



The prognostic value of tumor mutation burden in *EGFR*-mutant advanced lung adenocarcinoma, an analysis based on cBioPortal data base

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Background: Tumor mutation burden (TMB) is novel biomarker of promising predict value in prediction of immune checkpoint inhibitors (ICPis) in non-small cell lung cancer (NSCLC). However, the distribution of TMB in epidermal growth factor receptor (*EGFR*)-mutant advanced lung adenocarcinoma (LUAD) patients and the impact on overall survival (OS) time are not well demonstrated.

Methods: Information regarding gene mutations and patients' survival time in advanced LUAD was downloaded from The Cancer Genome Atlas (TCGA) database. The diversity of TMB in different *EGFR*-mutant types was observed and the predicted value of TMB for OS as well as other co-mutations were analyzed. The diversity of TMB was also observed in another Chinese cohort of advanced LUAD patients.

Results: The median TMB values of *EGFR* wild-type, other types of *EGFR* mutations, exon 19 deletions and L858R were 6.12, 5.66, 3.77 and 4.72, differences between wild-type and *EGFR* sensitive mutations (exon 19 deletion or L858R) were significant ($P < 0.001$ and $P < 0.01$). OS time of high TMB group was inferior to that of the low TMB group (24.03 months vs. not reached, $P = 0.0020$). TMB and TP53 together will make more accurate prediction of OS in *EGFR*-mutant advanced LUAD patients. Distribution of TMB in another Chinese cohort had the same trend.

Conclusions: In advanced LUAD patients, TMB was lower in patients with *EGFR*-mutant group than *EGFR* wild group. TMB was a negative prognostic biomarker of OS in *EGFR*-mutant LUAD patients, especially when TP53 was mutated together.

Keywords: Lung adenocarcinoma (LUAD); tumor mutation burden (TMB); overall survival (OS); prognosis; epidermal growth factor receptor mutation (*EGFR* mutation)

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Introduction

In the era of precision medicine, more and more genetic information detected by next generation sequence (NGS) is accumulating and changing the treatment of lung cancer

(1-3). In addition to traditional targets such as EGFR, anaplastic lymphoma kinase (ALK), ROS proto-oncogene 1 (ROS1), MET, HER-2, BRAF, etc. Large test panels such as MSK-IMPACT (4), FMI (5) and Burning Rock (6) can provide more information, especially TMB, which

can be only presented in the era of NGS. How to excavate the meaning of these massive data and apply it to daily treatment of lung cancer is of great practical significance.

TMB is a novel biomarker of promising predict value in prediction of immune checkpoint inhibitors (ICPis) in lung cancer. Many studies showed that in patients with high TMB, immunotherapy had a better effect (7-13). However, a considerable portion of non-small cell lung cancer (NSCLC) patients have *EGFR* mutations, especially in the East Asian population. The distribution of TMB in *EGFR*-mutant NSCLC patients and the prognostic and predictive value of TMB in *EGFR*-mutant NSCLC patients have not been well established in the first few years of clinical application of TMB.

Recently, TMB was reported to be negatively associated with clinical outcomes in metastatic *EGFR*-mutant lung cancer patients treated with *EGFR* tyrosine kinase inhibitor (TKI), including progression-free survival (PFS) and overall survival (OS) (14). However, in early stage resected NSCLC patients, the prognostic value of TMB was reverse as compared with late stage (15). Meanwhile, this study was based on Caucasian population, which have an obvious different *EGFR*-mutant rate compared with Chinese population. Further investigate TMB in *EGFR*-mutant NSCLC patient is meaningful.

Our previous study revealed that *TP53* had important prognostic value in *EGFR*-mutant NSCLC patients (16). And *TP53* mutation rates were diverse in patients with different TMB level (14). Whether *TP53* and TMB together could predict OS more accurately in *EGFR*-mutant NSCLC patients is still unknown.

