BRIEF REPORT

Prognostic Impact of Tumor Mutation Burden in Patients With Completely Resected Non-Small Cell Lung Cancer: Brief Report

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ABSTRACT

Introduction: Tumor mutation burden (TMB) is thought to be associated with the amount of neoantigen in the tumor and to have an important role in predicting the effect of immune checkpoint inhibitors. However, the relevance of TMB to prognosis is not yet fully understood. In this study, we investigated the clinical significance of TMB in patients with NSCLC and examined the relationship between TMB and prognosis.

Methods: We calculated TMB within individual tumors by whole-exome sequencing analysis using next-generation sequencing. We included that there were 90 patients with NSCLC who underwent surgery in the Hospital of Fukushima Medical University from 2013 to 2016. No patients received chemotherapy or immunotherapy before surgery. We assessed the correlation between TMB and prognosis.

Results: TMB greater than 62 was associated with worse overall survival (OS) of patients with NSCLC (hazard ratio [HR] = 6.633, $p = 0.0003$). Multivariate analysis showed poor prognosis with high TMB (HR = 12.31, $p = 0.019$). In patients with stage I NSCLC, higher TMB was associated with worse prognosis for both OS (HR = 7.582, $p = 0.0018$) and disease-free survival (HR = 6.07, $p = 0.0072$).

Conclusions: High TMB in NSCLC is a poor prognostic factor. If high TMB is a predictor of the efficacy of immune checkpoint inhibitors, postoperative adjuvant therapy with immune checkpoint inhibitors may contribute to improvement of recurrence and OS.

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Keywords: Tumor mutation burden; NSCLC; Prognosis; Immune-checkpoint inhibitor

Introduction

Progress in immunotherapy has been shown in the development of immune checkpoint inhibitors that target programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1).[1–3] For patients with NSCLC as well as melanoma or other types of cancer, discovery of definitive predictive biomarkers for treatment efficacy is one of the major issues.

The KEYNOTE 024 trial of first-line pembrolizumab for treatment of NSCLC showed that patients with a high level of PD-L1 expression (≥50%) in the tumor had a clear survival benefit.[1] Subgroup analysis of the Checkmate 026 trial of first-line nivolumab for treatment of NSCLC showed that tumor mutation burden (TMB), as

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well as PD-L1 expression, could be a predictive biomarker.[4] Furthermore, Roszik et al. reported that patients with high TMB showed better prognosis after treatment with pembrolizumab.[5]

Thus, TMB has attracted attention as a biomarker of immune checkpoint inhibitor efficacy; however, it is still unclear how TMB affects tumor behavior. In this study, we aimed to clarify the clinical and survival impact of TMB in patients with NSCLC.

Methods

Patients and Specimens

The study was approved by the Human Ethics Committee at Fukushima Medical University. We enrolled 90 consecutive patients who gave informed consent. Whole-exome sequencing (WES) was conducted in accordance with the Ethical Guidelines for Human Genome and Genetic Analysis Research. Patients had not received any chemotherapy or immunotherapy before surgery. Disease staging was evaluated according to TNM classification of lung cancer 7th edition. We divided two groups in terms of tumor size by 2.8 cm; that was the median value of our case series. The tumor size included not only ground-glass opacity (GGO) but also mixed GGO and only solid part. All analyzed tumor sizes were evaluated following the guidelines in the International Staging System for Lung Cancer, 7th edition; that is, by total size including GGO with solid part. Postoperative adjuvant therapy was performed for patients with tumor diameter >2.0 cm or with lymph node metastasis, except for patients with high risk for complications.

Fresh pairs of tumor and normal lung tissues were dissected from surgical specimens collected from 90 patients who underwent surgery in the Fukushima Medical University Hospital from 2013 to 2016. These pairs of tissues were used for WES analysis.

Sequencing Analysis

WES of 90 tumors and paired normal samples was performed using Ion Amplise Exome technology and Ion Proto platform (Thermo Fischer Scientific, Waltham, Massachusetts). The obtained DNA sequences were aligned to hg19 of the human genome. On average, the mean coverage depth was 123x, and 90.4% of target bases had a coverage of 20x. Sequence variants in the tumor were called using Ion Reporte 5.0 and CLC Genomics Workbench 8.0 software, and the number of nonsynonymous coding variants was counted. The obtained value was designated as the TMB. The Ion Amplise Colon and Lung Cancer Panel v2 and Ion Personal Genome Machine platform were used to detect tumor variants within the hotspot regions of *EGFR*, tumor protein p53 (*TP53*), *KRAS* and erb-b2 receptor tyrosine kinase 2 (*ERBB2*) genes.

