Immunohistochemistry of p53 surrogates *TP53* mutation as an accurate predictor for early-relapse of surgically resected stage I-III lung adenocarcinoma

Yasuyuki Kurihara, MD,[a] Takayuki Honda, MD, PhD,[b] Akira Takemoto, MD, PhD,[c] Katsutoshi Seto, MD, PhD,[a] Satoshi Endo, MD,[b] Kousuke Tanimoto, MD, PhD,[d] Susumu Kirimura, MD, PhD,[e] Masashi Kobayashi, MD, PhD,[a] Shunichi Baba, MD, PhD,[a] Yasuhiro Nakashima, MD, PhD,[a] Ryo Wakejima, MD, PhD,[a] Rie Sakakibara, MD, PhD,[b] Hironori Ishibashi, MD, PhD,[a] Johji Inazawa, MD, PhD,[d] Toshihiro Tanaka, MD, PhD,[c] Yasunari Miyazaki, MD, PhD,[b] and Kenichi Okubo, MD, PhD[a]

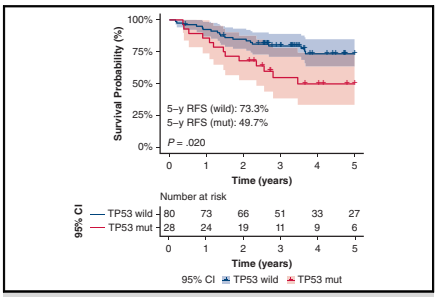
ABSTRACT

Introduction: *TP53* is a strong tumor suppressor gene; its deactivation contributes to carcinogenesis and influences clinical outcomes. However, the prognostic influence of p53 deactivation on early relapse in patients with surgically resected non–small cell lung cancer remains unclear.

Materials and methods: A cohort of 170 patients with primary stage I through III lung adenocarcinoma (LADC) and lung squamous cell carcinoma who underwent complete resection at Tokyo Medical and Dental University was screened for *TP53* mutations using panel testing, and association studies between *TP53* mutations and clinical data, including histology and postoperative recurrence, were performed. The association between *TP53* mutations and postoperative recurrence was validated using data from 604 patients with MSK-IMPACT from The Cancer Genome Atlas. Additional immunohistochemistry for p53 was performed on some subsets of the Tokyo Medical and Dental University population.

Results: Mutations in *TP53* were recurrently observed (35.9%; 61 out of 170) in the Tokyo Medical and Dental University cohort. In the histology-stratified analysis, patients with LADC histology showed *TP53* mutations that were associated with poor relapse-free survival (log-rank test; $P = .020$), whereas patients with lung squamous cell carcinoma histology showed *TP53* mutations that were not ($P = .99$). The poor prognosis of *TP53* mutation-positive LADCs was validated in The Cancer Genome Atlas-LADC cohort (log-rank test; $P = .0065$). Additional immunohistochemistry for p53 in patients with LADC histology in the Tokyo Medical and Dental University cohort showed a significant correlation between *TP53* mutations and abnormal IHC pattern of p53 (Cramer's correlation coefficient $V = 0.67$).

Conclusions: *TP53* mutation is a potential marker for worse prognosis in surgically resected LADC; immunohistochemistry for p53 could be a surrogate method to identify patients with LADC with a worse prognosis. (JTCVS Open 2024;20:183-93)



Relapse-free survival of postoperative patients with stage I through III lung adenocarcinoma.

CENTRAL MESSAGE

TP53 mutation is a potential marker for worse prognosis in surgically resected LADC; IHC for p53 could be a surrogate method to identify patients with LADC with a worse prognosis.

PERSPECTIVE

The assessments of recurrent risk and potential feasibility for AT in each patient are important, but the feasibility could not be accurately predicted at present. The study suggests p53 IHC serves as a reliable indicator of TP53 mutation status, a predictive factor for relapse of LADC in postoperative patients, that contributes to the sophistication of patient selection for AT.

From the Departments of [a]Thoracic Surgery, [b]Respiratory Medicine, and [e]Pathology, [c]Bioresource Research Center, and [d]Research Core, Tokyo Medical and Dental University, Tokyo, Japan.

Institutional Review Board Approval: The study was approved by the institutional review board of TMDU (M2022-018; June 3, 2022).

Informed Consent Statement: The need for consent was waived by the institutional review board of TMDU.

Drs Kurihara, Honda, and Takemoto contributed equally to this article.

Received for publication Feb 16, 2024; revisions received June 10, 2024; accepted for publication June 11, 2024; available ahead of print July 9, 2024.

Address for reprints: Kenichi Okubo, MD, PhD, Department of Thoracic Surgery, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8510, Japan (E-mail: okubo.thsr@tmd.ac.jp).

2666-2736

https://doi.org/10.1016/j.xjon.2024.06.005

Abbreviations and Acronyms

AT = adjuvant therapy
DBD = DNA-binding domain
IHC = immunohistochemistry
LADC = lung adenocarcinoma
LSQC = lung squamous cell carcinoma
NSCLC = non–small cell lung cancer
RFS = relapse-free survival
TCGA = The Cancer Genome Atlas
TMDU = Tokyo Medical and Dental University

Lung cancer is a significant cause of mortality among patients with solid tumors.[1] Its high recurrence rate, even after complete surgical resection for non–small cell lung cancer (NSCLC), is a possible reason for its poor prognosis. Therefore, the use of adjuvant therapy (AT) for postoperative patients with NSCLC with a high risk of recurrence should be considered to improve survival. However, the indications for AT in patients with postoperative NSCLC remain controversial. The execution rate of AT has been low in clinical practice[2,3] because the expected survival benefit of AT is not always uniform solely based on TNM stage but might depend on individual tumor or patient profiles.[4,5] Severe adverse events might cause not only treatment discontinuation but also may lead to harmful sequelae for patients' daily activities.[6] The assessments of recurrent risk and potential feasibility for AT in each patient are important; however, the feasibility of AT could not be accurately predicted at present. Therefore, tightening the indications for AT through accurate identification of postoperative patients with NSCLC at high risk of recurrence is still considered important.

