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Somatic mutation of LRP1B is associated with tumor mutational burden in patients with lung cancer

Dear Editor,

Immunocheckpoint inhibitors (ICI), such as nivolumab, atezolizumab and pembrolizumab, have shown significant clinical efficacy in advanced non-small cell lung cancer (NSCLC) [1–3]. One emerging biomarker for predicting response to ICI therapy is tumor mutational burden (TMB) [4]. Lung cancer patients with high-TMB (≥ 10 mutations per megabase, mut/MB) showed significantly longer progression-free survival (PFS) when treated with nivolumab plus ipilimumab than with chemotherapy (median PFS 7.2 months vs. 5.5 months), as confirmed by the Checkmate227 trial [5]. A recent retrospective study on blood TMB (bTMB), based on two large randomized trial samples from POPLAR and OAK, showed that when bTMB was higher than 16 mut/MB, the median overall survival (OS) of NSCLC patients receiving atezolizumab was significantly longer than that of patients receiving docetaxel (median OS 13.5 months vs. 6.8 months) [6]. Also, a large recent sample study showed that TMB was associated with improved survival in patients receiving ICI for multiple cancer types [7].

TMB detection is based on a large panel of next generation sequencing (NGS) or whole-exome sequencing. Several studies have recently reported that simpler methods, such as single gene mutation detection, could be used to predict TMB. [1,2,8,9]. Mutations in *LRP1B*, an important cancer suppressor gene, may lead to increased mutational loads throughout the genome in melanoma patients [8], predicting high-TMB. Furthermore, genomic alterations of *LRP1B* are important in the pathogenesis of lung cancer [10]. However, whether *LRP1B* mutations are related to TMB in lung cancer has not been reported.

Therefore, we conducted a retrospective study to elucidate whether *LRP1B* mutational status is associated with TMB in patients with lung cancer. The study was approved by the Ethics Committee of hospital and informed consents were obtained. Mutational load data, detected by NGS, of 60 patients with lung cancer were collected from July 2018 to April 2019. Mutations were detected in 520 genes closely associated to solid tumors using Illumina Novaseq 6000/Nextseq 500 Sequencing Platform and Burning Rock Dx Oncoscreen plus TM kit. *LRP1B* was subjected to whole exon sequencing. Mutations were defined as missense, stop gained, frameshift, splice region, synonymous, and intron mutations.

Results showed that *LRP1B* mutation frequency was 18.3% (11/60) (Table 1), including 4 cases (36.4%) with missense mutations, 4 (36.4%) with synonymous mutations, 1 (9.1%) with intron mutation, 1 (9.1%) with simultaneous missense and synonymous mutations, and 1 (9.1%) where missense, synonymous, intron and splice region mutations coexisted. *LRP1B* mutations were more common in squamous cell carcinoma or squamous differentiated carcinoma and in small-cell lung cancer (SCLC) or neuroendocrine carcinoma than in lung adenocarcinoma, with frequencies of 37.5% (3/8), p = 0.015; and 50.0% (7/14), $p = 2 \times 10^{-4}$ vs. 2.8% (1/36), respectively. *LRP1B* mutation rate was higher in smokers than in non-smokers [30.4% (7/23) vs. 11.1% (4/36)]. *LRP1B*-mutant patients had a lower median age than wild-type (60 vs 71, p = 0.049). *LRP1B* mutations had no correlation with gender, clinical stage, or other clinical characteristics (Table 1). In addition, *EGFR/ALK/ROS1/MET* (terms as sensitive mutation) and PD-L1 status was also analyzed (Table 1). Only one (1/29, 3.4%) *LRP1B*-

Table 1
Clinical characteristics of lung cancer patients undergoing *LRP1B* gene mutation detection.