Our study was conducted to reveal the distribution of TMB in different *EGFR*-mutant groups, especially in Chinese population to discuss the prognostic value of TMB in *EGFR*-mutant advanced lung adenocarcinoma (LUAD), as well as the correlation of TMB and other co-mutations such as *TP53* in this group of patients.

Methods

Information regarding TMB, *EGFR* alterations and patients' survival time in NSCLC was downloaded from The Cancer Genome Atlas (TCGA) database, an open access database that is available at <http://www.cbioportal.org> (17,18). Using the TCGA database, the test data of MSKCC-IMPACT (19) including 1,668 cases of LUAD patients were retrieved. Patients with *EGFR* mutation and survival data were analyzed to observe the prognostic

value of TMB. MSK-IMPACT is an FDA cleared NGS test in which tumor and normal DNA undergo targeted hybridization capture and deep-coverage NGS to detect somatic mutations, copy number changes, and select gene fusions in a custom gene panel of 341 (version 1), 410 (version 2), or 468 (version 3) genes (4). TMB was defined as the total number of non-synonymous single nucleotide or insertion/deletion mutations divided by the coding region captured in each panel (341 genes, 0.98 Mb; 410 genes, 1.06 Mb; 468 genes, 1.22 Mb). TMB value was presented in the form of mutation number per megabase pairs.

Another cohort of Chinese advanced LUAD patient was from Burning Rock Company. We use this cohort to further observe the distribution of TMB in advanced LUAD. All these patients were sequenced by two separated gene panels, which were presented as a custom gene panel of 295 or 520 genes. The detailed NGS library and sequencing protocol preparation was performed as previously described (6). TMB was defined as the number of somatic mutations excluding copy number variations (CNVs), fusions and large genome rearrangement (LGR) per megabase of genome examined. To be more specific, the mutations counted included missense, synonymous, frameshift, splice site and indel mutations whereas the genomic regions examined included all coding sequences extending 20 bp into the introns. The kinase domains of *EGFR* and *ALK* genes were excluded for TMB calculation. Thus, the total examined regions were 0.98 Mb for 295 panel and 1.26 Mb for 520 panel. TMB value was also presented in the form of mutation number per megabase pairs.

The data from TCGA database do not require ethical approval. This study was approved by the Committee on Ethics of Changzheng Hospital, Shanghai (No. 2017SL016).

Statistical methods

All the analyses were performed by SPSS 20.0. The continuous variables were described by median and range. The difference between the two groups was analyzed by Mann-Whitney U test. Comparison between three or more groups was analyzed by Kruskal-Wallis test with Dunn's post-test. Survival time was analyzed by Kaplan-Meier curves, where P value was determined by Log-rank test. Hazard's ratio and its 95% CI were determined by univariate and multivariate Cox proportional hazards model. All reported P values were two-tailed and $P < 0.05$ was considered statistically significant.

Results

The distribution of TMB in advanced LUAD patients

In the MSKCC-IMPACT study, there were 1,668 cases with advanced LUAD. Of the 1,668 cases, 1,258 cases were *EGFR* wild type patients, and 410 cases were with *EGFR* mutations. Among the 410 mutant patients, 172 cases had exon 19 deletions (19del), 131 cases had L858R, and 107 cases demonstrated other types of mutations. The median TMB values of *EGFR* wild-type, non-sensitive *EGFR* mutations, 19del and L858R were 6.12, 5.66, 3.77 and 4.72, respectively (Table 1). Difference between wild-type and *EGFR* 19del was significant ($P < 0.001$, Figure 1A).

We used another cohort of Chinese data from Burning Rock Company to further verify the distribution of TMB in advanced LUAD. A total of 292 advanced LUAD patients were included. In these patients, 168 cases were *EGFR* wild-

type patients, and 124 cases had *EGFR* mutations. Among the 124 *EGFR*-mutant patients, 44 cases had 19del, 28 cases had L858R, and 52 cases had other types of mutations. The median TMB values of *EGFR* wild-type, non-sensitive *EGFR* mutations, 19del and L858R were 6.10, 4.95, 4.10 and 3.10 respectively (Table 2). Differences between wild-type and each mutant group were significant (*EGFR*-19del vs. *EGFR* wild-type, $P < 0.001$; *EGFR*-L858R vs. *EGFR* wild-type, $P < 0.001$; other types of *EGFR* mutation vs. *EGFR* wild-type, $P < 0.05$, Figure 1B).

