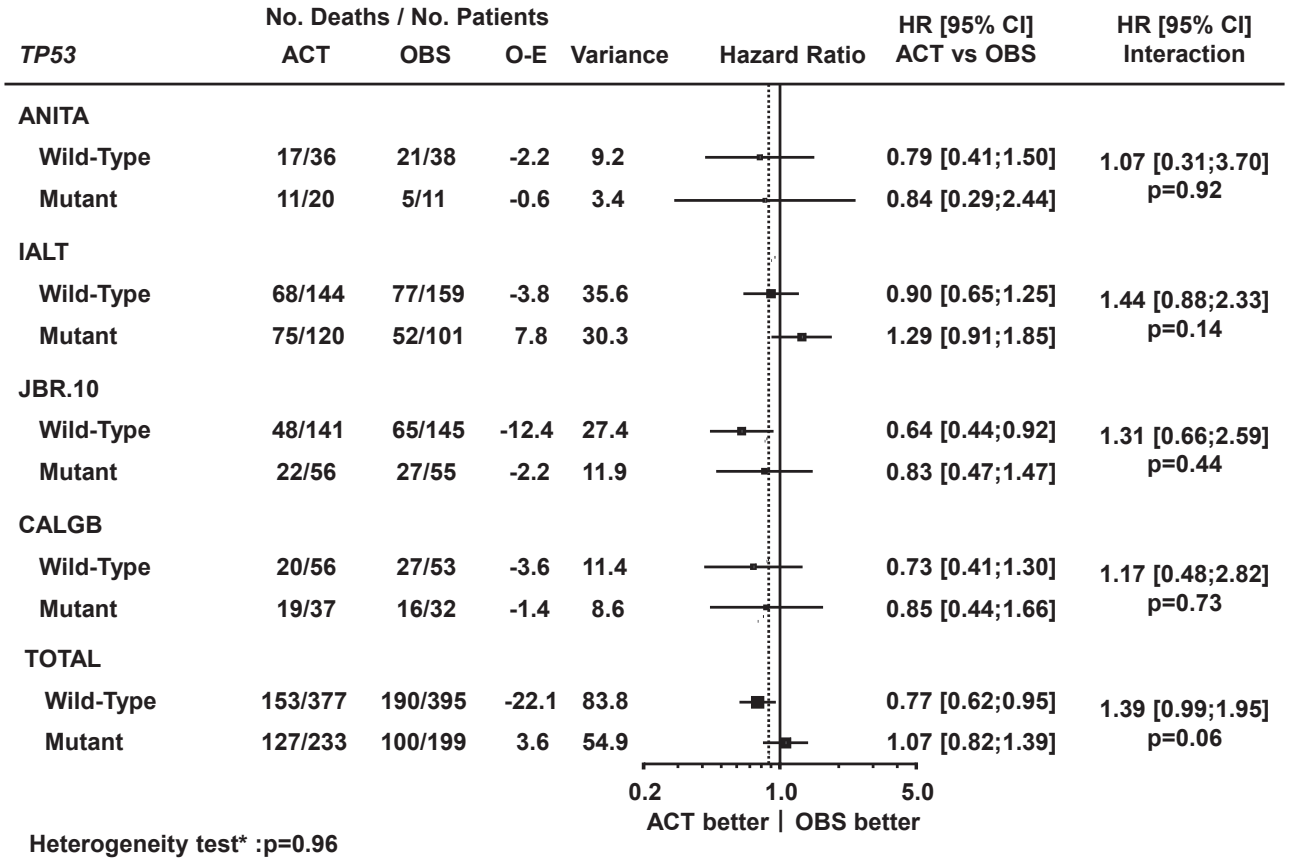

Figure 2. Forest plot of overall survival with chemotherapy (ACT) versus observation (OBS) by tumor protein p53 gene (*TP53*) mutation (wild type [WT] and mutant [MUT]). Hazard ratios (HRs) and 95% confidence intervals (CIs) are estimated using a multivariable Cox model. *Heterogeneity test refers to test for equality of the four *TP53**treatment interactions (one for each trial). Five patients were excluded owing to missing covariates. O-E, Difference between the number of deaths observed (O) and expected (E); ANITA, Adjuvant Navelbine International Trialist Association; IALT, International Adjuvant Lung Cancer Trial; JBR, J BRonchus; and CALGB, Cancer and Leukemia Group B.

analyses including radiotherapy as a covariate in the multivariable models showed similar results (data not shown).