Recent genomic analysis approaches, such as panel testing, have enabled oncologists to easily access representative genomic profiles of malignant tumors and extract helpful genomic information for therapeutic decisions for metastatic tumors.[7,8] Although only a few studies exist, some genomic characteristics, such as mutations in *TP53*, which controls a broad intercellular pathway, including cell cycle arrest, cell apoptosis, DNA repair, and genomic stability,[9] could be associated with worsened relapse-free survival (RFS) after surgery in patients with lung cancer.[10,11] These studies showed the potential utility of genomic analysis, especially of *TP53* mutations, for identifying patients with NSCLC at high recurrence risk. However, panel testing for tumors in clinical settings is currently widely used to identify helpful genomic information contributing to the sophistication of pharmacotherapy for patients with metastatic status, but not to predict recurrence.[8]

In gynecologic and gastrointestinal neuroendocrine neoplasms, p53 immunohistochemistry (IHC) accurately reflects the mutational status[12,13] even in small biopsied samples,[14] suggesting the potential availability of p53 IHC as a surrogate marker for *TP53* mutation. In addition, the rapidity and convenience of IHC will increase its value in terms of clinical utility. Because panel testing is not always available during the perioperative period, the results of p53 IHC could contribute to development of treatment strategies by thoracic surgeons. However, with respect to lung cancer, several previous studies have reported a low concordance and discrepancy between *TP53* mutation status and p53 IHC.[15-17] This was likely because next-generation sequencing technology used in recent panel testing was not adapted in these verification studies; thus, we hypothesized that *TP53* mutation detected using panel testing could be more matched to the results of p53 IHC. No integrated study of lung cancer showing the clinical influence of the gene mutation and the potential utility of IHC staining exists as yet; thus, we performed an association study between RFS after surgery and *TP53* mutation and validated it using The Cancer Genome Atlas (TCGA) data. The correlation between *TP53* mutation and p53 abnormal IHC staining was investigated.

MATERIALS AND METHODS

Patient Selection

A total of 170 of consecutive patients with lung adenocarcinoma (LADC) (n = 108) and lung squamous cell carcinoma (LSQC) (n = 62) aged 20 years or older, who underwent surgical resection without neoadjuvant therapy between April 2014 and March 2019 at Tokyo Medical and Dental University (TMDU) and whose specimens were preserved in the Bioresource Research Center of TMDU, were screened for 440 cancer-related genes, including *TP53* using panel testing (the TMDU cohort). Because LADC and LSQC are representative histologies in NSCLC and significant cancer-associated genes of these histologies were elucidated in previous comprehensive genomic analyses.[18] All patients were staged according to the seventh edition of the TNM classification.[19] The inclusion criteria were as follows: curative surgery with lobectomy and a definitive histologic diagnosis of LADC or LSQC. Clinical data collected from the medical records of each patient included age, gender, Eastern Cooperative Group Performance Status, history of chemotherapy-based AT, pathological findings, and RFS (defined as the time from surgery to first recurrence or death from any cause).[11] The study was conducted in accordance with the tenets of the Declaration of Helsinki (revised in 2013), was approved by the institutional review board of TMDU (M2022-018; June 3, 2022) and was granted a waiver of informed consent because of the anonymity of all patients whose data were used in the study.

DNA Screening

Detection and analysis of cancer-related Genomic mutations, including *TP53*. This study used genomic information analyzed using ACTOnco+ (ActMed Co, Ltd) and preserved at the Bioresource Research Center of TMDU. The detailed sequencing and data analysis protocols are described in the ACTOnco + Report supplied by ACT Genomics Co, LTD, as previously reported.[20] Briefly, genomic DNA from cancer cells was amplified using 4 pools of primer pairs targeting the coding exons of 440 cancer-related genes, including *TP53*. The

amplicons were ligated to barcoded adaptors. The quality and quantity of the amplified library were determined using a fragment analyzer (AATI) and Qubit (Invitrogen). Subsequently, the barcoded libraries were conjugated to sequencing beads using emulsion polymerase chain reaction and enriched using an Ion Chef system (Thermo Fisher Scientific) according to the Ion PI Hi-Q Chef Kit protocol (Thermo Fisher Scientific) or the Ion 540 Kit-Chef protocol (Thermo Fisher Scientific). Sequencing was performed using an Ion Proton or Ion S5 sequencer (Thermo Fisher Scientific).

The sequencer-generated raw reads were aligned to the hg19 reference genome employing the Ion Torrent Suite (version 5.10). Coverage depth was determined utilizing the Torrent Coverage Analysis Plug-in. Single-nucleotide variants and short insertions/deletions were identified through the Torrent Variant Caller plug-in (version 5.10). The coverage underwent downsampling to 4000. The Variant Effect Predictor (version 88) was utilized to annotate each variant by referencing databases COSMIC v.92 and dbSNP 151. Variants present in ToMMo 8.3 K or genomAD v2.1 were disregarded as polymorphisms and excluded from further analysis. Retention criteria encompassed variants with coverage $\geq$ 25 and allele frequency $\geq$5%, along with actionable variants possessing an allele frequency $\geq$2%. Variants documented in the Genome Aggregation Database r2.0.2 with a minor allele frequency >1% were categorized as polymorphisms. An in-house ACT Genomics database was consulted to identify technical errors. For subsequent analyses, variants included in dbSNP or COSMIC were considered clinically actionable and biologically significant. The outcomes of gene mutations were visualized using the *maftools* package in R version 4.1-2 (R Foundation for Statistical Computing).

External datasets from a Caucasian population extracted from TCGA. To validate the results obtained from the TMDU cohort, clinical and genomic data from patients with NSCLC in the MSK-IMPACT (Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets) study[21,22] of TCGA were obtained from cBioportal (https://www.cbioportal.org).

IHC of p53 and pathological evaluation. Formalin-fixed paraffin-embedded tumors were used in this study. A tissue microarray was conducted, and for samples that could not be assessed via tissue microarray, separate staining was carried out followed by re-evaluation. The IHC staining of p53 (DO-7; Leica Biosystem) was performed by an expert pathologist (A.T.) who blindly reviewed the specimens from mutation data. The scoring methodology involved multiplying the percentage of positively stained nuclei (0-4), assessed on a scale (0 = absent, 1 = <25%, 2 = 25%-50%, 3 = 50%-75%, and 4 = 75%-100%) by the intensity of staining (0-3), evaluated on a scale (0 = absent, 1 = <10%, 2 = 10%-50%, and 3 = 50%-100%), as previously described.[23] Abnormal staining patterns were defined as complete absence (score = 0) or overexpression (score = 4 or higher).

Statistical Analysis

Categorical variables were compared using either the χ^2 test or Fisher exact test. Numerical variables were compared using the Mann-Whitney *U* test. Survival analysis was conducted employing the Kaplan-Meier method. Concordance between the mutation status and IHC results was analyzed using Cramer's *V* test. All statistical analyses were carried out using R version 4.1-2). A Cramer's *V* value exceeding 0.25 denoted a very strong association.

RESULTS

Clinical and TP53 Genomic Characteristics of the TMDU Cohort

The TMDU cohort comprised 170 patients with NSCLC (LADC, n = 108 [63.5%] and LSQC, n = 62 [36.5%]) stages I through III, as summarized in Table 1. The TMDU cohort included 111 men (65.3%) and 59 women (34.7%) with a median age of 70 years (range, 29-87 years). Most patients in the TMDU cohort had a history of smoking (110 [64.7%]) and were current smokers (11 [6.5%]). In the TMDU cohort, 50 (29.4%) patients experienced recurrence.