Prognostic significance of TMB in advanced LUAD patients with *EGFR* sensitive mutations

In MSKCC-IMPACT cohort, 289 patients with both survival data and *EGFR* sensitive mutations (19del or L858R) data were analyzed to further reveal the prognostic value of TMB in *EGFR*-mutant advanced LUAD. In this specific population, the median TMB was 3.77. When we divided these patients into TMB high group (TMB ≥ 3.77) and TMB low group (TMB < 3.77), the median OS time was 24.03 months and not reached ($P = 0.0020$, Figure 2A). When we divided these patients into 3 groups by tertile (TMB high: TMB ≥ 5.10 , TMB median: $2.83 \leq$ TMB < 5.10 , TMB low: TMB < 2.83), the median OS time was 21.27 months, not reached and not reached ($P = 0.0135$, Figure 2B). Although median survival times were not reached, but the survival

Table 1 *EGFR* mutation and TMB in MSKCC panel

TMB	<i>EGFR</i> WT (n=1,258)	Non-sensitive mutation (n=107)	19del (n=172)	L858R (n=131)	Total (n=1,668)
Percent	75%	6%	10%	8%	100%
Median TMB/M	6.12	5.66	3.77	4.72	5.66

EGFR, epidermal growth factor receptor; TMB, tumor mutation burden; WT, wild type.

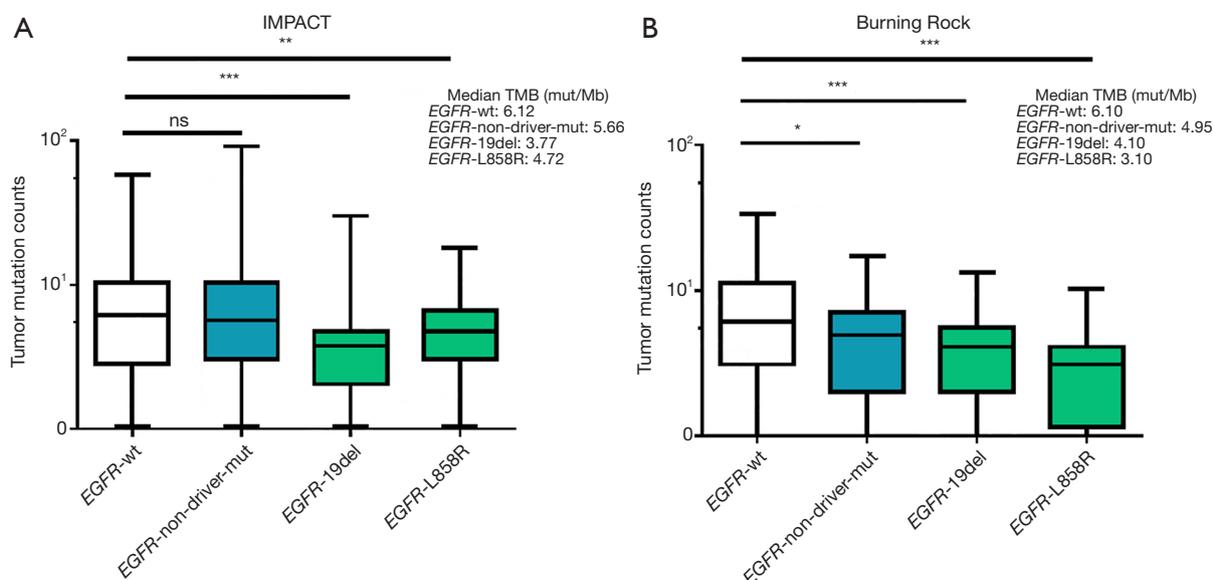


Figure 1 TMB distribution in MSKCC-IMPACT and Burning Rock cohorts. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. TMB, tumor mutation burden; *EGFR*, epidermal growth factor receptor.