TP53 has been shown consistently to be one of the most frequently mutated genes in lung cancers irrespective of histological type, with the vast majority of mutations clustering in exons 5 to 8.[9,12,25,26] According to the International Agency for Research on Cancer *TP53* mutation database, mutations in these regions represent more than 92.5% of all mutations in NSCLC (2102 mutations in 2292 cases matching the definition of this study).[27] In the present study, mutations outside exons 5 to 8 were not systematically analyzed because of the lack of suitable materials for all tumors. Therefore, it is possible that a few cases with mutations outside these exons have been misclassified. The definition of *mutant TP53* in this study is conservative because bias that may result from misclassification of rare mutants as *wild type* might only attenuate the significance of our results.

Whether p53 IHC staining result is a useful marker for p53 status in addition to or as a surrogate for mutation detection has long been debated.[5,26] In this study, despite an overall strong correlation ($p < 0.0001$), the results of IHC staining were positive for 33% of WT *TP53* but negative for 39% of tumors with MUT *TP53*. Whereas MUT *TP53* tumors with a negative IHC staining result were almost entirely explained by mutations causing a null p53 phenotype (truncated or abnormally spliced protein), cases with WT *TP53* in which the result of IHC staining is positive may result from multiple mechanisms (including abnormal accumulation of WT p53 in active or inactive forms). However, there was no significant difference between the four groups on the basis of combination of p53 IHC staining and *TP53* analysis results on survival, and *TP53* had no prognostic effect after controlling for IHC in a multivariable analysis. This observation stresses that mutation, whatever the IHC staining status, is the defining molecular parameter that underscores the implication of *TP53* in NSCLC.

Table 5. Predictive Effect of *TP53* Mutation on Disease-Free Survival

	Adjuvant Chemotherapy (Events per Patients)	Observation (Events per Patients)	HR (Adjuvant Chemotherapy vs. Observation) (95% CI)	Test for Interaction *TP53**treatment
All cases				
WT n = 772	179 of 377	218 of 395	0.75 (0.62-0.92) $p = 0.005$	
MUT N=432	138 of 233	115 of 199	0.98 (0.76-1.26) $p = 0.86$	
HR (MUT vs. WT) (95% CI)	1.31 (1.04-1.64) $p = 0.02$	1.01 (0.80-1.27) $p = 0.96$		$p = 0.11$
Squamous Cell Carcinoma				
WT n = 307	63 of 154	79 of 153	0.69 (0.50-0.96) $p = 0.03$	
MUT n = 215	70 of 117	58 of 98	1.00 (0.70-1.42) $p = 0.99$	
HR (MUT vs. WT) (95% CI)	1.56 (1.11-2.20) $p = 0.01$	1.08 (0.77-1.52) $p = 0.66$		$p = 0.14$
Adenocarcinoma				
WT n = 361	91 of 171	110 of 190	0.81 (0.61-1.07) $p = 0.14$	
MUT n = 143	37 of 71	40 of 72	0.82 (0.52-1.28) $p = 0.38$	
HR (MUT vs. WT) (95% CI)	0.99 (0.68-1.46) $p = 0.97$	0.98 (0.68-1.41) $p = 0.92$		$p = 0.96$
Other NSCLC histologic diagnoses				
WT n = 104	25 of 52	29 of 52	0.73 (0.42-1.25) $p = 0.24$	
MUT = 74	31 of 45	17 of 29	1.23 (0.68-2.24) $p = 0.50$	
HR (MUT vs. WT) (95% CI)	1.49 (0.87-2.55) $p = 0.14$	0.88 (0.48-1.62) $p = 0.68$		$p = 0.20$
Heterogeneity test[a]	$p = 0.20$	$p = 0.83$		
Interaction test[b]				$p = 0.48$

[a]Test for equality of the three hazard ratios (MUT vs. WT) between histologic diagnosis subtypes in the adjuvant chemotherapy and observation arms, separately.
[b]*p* Value of the third-order interaction (histologic diagnosis**TP53**treatment) (i.e., equality of TP53*treatment interaction between histology subtypes. The *p* value of the nullity of the three interaction terms was 0.28. Five patients were excluded owing to missing covariates.
TP53, tumor protein p53 gene; HR, hazard ratio of event, HR (MUT vs. WT), hazard ratio for mutant vs. wild-type; CI, confidence interval; NSCLC, non-small cell lung cancer.