TABLE 1. Clinicopathologic characteristics of the selected patients

Characteristic	TMDU cohort (N = 170)
Age (y)	69 (29-87)
Gender	
Male	111 (65.3)
Female	59 (34.7)
Smoking history	
Never	49 (28.8)
Ever	110 (64.7)
Current	11 (6.5)
ECOG-PS	
0	167 (98.2)
1	3 (1.8)
Histology	
LADC	108 (63.5)
LSQC	62 (36.5)
Stage	
I	105 (61.8)
II	37 (21.8)
III	28 (16.5)
TP53 mutation	
No	109 (64.1)
Yes	61 (35.9)
AT	
No	120 (70.6)
Yes	50 (29.4)
Recurrence	
No	120 (70.6)
Yes	50 (29.4)

Values are presented as median (range) or n (%). *TMDU*, Tokyo Medical and Dental University; *ECOG-PS*, Eastern Cooperative Oncology Group Performance Status; *LADC*, lung adenocarcinoma; *LSQC*, lung squamous cell carcinoma; *AT*, adjuvant therapy.

The genomic profile of the TMDU cohort is shown in Figure 1. Mutations in *TP53* (n = 61 [35.9%]), *EGFR* (n = 44 [25.9%]), and *KRAS* (n = 18 [10.6%]) were recurrently detected in the TMDU cohort. These data agree with those reported in previous studies on LADC and LSQC in East-Asian cohorts.[24,25]

Association Between *TP53* Mutation Status and RFS in the TMDU Cohort and its Validation Using the TCGA Data

In the association study conducted on the TMDU cohort between *TP53* mutations and RFS, a noteworthy negative correlation was revealed (log-rank test; P = .048) (Figure E1). Considering the morphological and genomic distinctions between LADC and LSQC, additional association studies were stratified by histologic type. These

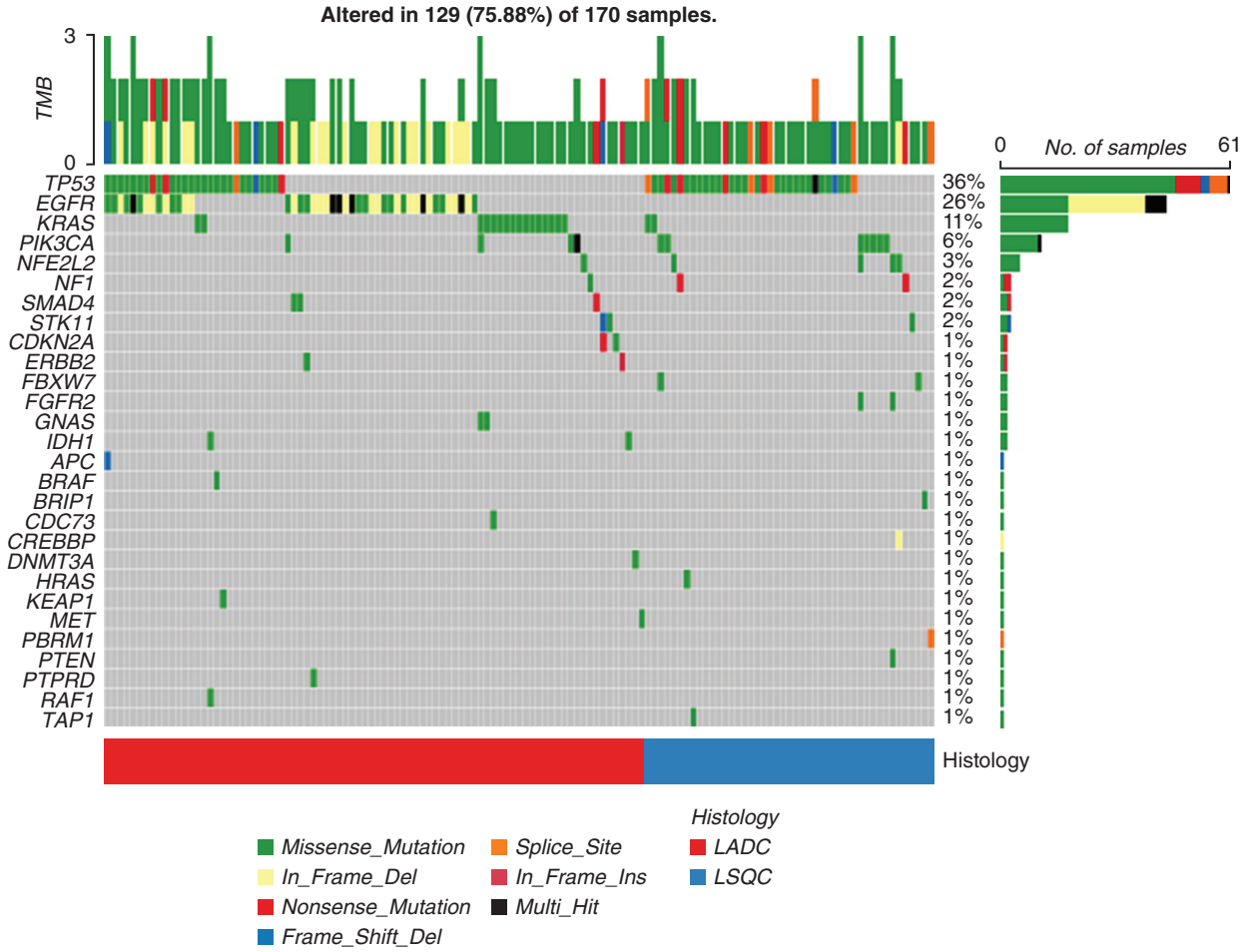


FIGURE 1. Genomic profile of the Tokyo Medical and Dental University (*TDMU*) cohort. *LADC*, Lung adenocarcinoma; *LSQC*, lung squamous cell carcinoma.

analyses indicated that patients with LADC histology displayed *TP53* mutations that were associated with poor RFS (log-rank test; $P = .020$), whereas patients with LSQC histology did not exhibit *TP53* mutations with such an association (log-rank test; $P = .99$) (Figure 2). Notably, within the LADC subset, there were no significant differences observed between *TP53* mutant and *TP53* wild-type regarding potential prognostic factors such as age and the presence of adjuvant therapy in the TMDU cohort (Table E1). This outcome implies that *TP53* mutations could potentially exert a stronger association with the prognosis of patients with LADC than with the prognoses for those with LSQC and details of *TP53* mutations detected in LADC patients are summarized in Table E2. Additionally, another genomic feature named tumor mutation burden showed a slightly higher trend in *TP53* mutant samples than in *TP53* wild-type samples (Figure E2). Data from MSK-IMPACT within TCGA, comprising 604 patients who underwent surgical resection for LADC, were extracted and employed as a validation cohort, specifically representing a Caucasian population. The statistical significance observed in the TMDU cohort regarding RFS in relation to the presence of *TP53* mutations was also noted in the TCGA cohort (log-rank test; $P = .0065$) (Figure E3).