time was significantly longer in patients with low or median TMB than high TMB. However, the survival curve of high TMB and median TMB were overlapping. If these patients were divided into 4 groups by quartile ($TMB \geq 6.12$, $3.77 \leq TMB < 6.12$, $2.83 \leq TMB < 3.77$, $TMB < 2.83$), the median OS time was 21.27 months, not reached, not reached and not reached ($P=0.0180$, *Figure 2C*). Similarly, although median survival times were not reached, but the survival time was significantly longer in patients with low or intermediate TMB than high TMB. The survival curve of the top two TMB groups were overlapping. As above, we use median TMB as cutoff value to divide TMB into high

or low groups for further analyses.

We further carried out multivariate analysis to determine the prognostic value of TMB. Due to limited information by the MSKCC-IMPACT database, only gender and smoking status were obtained. However, all the sequencing data was from advanced NSCLC patients, so tumor staging of these patients was almost the same. As our former article had mentioned, *TP53*, *EGFR*, *SMARCA4*, *STK11*, gender and smoking status were statistically significant factors in univariate analysis in advanced NSCLC (16). Hence, we further performed univariate and cox regression analysis.

In univariate analysis, only *TP53*, TMB, *STK11* and gender was statistically significant, these 4 factors were included in multivariate analysis, and all these factors was statistically significant. We confirmed that TMB was an independent prognostic factor in *EGFR*-mutant advanced LUAD (*Table 3*).

Correlation of *TP53* gene mutation and high TMB in advanced *EGFR*-mutant LUAD

As *TP53* was the most common mutated gene in NSCLC,

Table 2 *EGFR* mutation and TMB in Burning Rock panel

TMB	<i>EGFR</i> WT (n=168)	Non-sensitive mutation (n=52)	19del (n=44)	21 L858R (n=28)	Total (n=292)
Percent	58%	18%	15%	10%	100%
Median TMB/M	6.10	4.95	4.10	3.10	4.80

EGFR, epidermal growth factor receptor; TMB, tumor mutation burden; WT, wild type.