Statistical Analysis

Kaplan–Meier analysis and log-rank tests were performed using GraphPad Prism software. Adjusted hazard ratios (HRs) were determined using a Cox proportional hazards regression model.

Results

Patient characteristics are presented in Table 1. There were 63 men and 27 women with a median age of 70 years (range, 40 to 87 years). Sixty-two (68.9%) patients had adenocarcinoma and 28 (31.1%) had squamous cell carcinoma. Median tumor size was 2.8 cm. Twenty-four patients were positive for *EGFR* mutation and histologically all of them had adenocarcinoma. Sixty-seven (74.4%) patients had stage 1 NSCLC. Twenty-three (25.6%) patients were administered postoperative adjuvant chemotherapy. Adjuvant therapy without platinum included tegafur/uracil and tegafur/gimeracil/

Table 1. Patient Characteristics

	N = 90	
Median age, yrs (range)	70 (40-87)	
Gender		
Male / female	63 (70.0%) / 27 (30.0%)	
Smoking status		
Never smoker	28	(31.1%)
Former or current smoker	62	(68.9%)
Median brinkman Index (range)	675 (45-2580)	
Tumor size, cm (range)	2.8 (0.8-11.0)	
Histology		
Adenocarcinoma	63	(70.0%)
Squamous cell carcinoma	27	(30.0%)
EGFR-mutation		
Exon21;L858R	14	(15.5%)
Exon19 deletion	8	(8.9%)
Others	2	(2.2%)
Wild-type/Unknown	66	(73.3%)
Pathological stage		
IA	43	(47.8%)
IB	24	(26.7%)
IIA	4	(4.4%)
IIB	8	(8.9%)
IIIA	9	(10.0%)
IIIB	2	(2.2%)
Adjuvant therapy		
Platinum	8	
Others	15	
Recurrence	22	(24.4%)
Treatment for recurrence		
Platinum	10	
EGFR-TKI	5	
Others	5	
Best supportive care	2	
Death	15	(16.7%)
Median TMB (range)	62	(10-502)

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; TMB, tumor mutation burden.

oteracil. The median follow-up was 792 days (range, 87 to 1,469 days). Twenty-two (24.4%) patients had tumor relapse, and 20 underwent treatment with cytotoxic chemotherapy, epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), immune checkpoint inhibitor, or radiation therapy. Two cases were administered nivolumab for recurrence after cytotoxic chemotherapy or EGFR-TKI; however, both developed adverse effects and discontinued treatment before evaluation. There were 15 (16.7%) deaths; 4 of which were not a result of lung cancer progression (2 pneumonia, 1 emphysema, and 1 cardiac failure).

The median TMB was 62 (range, 10 to 502). We designated TMB $\geq$ 62 as high and TMB $<$ 62 as low, considering the median value as a boundary. Univariate analysis showed that TMB $\geq$ 62 was a poor prognostic factor in overall survival (OS), along with squamous cell carcinoma and tumor size $\geq$ 2.8 cm. Multivariate analysis showed that high TMB was an independent prognostic factor in OS (Table 2). Univariate analysis showed that tumor size $\geq$ 2.8, stage 2 or 3, and lymph node metastasis–positive were poor prognostic factors in disease-free survival (DFS) (Supplementary Table 1).

The 3-year survival rate of patients with high and low TMB was 58.9% and 90.3%, respectively (p = 0.0005) (Fig. 1*A*), although there was no significant difference in DFS (p = 0.0812) (Fig. 1*B*). Furthermore, even in stage 1 patients, there were significant differences in OS and DFS between high- and low-TMB groups (Fig. 2). In the 23 patients who underwent postoperative adjuvant chemotherapy, DFS was shorter in the TMB-high group despite chemotherapy (HR = 6.046, 95% confidence interval [CI]: 1.172 to 31.18, p = 0.0336) (Supplementary Fig. 1). In the 16 patients with stage 1 cancer who underwent postoperative adjuvant chemotherapy, OS and DFS were similar to those of all patients administered adjuvant therapy (Supplementary Fig. 2)

Discussion

We showed that high TMB in patients with NSCLC can be a poor prognostic factor. We also indicated that recurrence rate was higher in the high-TMB group with stage 1 NSCLC. A large-scale examination of TMB has been reported by the Genome Project Team of the National Cancer Institute.[6,7] They analyzed the number of somatic mutations in patients with lung cancer and its variations using WES. They analyzed the frequency and type of driver mutations in detail, but the relationship between TMB and prognosis was not mentioned. It should be noted that TMB in our examination was less than in their study. The reason for this is that we counted nonsynonymous variants only.