Characteristic	Total (n)	LRP1B somatic mutation n (%)	LRP1B WT n (%)	P value*
Patients number (Total)	60	11 (18.3)	49 (81.7)	–
Median Age (Range)	62 (44-86)	60 (52-76)	71 (44-86)	0.049
Gender				0.093
Male	34	9 (26.5)	25 (73.5)	
Female	26	2 (7.7)	24 (92.3)	
Smoking Status&				0.089
Smoker	23	7 (30.4)	16 (69.6)	
Non-smokers	36	4 (11.1)	32 (88.9)	
ECOG-PS score&				0.234
0	6	1 (16.7)	5 (83.3)	
1	38	5 (13.2)	33 (86.8)	
≥2	15	5 (33.3)	10 (66.7)	
Stage&				0.468
I-IIIA	3	1 (33.3)	2 (66.7)	
IIIB-IV	56	10 (17.9)	46 (82.1)	
Lymph node&				0.429
Metastasis	46	10 (21.7)	36 (78.3)	
No metastasis	12	1 (8.3)	11 (91.7)	
Pathological subtype				
Adenocarcinoma	36	1 (2.8)	35 (97.2)	
Squamous cell carcinoma or squamous differentiation	8	3 (37.5)	5 (62.5)	0.015
SCLC or Neuroendocrine	14	7 (50.0)	7 (50.0)	2×10^{-4}
Sarcomatoid carcinoma	2	0 (0.0)	2 (100.0)	
Sensitive gene mutation	29	1 (3.4)	28 (96.6)	—
EGFR mutation	19	0 (0.0)	19 (100.0)	
ALK rearrangement	3	0 (0.0)	3 (100.0)	
ROS1 rearrangement	1	0 (0.0)	1 (100.0)	
MET amplification / Exon 14 Splice Site Mutation	6	1 (16.7)	5 (83.3)	
PD-L1 expression (%)	8	3 (37.5)	5 (62.5)	—
< 1%	4	2 (50.0)	2 (50.0)	
1–49%	3	1 (33.3)	2 (66.7)	
≥50%	1	0 (0.0)	1 (100.0)	

* P values were calculated by *T*-test, Chi-square test or Fisher's exact test.
& Information was not available for some cases.

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Table 2
Relationship between *LRP1B* mutational status and TMB value.

		LRP1B		Total (N)	P value
		Mutation	WT		
TMB	High (≥10)	10	10	20	2×10^{-5}
	Low (< 10)	1	39	40	
Total (N)		11	49	60	
Median TMB (Tissue)		17.1	6.0		0.009
Median TMB (Blood)		17.5	2.4		0.018

P values were calculated by Chi-square test or Fisher's exact test.

mutant patient had concomitant sensitive mutations (*MET* exon 14 splice site mutation with bTMB value 9.5), whereas 96.6% (28/29) sensitive mutations in *LRP1B* wild-type patients. 8 of 60 patients underwent PD-L1 detection (Ventana) which did not meet the criteria for a statistical analysis.

LRP1B mutation frequency was 90.9% (10/11) when accompanying mutated *TP53,* and it was 69.4% (34/49) in *LRP1B* wild-type. *LRP1B*-mutant patients had higher median TMB values than wild-type patients (tissue: 17.1 vs. 6.0, p = 0.009; blood: 17.5 vs. 2.4, p = 0.018) (Table 2). A high-TMB was found along with missense, splice region, synonymous, and introns mutations of *LRP1B*, suggesting that high-TMB is not related to particular *LRP1B* mutation types, and that *LRP1B* complete sequence mutations may be related to an increased mutational load of the whole genome. Taking TMB = 10 as cut-off value [5], the positive rate of high-TMB (TMB > 10) among *LRP1B*-mutant patients was 90.9% (10/11), while that of wild-type patients was 20.4% (10/49), $p = 2 \times 10^{-5}$ (Table 2). In addition, using TMB ≥ 10 as the gold standard, the AUC (area under the ROC curve) of *LRP1B* mutation was 0.737, p = 0.003, 95% confidence interval: (0.587–0.888), with a sensitivity of 50.0% and a specificity of 97.5% (Fig. 1). The Kappa test value for consistency of the analysis of *LRP1B* mutations and TMB > 10 was 0.535, $p = 7 \times 10^{-6}$. All data were analyzed using the SPSS 19.0 software.