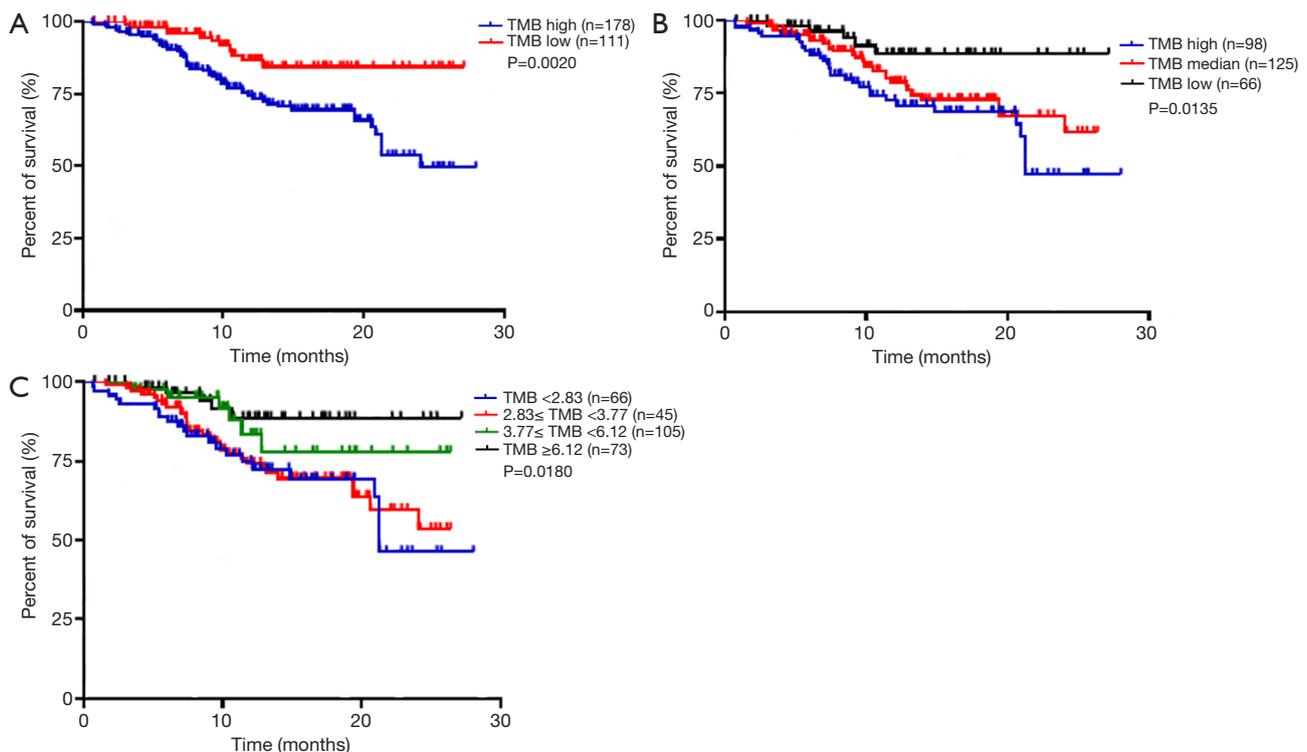


Figure 2 The prognostic value of TMB in MSKCC cohort. (A) TMB high and low groups; (B) TMB high, median and low groups; (C) TMB in 4 groups by quartile. TMB, tumor mutation burden.

Table 3 Multivariate Cox proportional hazard analyses of clinicopathological factors for OS in advanced NSCLC

Features	Group	Univariate		Multivariate	
		HR with 95% CI	P value	HR with 95% CI	P value
Gender	Male	Reference	–	–	–
	Female	0.568 (0.342–0.943)	0.029	0.529 (0.317–0.883)	0.015
Smoking	Never	Reference	–	–	–
	Previous/current smoker/unknown	1.160 (0.751–1.791)	0.504	–	–
EGFR	19del	Reference	–	–	–
	L858R	1.084 (0.658–1.786)	0.752	–	–
SMARCA4	Wild	Reference	–	–	–
	Mutated	1.708 (0.732–3.986)	0.216	–	–
STK11	Wild	Reference	–	–	–
	Mutated	5.278 (1.896–14.694)	0.010	4.112 (1.451–11.653)	0.008
TMB	High	Reference	–	–	–
	Low	0.374 (0.195–0.917)	0.003	0.450 (0.232–0.873)	0.018
TP53	Wild	Reference	–	–	–
	Mutated	2.381 (1.291–4.393)	0.005	2.243 (1.197–4.200)	0.012

OS, overall survival; NSCLC, non-small cell lung cancer; HR, hazard ratio; CI, confidence interval.

so we observed the correlation of *TP53* and TMB in advanced *EGFR*-mutant LUAD. We found that in TMB high group, *TP53* mutation rate was 76.4% (136 in 178), in TMB low group, *TP53* mutation rate was 47.7% (53 in 111), the difference was statistically significant ($P < 0.001$).

Prognostic value of TMB and *TP53* in advanced *EGFR*-mutant LUAD

In our previous study, we found that *TP53* was an important prognostic factor in advanced LUAD, and the different mutation site of *TP53* has different prognostic value (16). At the same time, *TP53* mutation rate was higher in TMB high group, so we were wondering whether TMB combined with *TP53* may be a better prognostic factor in advanced *EGFR*-mutant LUAD. Thus, we made further statistically analysis.