Concordance Between *TP53* Mutation and Abnormal Staining of p53 IHC

Because our association studies between *TP53* mutation and RFS in patients with LADC showed that *TP53* mutation was a prognostic marker for surgically resected LADC, IHC of p53 for patients with LADC histology in the TMDU cohort was performed to investigate a potential utility for surrogating *TP53* mutation, and representative cases are shown in Figure E4. A recent study showed not only complete absence but also overexpression patterns of p53 IHC strongly predictive of an underlying *TP53* mutations reflecting[26] that the majority of *TP53* mutations are missense mutations producing mutant proteins that accumulate in the nucleus of tumor cells and can be detected through positive IHC.[27] Our scoring system was based on a previous study[23] in which abnormal staining patterns were defined as complete absence or overexpression using lung cancer tissue samples.

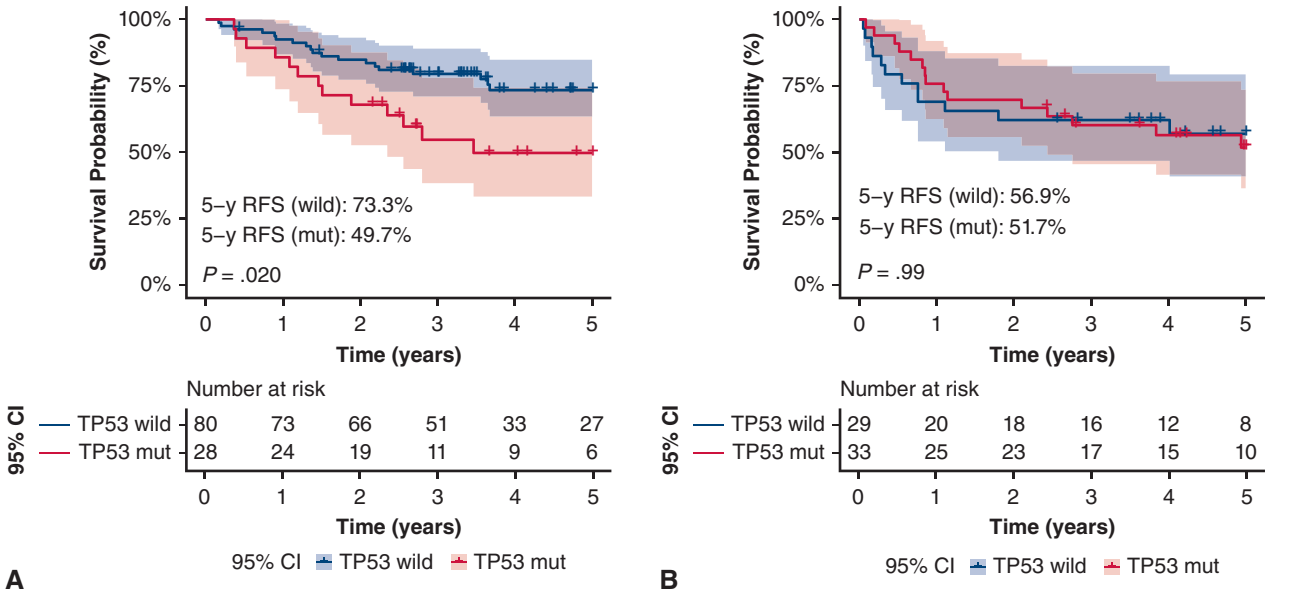


FIGURE 2. Relapse-free survival of patients with primary stage I through III lung adenocarcinoma (*LADC*) (A) and lung squamous cell carcinoma (*LSQC*) (B) who underwent lung resection.

Cramer's *V* test showed high concordance between *TP53* mutation and p53 abnormal IHC staining (Table 2). This result suggested that abnormal p53 IHC staining could be a surrogate marker for predicting early relapse in surgically resected LADC. Patients with LADC in the TMDU cohort were re-sorted according to the results of p53 staining. Patients with LADC showing abnormal p53 staining exhibited worse RFS than did those showing normal p53 staining (Figure E5). To adjust potential confounding factors for RFS, additional Cox-regression multivariable analysis was performed to demonstrate that not only tumor stage (hazard ratio, 7.3; 95% CI, 3.35-15.8; $P < .001$) but also abnormal p53 IHC (hazard ratio, 2.2; 95% CI, 1.08-4.22; $P = .030$) was significantly and independently associated with poor RFS (Table 3).

DISCUSSION

In this study, we validated *TP53* mutation was associated with early relapse in patients with surgically resected LADC, and IHC of p53 showed high concordance with the mutation status, suggesting that IHC of p53 could be a surrogate marker of early relapse in patients with resected LADC. After adjusting for potential confounding factors, abnormal p53 IHC was still found to be independently associated with poor RFS. This study may contribute in providing a convenient and reproducible methods to identify poor RFS, thereby aiding thoracic surgeons in the development of accurate treatment strategies during the perioperative period.

The transcription factor p53 is encoded by a tumor suppressor gene involved in the response to DNA damage, oxidative stress, and oncogenic hyperproliferation.[28] Its gene *TP53* is the most frequently mutated gene in human cancers, including lung cancer. The 2 primary histological categories of NSCLC consist of LADC and LSQC, with *TP53* identified as the most prominently mutated gene in both classes.[18] Previous reports have indicated the potential malignancy associated with *TP53* mutations, which suggests their contribution to a poorer prognosis. Moreover, these mutations have been linked to strong carcinogenic effects. Translational studies of *TP53* mutations in lung cancer reported that the mutations could have a clinical influence on the drug resistance of NSCLC[29] and early relapse in surgically resected NSCLC.[10,11] In our study, we also validated the negative prognostic influence of *TP53* mutations in LADC, showing an association between these mutations and early relapse after surgery both in our Japanese and TCGA cohorts, which mainly consisted of Caucasian populations. Contrary to LADC, *TP53* mutations could not serve as a prognostic indicator for LSQC. Several reasons may explain this phenomenon. Patients with LSQC are up to 94% more likely to harbor *TP53* mutations but this mutation is not always associated with prognosis in certain cancers such as LSQC in TCGA cohort.[30] Carcinogens from smoking form DNA adducts resulting in aberrant function of various cancer-associated

TABLE 2. Association of *TP53* mutation and p53 immunohistochemistry (IHC) pattern in lung adenocarcinoma