This study is the first to report that single gene *LRP1B* mutations are correlated with high-TMB in lung cancer patients. *LRP1B*-mutant patients have both higher TMB values and prevalence of high-TMB than *LRP1B* wide-type patients. The discrepancy of high-TMB prevalence between *LRP1B*-mutant and wild-type patients was as high as 70.5%, a correlation also observed in melanoma studies [8]. ROC curve and Kappa analysis confirmed the high specificity and moderate consistency of *LRP1B* mutational status for predicting TMB > 10. The possibility of a low-TMB in *LRP1B*-mutant patients is extremely low, but low-TMB does exist in wild-type patients. Furthermore, we found a trend that high-TMB patients had longer PFS than low-TMB patients when treated with nivolumab/pembroluzimab. Seven patients with TMB > 10 (undetected or low PD-L1) were treated with nivolumab/pembrulumab, had a median PFS of 3.5 months (range from 1.5 to > 7 months). However, three patients with TMB < 10 (1 patient with high expression of PD-L1 and 2 without detection) had a median PFS of 2 months (range from 1 to 2 months). *LRP1B* mutation frequency was positively correlated with tumor grade in Li Ding's study [10], and here we found *LRP1B* was associated with pathological types, age and smoking status. Our data was consistent with the data from UALCAN database that *LRP1B* expression in smokers was significantly higher than that in non-smokers with lung squamous cell carcinoma (p = 0.021) [11]. Interestingly, we found that in *LRP1B*-mutant patients, sensitive mutations such as *EGFR/ALK/ROS1/MET* were rare. Since high-TMB was one of resistant mechanism for *EGFR*-TKI treatment [12,13], our finding illustrate a hypothesis that the *LRP1B* might be relatively exclusive to current driver-gene mutations and a more potent for either immunotherapy or targeted therapy.

In summary, we found that *LRP1B* single gene mutations are correlated with high-TMB in lung cancer, providing new evidence for the searching of simpler, complementary, or replacement methods to TMB as ICI therapy marker. However, further prospective studies are needed to explore the relationship between *LRP1B* mutations and the therapeutic effect and prognosis of ICI.

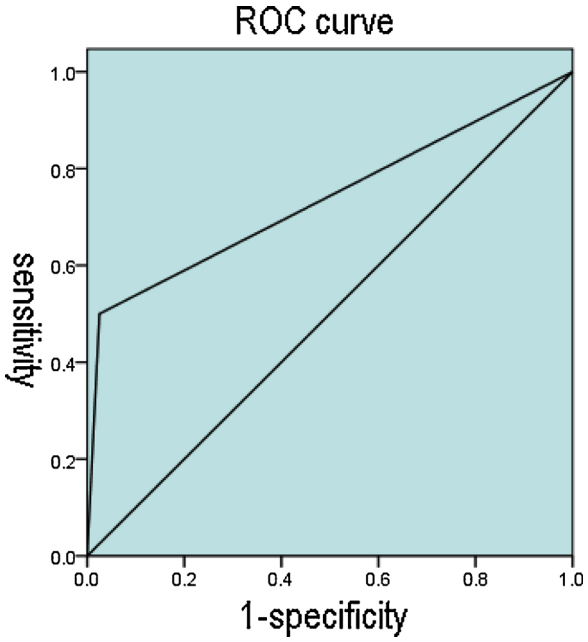

Fig. 1. High specificity of LRP1B mutational status for predicting TMB > 10. ROC curve analysis was used to determine the sensitivity and specificity of LRP1B mutations for the differential diagnosis of TMB > 10. The area under the ROC curve was 0.737, p = 0.003, 95% confidence interval: (0.587–0.888).

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Disclosure statement

Dr. Yan Liu and Shi Yan contributed to the gene mutational detection. Dr. Ying Liu and Xianhong Liu contributed with cases of patients. The authors have no conflict of interests to declare.

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Shaowei Lan[a,b,1], Hui Li[a,b,1]

[a] *Translational Oncology Research Lab, Changchun, 130012, China*

[b] *Jilin Provincial Key Laboratory of Molecular Diagnostics for Lung Cancer, Changchun, 130012, China*

Ying Liu[c], Lixia Ma[c], Xianhong Liu[c]

[c] *Department of Medical Thoracic Oncology, Jilin Provincial Cancer Hospital, Changchun, 130012, China*

Yan Liu[a,b], Shi Yan[a,b]

[a] *Translational Oncology Research Lab, Changchun, 130012, China*

[b] *Jilin Provincial Key Laboratory of Molecular Diagnostics for Lung Cancer, Changchun, 130012, China*

Ying Cheng[a,b,c,*]

[a] *Translational Oncology Research Lab, Changchun, 130012, China*

[b] *Jilin Provincial Key Laboratory of Molecular Diagnostics for Lung Cancer, Changchun, 130012, China*

[c] *Department of Medical Thoracic Oncology, Jilin Provincial Cancer Hospital, Changchun, 130012, China*

E-mail address: chengying@csco.org.cn.

[*] Corresponding author at: Jilin Provincial Cancer Hospital, 1018 Huguang Road, Changchun, Jilin, China.

[1] Shaowei Lan and Hui Li contributed equally to this work.