When we consider TMB and *TP53* mutation together, we can classify these patients into four groups. Group A, TMB high and *TP53* wild type. Group B, TMB high and *TP53* mutant type. Group C, TMB low and *TP53* wild type. Group D, TMB low and *TP53* mutant type. The OS disparity of them was statistically significant ($P = 0.0009$). However, the survival curves of group A, group C and

group D were overlapping, each of the 3 groups has better OS than group B (Figure 3A). When we integrate group A, group C and group D together, we can further classify these patients into two groups. Group A, TMB high and *TP53* mutant type. Group B, non-TMB high and *TP53* mutant type. The survival curve of these two groups was separated and statistically significant ($P < 0.0001$, Figure 3B). It is likely that TMB high and *TP53* mutation were both important for the prediction of OS in advanced *EGFR*-mutant LUAD. The former viewpoint of judge the prognosis only by *TP53* or TMB may mis-divide some patients with better prognosis into worse prognosis group.

Discussion

With the wide application of targeted drugs, the treatment of lung cancer has entered the era of precision treatment. As a therapeutic target with the highest mutation rate in LUAD, the total mutation rate of *EGFR* in east Asian LUAD was as high as 50.2% (20), and its expression was associated with poor prognosis (21). In addition, the drug-sensitive mutation rate of *EGFR* is high. 19del accounted for 45%, exon 21 L858R mutation accounted for 40%, and the remaining 10% of mutations involved exons 18 and

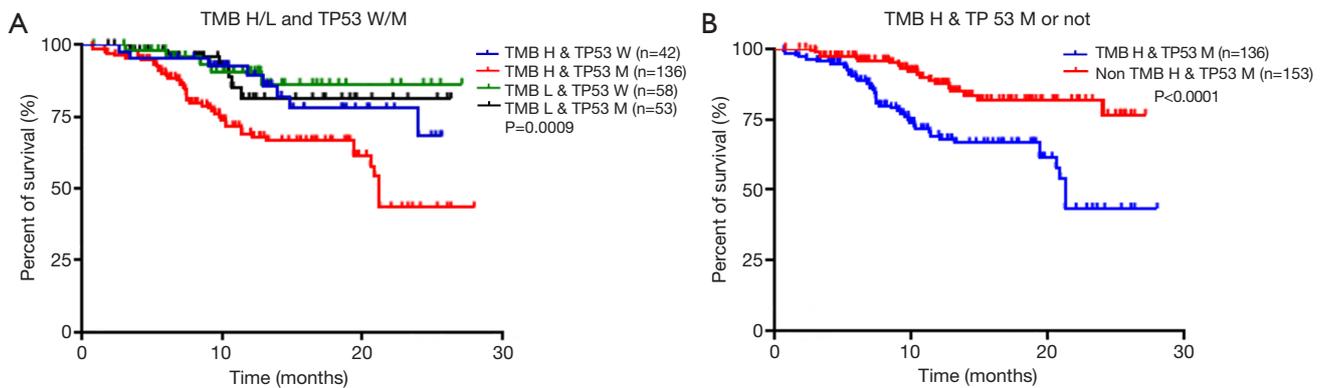


Figure 3 The prognostic value of TMB and *TP53* together in MSKCC cohort. (A) Patients in 4 groups; (B) patients in 2 groups. TMB H: TMB high; TMB L: TMB low; *TP53* W: *TP53* wild type; *TP53* M: *TP53* mutant type. TMB, tumor mutation burden.

20 (21). Therefore, *EGFR* is the most important target in lung cancer therapy. The I subclass of the receptor tyrosine kinase (RTK) superfamily consists of *ERBB* or epidermal growth factor (EGF) receptors and includes four members: *EGFR/ERBB1*, *ERBB2*, *ERBB3*, and *ERBB4* (22,23). All members have an extracellular ligand binding region, a single transmembrane region and a cytoplasmic tyrosine kinase domain (22,24). The extracellular partial binding of *EGFR* ligand and receptor successfully activates the active dimer, whose autophosphorylation activates the RAS-RAF-MEK-mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3-kinase (PI3K)-Akt pathways (25). MAPK signaling pathway is mainly involved in the regulation of cell proliferation, differentiation, survival and invasion. PI3K/Akt pathway controls various important cell functions including glucose metabolism, cell proliferation and survival (26).