TP53	p53 IHC		Total
	Normal	Abnormal	
Wild-type	63	17	80
Mutation	1	27	28
Total	64	44	108
Cramer's *V*			0.67

TABLE 3. Univariable and multivariable Cox regression analysis for relapse-free survival in the patients with lung adenocarcinoma of the Tokyo Medical and Dental University cohort

Parameter	Univariable			Multivariable		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age ≥70 vs <70 y	1.1	0.53-2.13	.87			
Gender, male vs female	0.92	0.46-1.85	.81			
Smoking history, ever or current vs never	0.69	0.34-1.38	.29			
Histologic subtype, solid or with micropapillary pattern vs others	1.2	0.56-2.35	.71			
Stage, II and III vs I	**7.3**	**3.38-15.9**	**<.001**	**7.3**	**3.35-15.8**	**<.001**
AT, yes vs no	1.2	0.56-2.39	.70			
p53 IHC, abnormal vs normal	**2.2**	**1.11-4.49**	**.025**	**2.2**	**1.08-4.42**	**.030**

The variables with *P*-value <.05 in univariable analysis were included in multivariable analysis. Those with *P*-value <.05 in multivariable analysis were shown in bold. *AT*, Adjuvant therapy; *IHC*, immunohistochemistry.

genes. Chromosomal aberrations in LSQC could be grouped into a broad category, compromising not only loss of cell cycle regulation (*TP53, RB1, CDKN2A, MYC*) but also expression of squamous cell differentiation pathways (*NOTCH, SOX2, TP63*), upregulation of oncogenic signaling through the RAS and PI3K pathways, and abnormalities in epigenetic regulators (*KMT2D, NSD1, KDM6A*). LSQC could be influenced by a wide variety of biological pathways and thus may not be determined only by loss of p53 function due to *TP53* mutations.

Previous studies of gynecologic and gastrointestinal neuroendocrine neoplasms, except lung cancer, reported high accuracy when using p53 IHC to predict *TP53* mutations.[12,13] However, our study also showed high concordance between abnormal IHC and these mutations, in contrast to previous studies of lung cancer.[15-17] Additionally, the prognostic influence of *TP53* mutations and/or abnormal p53 IHC had not been integrated, varying from previous reports,[15-17] but our study integrated the clinical importance of both *TP53* mutations and p53 IHC, although subgroup analysis according to tumor stage could not stratify RFS of stage I LADC, for which stage the indications for adjuvant therapy are still not straightforward (data not shown). Several factors could explain the difference between the previous results and our findings. The evaluation of p53 by IHC differed according to the study. In previous reports,[15-17] *TP53* mutation detection was mainly performed using the Sanger sequencing-based assay and preferentially screened at least exons 5 to 8, which comprise the DNA-binding domain (DBD) categorized as a classical hotspots.[31] The unbiased screening using next-generation sequencing showed approximately 20% of point mutations and insertions/deletions occurring outside the DBD hotspot,[32] in fact, 17.9% of gene aberrations outside the DBD hotspot were also observed in the TMDU cohort (data not shown). These gene aberrations outside the DBD hotspot of *TP53* could also interfere with p53 activities[32] supporting our results of worse prognosis of LADC patients with *TP53* mutations than of those with *TP53* wild type.

Recent evidence of AT shows that not only platinum-based chemotherapy but also molecular-targeted or immune-oncology drugs have a significant benefit for the survival of patients after NSCLC surgery,[33-35] suggesting the importance of seamless screening of biomarkers for molecular-targeted or immune-oncology drugs after surgery. However, not all of the patients with LADC carry druggable driver mutations or high programmed death-ligand 1 (PD-L1) expression, and nonnegligible frequencies of adverse events would develop during therapies.[34,35] p53 IHC data could help oncologists select LADC patients for AT while considering the risks and benefits of AT. In fact, in the TMDU cohort, LADC patients with *TP53* mutations showed a higher tumor mutation burden than did those with *TP53* wild-type. These data would further contribute to the increase in sophistication of patient selection for AT using immuno-oncology drugs.

A few limitations were identified in our study. Primarily, it was a retrospective analysis encompassing a restricted number of patients. Secondly, our study lacked assessment of the interplay between driver oncogene and PD-L1 status. Our results on p53 IHC could not simply be applied when LADC shows positive of driver mutation such as *EGFR* of high PD-L1 status expression. Our results should be carefully interpreted. Additionally, we did not validate the association between p53 IHC results and AT efficacy. Acknowledging these constraints, there is a need for further prospective studies to thoroughly investigate and ascertain the utility of p53 IHC.

CONCLUSIONS

Our study revealed that individuals diagnosed with LADC and possessing *TP53* mutations experienced a

shorter recurrence-free survival than did those lacking these mutations. We noted a significant correlation between *TP53* mutation and abnormal p53 IHC results, suggesting that p53 IHC might serve as a convenient surrogate marker to identify the mutation status.

Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing articles for which they may have a conflict of interest. The editors and reviewers of this article reported no conflicts of interest.

The authors thank Editage (www.editage.jp) for English language editing.