Rizvi et al. were the first to report, in 2015, the relationship between TMB and the efficacy of immune checkpoint inhibitors.[8] They showed that in NSCLC patients with high TMB, administration of anti–PD-1 antibody pembrolizumab resulted in prolonged prognosis compared with patients with low TMB. Even then, Roszik et al. reported analysis using The Cancer Genome Atlas mutation data.[5] Patients with melanoma with low TMB had poor OS after therapy with cytotoxic T-lymphocyte–associated antigen 4 antibody ipirimumab and adoptive T-cell immunotherapy. Patients with pulmonary adenocarcinoma with low TMB also had low response rate and poor OS after therapy with pembrolizumab. Carbone et al. indicated that patients with high TMB and PD-L1 expression level $\geq$50% had a higher response rate (75%) than those with only one of these factors.[4] What is common to these results is that high TMB in patients

Table 2. Univariate and Multivariate Analysis of Prognostic Factors of Overall Survival

	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Age > 70 yrs	1.650	0.598-4.554	0.3335			
Male	2.690	0.9286-7.790	0.0682			
Smoker	2.771	0.9627-7.976	0.0588			
Sq	4.099	1.197-14.03	0.0246	1.139	0.384-3.379	0.815
Tumor size>2.8cm	3.707	1.332-10.32	0.0121	3.657	0.815-16.42	0.091
Stage2-3	1.392	0.4438-4.368	0.5704			
LN+	0.910	0.2641-3.136	0.8813			
EGFR mutation-	1.734	0.514-5.849	0.7872			
TP53 mutation+	1.886	0.6729-5.287	0.2276			
KRAS mutation+	0.536	0.1218-2.355	0.4086			
ERBB2 mutation+	1.190	0.2434-5.816	0.8300			
TMB > 62	6.633	2.387-18.43	0.0003	12.311	1.517-99.89	0.019
Adjuvant therapy	1.593	0.5204-4.874	0.4149			

Sq, squamous cell carcinoma; LN, lymph node; TMB, tumor mutation burden; HR, hazard ratio; CI, confidence interval; *TP53*, tumor protein p53; *ERBB2*, erb-b2 tyrosine kinase 2.

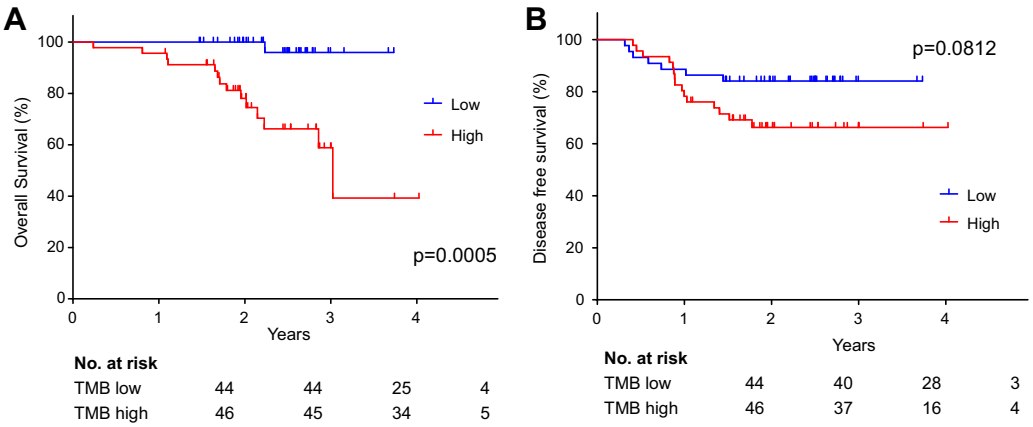

Figure 1. Kaplan-Meier survival curves for patients with high versus low TMB. (*A*) OS between patients with high and low TMB. There was a significant difference in OS ($p = 0.0005$) between the two arms. (*B*) DFS between patients with high and low TMB. There was no difference in event number (15 versus 7) or DFS ($p = 0.0812$) between the two arms. DFS, disease-free survival; OS, overall survival; TMB: tumor mutation burden.

with NSCLC can predict effectiveness of immune checkpoint inhibitors. However, it does not indicate what meaning TMB has for NSCLC. Our multivariate analysis led us to conclude that high TMB is a poor prognostic factor in surgically treated NSCLC.