The tendency toward a benefit from ACT among patients with WT *TP53* is compatible with the hypothesis that suppressor activities associated with WT p53 may contribute to better long-term benefit from ACT. One possible mechanism is that tumors with MUT *TP53*, particularly SCC, may escape DNA damage–dependent cell senescence and apoptosis induced by ACT. This interpretation is consistent with the reported association between WT *TP53* genotype and positive response to induction chemotherapy using cisplatin and etoposide in advanced NSCLC.[5] On the other hand, the tendency toward association between MUT *TP53* and worse outcomes with ACT is suggestive of a negative effect of some mutants on long-term benefit from ACT. The strongest negative effect was observed for mutations causing amino acid substitution in the hydrophobic core domain of the p53 protein, outside the DNA- binding surface (missense non-DBM mutations). These mutations are thought to inactivate p53 by disrupting the correct folding of a hydrophobic core made of two β sheets that support the DNA-binding surface of the protein.[28] An interesting hypothesis to explain this association is that these mutations may exert a GOF effect that interferes with response to cisplatin-based chemotherapy. Several molecular mechanisms underlying mutant *TP53* GOF have been proposed. For example, structurally altered mutant p53 proteins may exert "prion-like" effects by binding to other suppressor proteins such as p63, interfering with its capacity to control squamous cell differentiation.[29–31] Of note, the P63 locus (3q27-28) is frequently amplified in lung SCC, in which the deltaNp63 protein isoform is almost systematically overexpressed. This protein promotes the renewal of progenitors of squamous mucosa and is therefore an important molecular factor underlying squamous differentiation in the bronchial epithelium.[32–34] Other mechanisms of mutant p53 GOF effects include perturbation of steroid metabolism through interaction with the transcription factor sterol

regulatory element binding protein[35] or enhanced cancer cell metabolism through direct inhibition of 5′ adenosine monophosphate–activated protein kinase.[36] In lung cancer, several forms of mutant p53 have been suggested to exert a GOF effect by enhancing the expression of the receptor tyrosine kinase Axl.[37]

An alternative explanation, however, should be considered. Missense non-DBM mutations have been found to be particularly common in SCC specimens from heavy smokers, occurring at sites of adduct formation by carcinogens from tobacco smoke such as polycyclic aromatic hydrocarbons.[38] For example, mutations at codons 157 and 158, both belonging to the "missense non-DBM" mutation class, represent a mutation hotspot in SCC of smokers that is not observed in other cancers or in lung cancers of never-smokers.[39,40] Thus, it is possible that this class of mutation may be associated with a more aggressive tumor phenotype, not by virtue of specific functional characteristics but by virtue of the fact that they represent biomarkers of mutagenic exposure to tobacco smoke. Importantly, large-scale analyses of lung cancer genomes of smokers have shown that these tumors contain up to 10-fold more genetic alterations than do those of non-smokers,[41,42] attesting to the overwhelming mutational power of exposure to cigarette smoke. These tumors may contain extremely rearranged genomes and therefore exhibit a phenotypic plasticity under challenge by ACT, which enhances their capacity to resist and ultimately escape the effects of therapy. In addition, these tumors may also contain sweeping epigenetic changes causing profound changes in gene expression patterns.[43–45] These profound genetic, genomic, and epigenetic alterations may combine to promote the emergence of very aggressive tumor phenotypes.

In summary, *TP53* mutation is not prognostic and has only a marginal predictive effect for ACT in patients with completely resected NSCLC. Thus, at this time, it cannot be recommended as tool to select NSCLC patients for ACT. However, our results show differences in outcomes among patients with *TP53* mutations according to tumor histology as well as according to type and position of the mutation. These results suggest that specific mutations may be associated with a differential outcome from chemotherapy. The possibility that specific types of mutations may be associated with biological or genetic characteristics that predict poor outcome from ACT requires further investigation.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2016.02.002.

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