References

1. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer*. 2019;144(8):1941-1953. https://doi.org/10.1002/ijc.31937
2. West H, Hu X, Zhang S, et al. Treatment patterns and outcomes in resected early-stage non-small cell lung cancer: an analysis of the SEER-Medicare data. *Clin Lung Cancer*. 2023;24(3):260-268. https://doi.org/10.1016/j.cllc.2022.12.005
3. Lee JM, Wang R, Johnson A, Ogale S, Kent M, Lee JS. Real-world adjuvant chemotherapy patterns and outcomes among elderly patients with resected early non-small-cell lung cancer in the USA. *Futur Oncol*. 2023;19(1):37-47. https://doi.org/10.2217/fon-2022-0845
4. Pathak R, Goldberg SB, Canavan M, et al. Association of survival with adjuvant chemotherapy among patients with early-stage non-small cell lung cancer with vs without high-risk clinicopathologic features. *JAMA Oncol*. 2020;6(11):1741-1750. https://doi.org/10.1001/jamaoncol.2020.4232
5. Pignon J-P, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *J Clin Oncol*. 2008;26(21):3552-3559. https://doi.org/10.1200/JCO.2007.13.9030
6. Tao JJ, Visvanathan K, Wolff AC. Long term side effects of adjuvant chemotherapy in patients with early breast cancer. *Breast*. 2015;24(0 2):S149-S153. https://doi.org/10.1016/j.breast.2015.07.035
7. Carr TH, McEwen R, Dougherty B, et al. Defining actionable mutations for oncology therapeutic development. *Nat Rev Cancer*. 2016;16(5):319-329. https://doi.org/10.1038/nrc.2016.35
8. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*. 2017;23(6):703-713. https://doi.org/10.1038/nm.4333
9. Kastenhuber ER, Lowe SW. Putting p53 in context. *Cell*. 2017;170(6):1062-1078. https://doi.org/10.1016/j.cell.2017.08.028
10. Goto T, Kunimasa K, Hirotsu Y, et al. Association of mutation profiles with postoperative survival in patients with non-small cell lung cancer. *Cancers (Basel)*. 2020;12(11):3472. https://doi.org/10.3390/cancers12113472
11. Jones GD, Brandt WS, Shen R, et al. A genomic-pathologic annotated risk model to predict recurrence in early-stage lung adenocarcinoma. *JAMA Surg*. 2021;156(2):e205601. https://doi.org/10.1001/jamasurg.2020.5601
12. Yemelyanova A, Vang R, Kshirsagar M, et al. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis. *Mod Pathol*. 2011;24(9):1248-1253. https://doi.org/10.1038/modpathol.2011.85
13. Li J, Wang J, Su D, et al. p53 Immunohistochemistry patterns are surrogate biomarkers for TP53 mutations in gastrointestinal neuroendocrine neoplasms. *Gastroenterol Res Pract*. 2021;2021:2510195. https://doi.org/10.1155/2021/2510195
14. Singh N, Piskorz AM, Bosse T, et al. p53 immunohistochemistry is an accurate surrogate for TP53 mutational analysis in endometrial carcinoma biopsies. *J Pathol*. 2020;250(3):336-345. https://doi.org/10.1002/path.5375
15. Scoccianti C, Vesin A, Martel G, et al. Prognostic value of TP53, KRAS and EGFR mutations in nonsmall cell lung cancer: the EUELC cohort. *Eur Respir J*. 2012;40(1):177-184. https://doi.org/10.1183/09031936.00097311
16. Tsao M-S, Aviel-Ronen S, Ding K, et al. Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer. *J Clin Oncol*. 2007;25(33):5240-5247. https://doi.org/10.1200/JCO.2007.12.6953
17. Mitsudomi T, Oyama T, Nishida K, et al. p53 nuclear immunostaining and gene mutations in non-small-cell lung cancer and their effects on patient survival. *Ann Oncol*. 1995;6(suppl 3):S9-S13. https://doi.org/10.1093/annonc/6.suppl_3.s9
18. Campbell JD, Alexandrov A, Kim J, et al. Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nat Genet*. 2016;48(6):607-616. https://doi.org/10.1038/ng.3564
19. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol*. 2007;2(8):706-714. https://doi.org/10.1097/JTO.0b013e31812f3c1a
20. Honda T, Seto K, Endo S, et al. The possibility of mutations of RAS signaling genes and/or TP53 in combination as a negative prognostic impact on pathological stage I non-small cell lung cancer. *Cancer Med*. 2023;12(19):19406-19413. https://doi.org/10.1002/cam4.6535
21. Jamal-Hanjani M, Wilson GA, McGranahan N, et al. Tracking the evolution of non-small-cell lung cancer. *N Engl J Med*. 2017;376(22):2109-2121. https://doi.org/10.1056/NEJMoa1616288
22. Abbosh C, Birkbak NJ, Wilson GA, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature*. 2017;545(7655):446-451. https://doi.org/10.1038/nature22364
23. Ma X, Le Teuff G, Lacas B, et al. 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. *J Thorac Oncol*. 2016;11(6):850-861. https://doi.org/10.1016/j.jtho.2016.02.002
24. Saito M, Shiraishi K, Kunitoh H, Takenoshita S, Yokota J, Kohno T. Gene aberrations for precision medicine against lung adenocarcinoma. *Cancer Sci*. 2016;107(6):713-720. https://doi.org/10.1111/cas.12941
25. Kim Y, Hammerman PS, Kim J, et al. Integrative and comparative genomic analysis of lung squamous cell carcinomas in East Asian patients. *J Clin Oncol*. 2014;32(2):121-128. https://doi.org/10.1200/JCO.2013.50.8556
26. Köbel M, Ronnett BM, Singh N, Soslow RA, Gilks CB, McCluggage WG. Interpretation of P53 immunohistochemistry in endometrial carcinomas: toward increased reproducibility. *Int J Gynecol Pathol*. 2019;38(suppl 1):S123-S131. https://doi.org/10.1097/PGP.0000000000000488
27. Olivier M, Taniere P. Somatic mutations in cancer prognosis and prediction: lessons from TP53 and EGFR genes. *Curr Opin Oncol*. 2011;23(1):88-92. https://doi.org/10.1097/cco.0b013e3283412dfa
28. Levine AJ, Oren M. The first 30 years of p53: growing ever more complex. *Nat Rev Cancer*. 2009;9(10):749-758. https://doi.org/10.1038/nrc2723
29. Kim Y, Lee B, Shim JH, et al. Concurrent genetic alterations predict the progression to target therapy in EGFR-mutated advanced NSCLC. *J Thorac Oncol*. 2019;14(2):193-202. https://doi.org/10.1016/j.jtho.2018.10.150
30. Li VD, Li KH, Li JT. TP53 mutations as potential prognostic markers for specific cancers: analysis of data from The Cancer Genome Atlas and the International Agency for Research on Cancer TP53 Database. *J Cancer Res Clin Oncol*. 2019;145(3):625-636. https://doi.org/10.1007/s00432-018-2817-z
31. Kennedy MC, Lowe SW. Mutant p53: it's not all one and the same. *Cell Death Differ*. 2022;29(5):983-987. https://doi.org/10.1038/s41418-022-00989-y
32. Bouaoun L, Sonkin D, Ardin M, et al. TP53 variations in human cancers: new lessons from the IARC TP53 database and genomics data. *Hum Mutat*. 2016;37(9):865-876. https://doi.org/10.1002/humu.23035
33. Arriagada R, Auperin A, Burdett S, et al. Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data. *Lancet*. 2010;375(9722):1267-1277. https://doi.org/10.1016/S0140-6736(10)60059-1
34. Wu Y-L, Tsuboi M, He J, et al. Osimertinib in resected EGFR-mutated non-small-cell lung cancer. *N Engl J Med*. 2020;383(18):1711-1723. https://doi.org/10.1056/NEJMoa2027071
35. Felip E, Altorki N, Zhou C, et al. Adjuvant atezolizumab after adjuvant chemotherapy in resected stage IB-IIIA non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase 3 trial. *Lancet*. 2021;398(10308):1344-1357. https://doi.org/10.1016/S0140-6736(21)02098-5