The previous studies reported above were for patients with advanced NSCLC, whereas our study was in patients with early-stage NSCLC treated with surgery. OS and DFS data may be influenced not only by surgery but also by various other treatments. For example, postoperative adjuvant chemotherapy was performed in the group with tumor size >2.0 cm and/or lymph node metastasis at the time of surgery, except for patients with high risk for complications, and treatment such as conventional chemotherapy, radiotherapy, or immune checkpoint inhibitor was added to the relapse group. There was no difference in DFS among all the patients, but there was a significant difference in DFS in patients with stage 1 NSCLC. DFS was shorter in the high-TMB group among patients administered adjuvant chemotherapy. As a limitation, our study was the small sample size from a single institution and it may not be representative of all patients with NSCLC. Thus, it may make subanalysis difficult. In line with this background, High TMB tended to be a poor prognostic factor for DFS, although there was no significant difference. However, in this small-sized clinical study, when we consider the association between chemotherapy and TMB, high TMB was a significantly poor prognostic factor for DFS. This means that high TMB may be involved in resistance to previous adjuvant therapy and could associated with refractoriness to chemotherapy. Assuming that high TMB is a predictive biomarker for the effect of immune checkpoint inhibitors, it is worth considering adjuvant

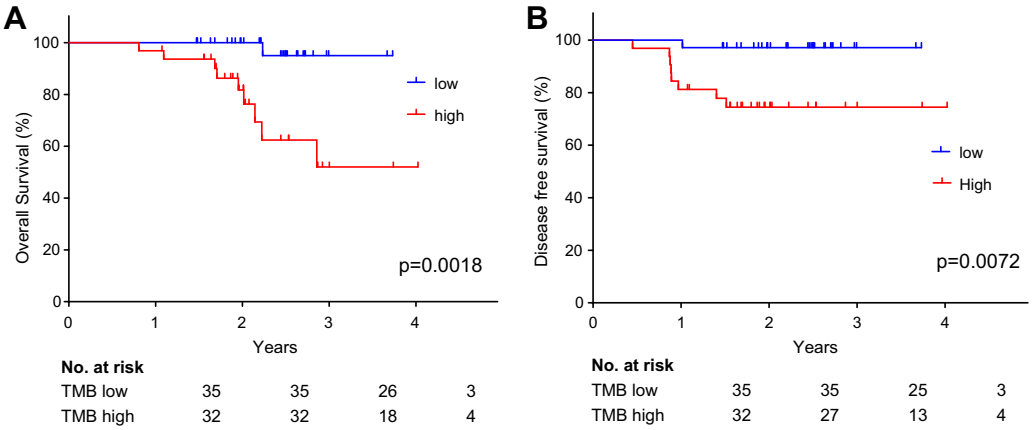

Figure 2. Kaplan-Meier survival curves for patients with high versus low TMB in stage 1 NSCLC. (*A*) OS between patients with high and low TMB in stage 1 NSCLC. There was a significant difference in OS ($p = 0.0018$) between the two arms. (*B*) DFS between patients with high and low TMB in stage 1 NSCLC. There was a significant difference in event number (8 versus 1) and DFS ($p = 0.0072$) between the two arms. DFS, disease-free survival; OS, overall survival.

therapy with immune checkpoint inhibitors in patients with high TMB at the time of surgery.

Adjuvant therapy with immune checkpoint inhibitors has never been reported; however, a number of clinical trials of postoperative adjuvant therapy with immune checkpoint inhibitors are ongoing. We could find several clinical trials investigating postoperative effects of nivolumab (NCT02595944), pembrolizumab (NCT03254004), and anti–PD-L1 antibody durvalumab/tremelimumab (NCT03130764), and so on. TMB as a biomarker for immune checkpoint inhibitor efficacy has been analyzed in patients administered anti–PD-1/PD-L1 inhibitors. Our current study suggests that high TMB is a poor prognostic factor in patients with completely resected NSCLC. Therefore, we believe that there is a possibility of using immune checkpoint inhibitors as adjuvant therapy, and our results may be important in patient selection. Currently, it is difficult to explain why high TMB was associated with poor survival. We must investigate further the detailed mechanism of how TMB contributes to malignant potential.

In conclusion, we revealed that high TMB could be a poor prognostic factor in patients with completely resected NSCLC. In early-stage NSCLC, the high recurrence rate in patients with high TMB suggests that TMB is related to the degree of tumor malignancy. If high TMB is a predictive biomarker for efficacy of immune checkpoint inhibitors, postoperative adjuvant therapy with immune checkpoint inhibitors may contribute to improvement of recurrence and OS.

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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 https://doi.org/10.1016/j.jtho.2018.04.003.

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