Key Words: lung adenocarcinoma, *TP53*, immunohistochemistry, early relapse

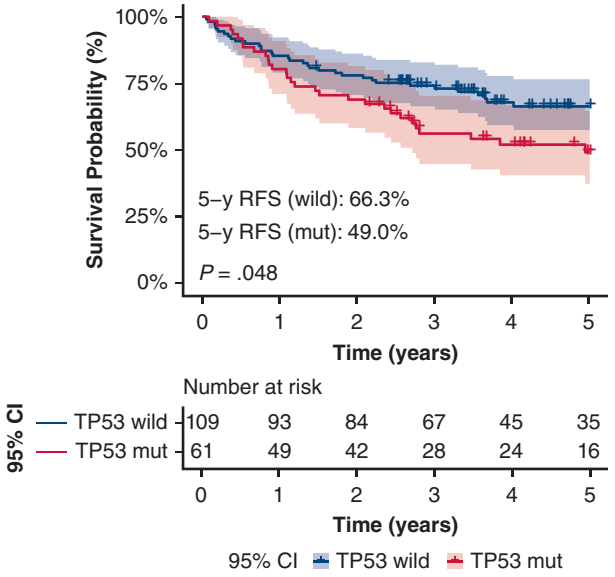


FIGURE E1. Relapse-free survival of patients with primary stage I through III lung carcinoma in the Tokyo Medical and Dental University cohort is displayed using Kaplan-Meier curve. *P* values are derived from log-rank test and the number at risk tables of each group are also displayed.

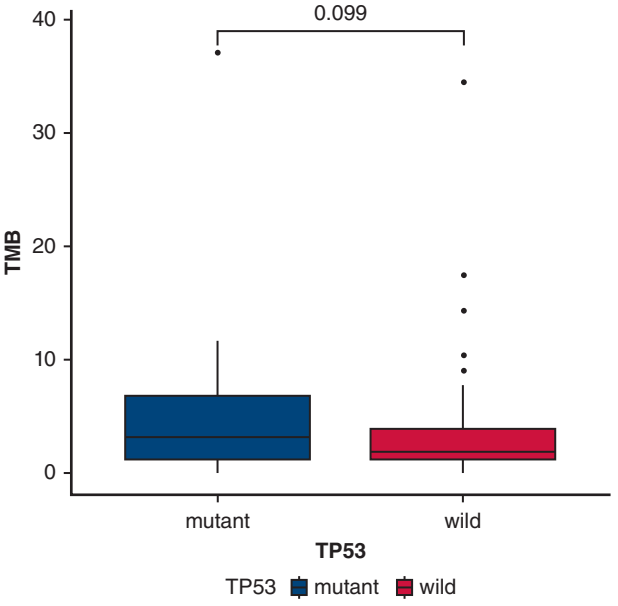


FIGURE E2. Tumor mutation burdens between lung adenocarcinoma with mutant and wild-type *TP53*. *P* values was calculated using Mann-Whitney *U* test. Boxplot shows the 50th, 25th, and 75th percentiles (*middle, bottom, and top lines of the boxes*, respectively) and values outside the 95th percentile (shown as *dots*).

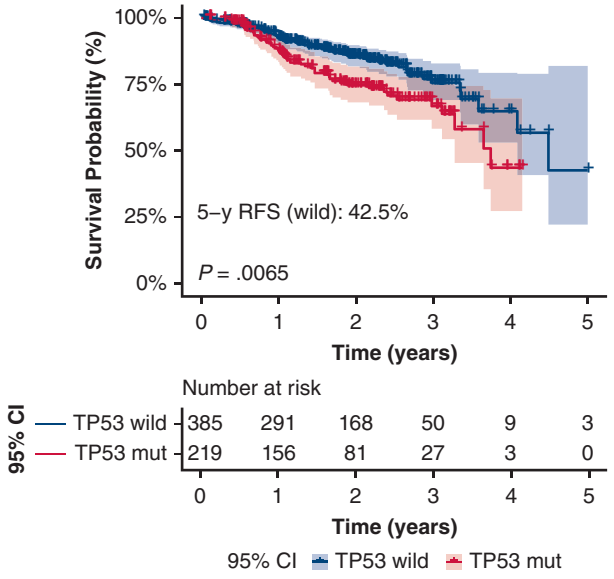


FIGURE E3. Relapse-free survival of patients with primary stage I through III lung adenocarcinoma in the The Cancer Genome Atlas cohort is displayed using Kaplan-Meier curve. *P* values are derived from log-rank test and the number at risk tables of each group are also displayed.

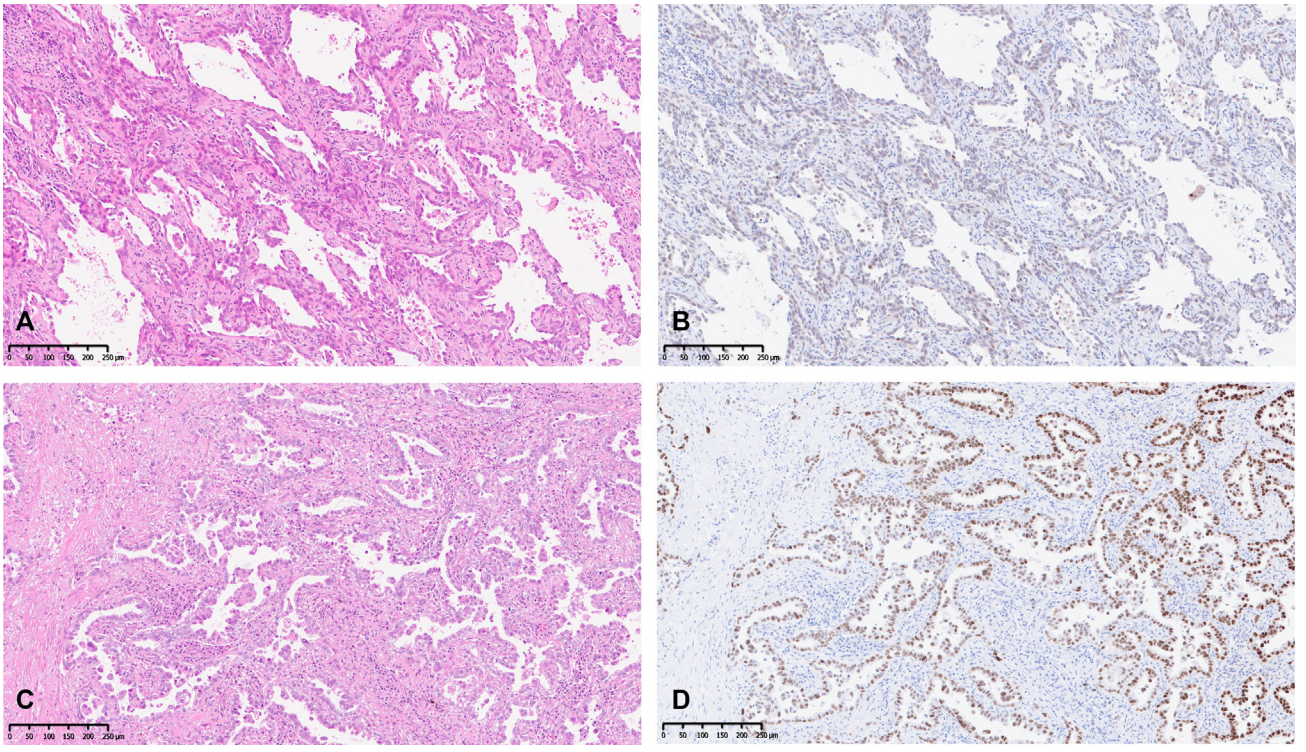


FIGURE E4. Representative cases of immunohistochemistry staining for lung adenocarcinoma (*LADC*) in the Tokyo Medical and Dental University cohort. Pathological images of hematoxylin-eosin (HE) (A) and p53 (B) stain for LADC with wild-type *TP53* and HE (C) and p53 (D) for LADC with *TP53* mutation.

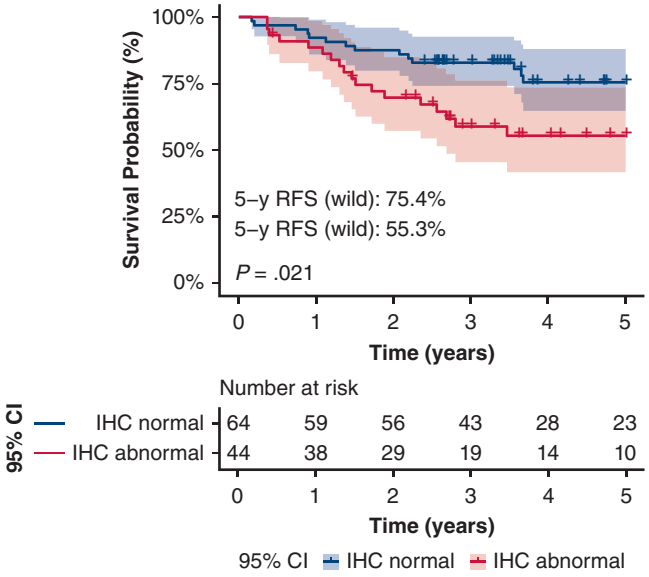


FIGURE E5. Relapse-free survival of patients with primary stage I through III lung adenocarcinoma stratified according to p53 IHC staining is displayed using Kaplan-Meier curve. *P* values are derived from log-rank test and the number at risk tables of each group are also displayed. *IHC*, Immunohistochemistry.

TABLE E1. Clinicopathological characteristics of the patients with lung adenocarcinoma (LADC) in the Tokyo Medical and Dental University (TMDU) cohort

Characteristics	LADC in TMDU cohort (n = 108)		*P* value
	TP53 Mutant (n = 28)	TP53 Wild-type (n = 80)	
Age (y)	69.5 (49-84)	70 (29-87)	.62
Gender (%)			1
Male	17	47	
Female	11	330	
Smoking history			.87
Never	10 (35.7)	33 (41.3)	
Ever	17 (60.7)	44 (55.0)	
Current	1 (3.6)	3 (3.8)	
ECOG-PS			1
0	27 (96.4)	79 (98.8)	
1	1 (3.6)	1 (1.3)	
Histology subtype			.13
Solid or with micropapillary pattern	20 (71.4)	42 (52.5)	
Others	8 (28.6)	38 (47.5)	
Stage			.27
I	16 (57.1)	56 (70.0)	
II	6 (21.4)	16 (20.0)	
III	6 (21.4)	8 (10.0)	
AT			.33
No	15 (53.6)	61 (76.3)	
Yes	13 (46.4)	19 (23.8)	

Values are presented as median (range) or n (%). *ECOG-PS*, Eastern Cooperative Oncology Group Performance Status; *AT*, adjuvant therapy.

TABLE E2. Detailed descriptions of *TP53* mutations observed in the patients with lung adenocarcinoma of the Tokyo Medical and Dental University cohort

Case	HGVSc	HGVSp	Consequences	Pathogenicity	p53 IHC
1	c.902delC	p.Pro301GlnfsTer44	frameshift_variant	LOF	Overexpression
2	c.817C>A	p.Arg273Ser	missense_variant	LOF	Complete absence
3	c.818G>T	p.Arg273Leu	missense_variant	GOF	Overexpression
4	c.451C>T	p.Pro151Ser	missense_variant	GOF	Overexpression
5	c.517G>T	p.Val173Leu	missense_variant	GOF	Overexpression
6	c.517G>T	p.Val173Leu	missense_variant	GOF	Overexpression
7	c.818G>T	p.Arg273Leu	missense_variant	GOF	Overexpression
8	c.818G>T	p.Arg273Leu	missense_variant	GOF	Overexpression
9	c.746G>C	p.Arg249Thr	missense_variant	GOF	Overexpression
10	c.329G>T	p.Arg110Leu	missense_variant	LOF	Overexpression
11	c.380C>T	p.Ser127Phe	missense_variant	LOF	Overexpression
12	c.578A>T	p.His193Leu	missense_variant	LOF	Overexpression
13	c.569C>G	p.Pro190Arg	missense_variant	LOF	Overexpression
14	c.422G>A	p.Cys141Tyr	missense_variant	LOF	Overexpression
15	c.853G>A	p.Glu285Lys	missense_variant	LOF	Overexpression
16	c.329G>T	p.Arg110Leu	missense_variant	LOF	Overexpression
17	c.427G>A	p.Val143Met	missense_variant	LOF	Overexpression
18	c.713G>C	p.Cys238Ser	missense_variant	LOF	Overexpression
19	c.832C>T	p.Pro278Ser	missense_variant	LOF	Overexpression
20	c.1009C>T	p.Arg337Cys	missense_variant	LOF	Overexpression
21	c.730G>A	p.Gly244Ser	missense_variant	LOF	Overexpression
22	c.821T>C	p.Val274Ala	missense_variant	LOF	Overexpression
23	c.722C>G	p.Ser241Cys	missense_variant	LOF	Overexpression
24	c.743G>T	p.Arg248Leu	missense_variant	LOF	Overexpression
25	c.96+1G>C	-	splice_donor_variant	LOF	Complete absence
26	c.574C>T	p.Gln192Ter	stop_gained	LOF	Complete absence
27	c.202G>T	p.Glu68Ter	stop_gained	LOF	Complete absence
28	c.892G>T	p.Glu298Ter	stop_gained	LOF	Normal

HGVS, Human Genome Variation Society; *IHC*, immunohistochemistry; *LOF*, loss of function; *GOF*, gain of function.