With the wide application of NGS, TMB has been paid more and more attention to the role of cancer screening, surveillance, and therapy (27). The definition of TMB is the total number of mutations per coding area of a tumor genome (28). It was also called as tumor mutation load (TML) or tumor mutational burden (8,9). TMB showed differences in different races, and the difference in TMB in different races was associated with the incidence of cancer (29). Somatic mutations in tumor cells may produce neoantigens, and the recognition of neoantigens by T-cells seems to be important for the activity of programmed cell death protein 1 (PD-1) pathway inhibitors (9). Several studies have suggested that tumors harboring high levels of somatic mutations might be highly sensitive to ICPis (12,30). TMB is not only related to immunotherapy, but also can

predict the efficacy of different chemotherapy regimens. It was found that in low TMB group of colorectal cancer patients, PFS was longer in regimens containing irinotecan than oxaliplatin (11.9 vs. 6.5 months, $P<0.001$) (31). In addition to the predictive value, TMB was thought to be associated with cancer prognosis, such as head and neck squamous cancer, lung cancer and so on (29).

NSCLC was found to have higher TMB than many other cancer types except melanoma, where ICPis was first used (10). In NSCLC, TMB was also an important factor in the predictive and prognostic aspects. Many studies have found that TMB was positively related to the effect of ICPis (9,32). Rizvi *et al.* demonstrated that the median number of nonsynonymous mutations was higher in patients with durable clinical benefit (DCB) (partial or stable response lasting for >6 months) vs. those with no durable benefit (NDB) (12). McGranahan found that sensitivity to PD-1 and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) blockade in patients with advanced NSCLC was enhanced in tumors enriched for clonal neoantigens. At the same time, a high neoantigen burden, which was defined as the upper quartile of neoantigen load, was associated with significantly longer OS in LUAD (33). In NSCLC patients, without considering *EGFR* mutation, a 51 gene panel sequencing indicated that the prognosis of the patients with high TMB was inferior to those with low TMB (34). In Chinese population, the results also demonstrated that greater burden of genetic alterations was associated with worse OS rate, and multivariable analysis demonstrated that the mutation burden was an independent prognostic factor for the patients (34). This disparity of OS in LUAD of high TMB may be caused by the administration of ICPis in the

study population.

In *EGFR*-mutant advanced NSCLC patients, results of ICPis remained controversial. Although the preclinical results indicated that programmed cell death-ligand 1 (PD-L1) expression was higher in *EGFR*-TKI resistant cells harboring *EGFR*-T790M mutation and suggested that PD-1/PD-L1 blockade may be a promising approach in pre-treated *EGFR*-mutant advanced NSCLC patients (35). But these results were not replicated in the clinical trials (36,37). Furthermore, a study in Korea found that lung cancer patients with T790M mutation after using *EGFR*-TKI had lower TMB, confirming the lower efficacy on ICPis from the point of mechanism (38). Recently, a meta-analysis suggested that the use of ICPis in pre-treated *EGFR*-mutant advanced NSCLC patients had similar OS but worse PFS when compared with docetaxel, whereas both OS and PFS were in favor of ICPis in the wild-type *EGFR* population (39). In consideration of the positive predictive value of TMB to the effect of ICPis, TMB in *EGFR*-mutant advanced NSCLC patients may be inferior to those with wild-type *EGFR*, so ICPis is unlikely to be effective in *EGFR*-mutant advanced NSCLC patients. In patients with *EGFR* mutations, we already know that tumor burden was associated with the prognosis of patient (40-42). Recently, TMB was proved to be an adverse prognostic factor in *EGFR*-mutant late stage NSCLC patients (14), but the prognostic value was opposite in resected early stage NSCLC patients (15). Meanwhile, *EGFR*-mutant late stage NSCLC patients with higher TMB benefitted less in administration of *EGFR*-TKIs (14). However, the diversity of TMB in *EGFR*-mutant Chinese NSCLC patients is still unknown, which has a totally different *EGFR* mutation rate compared with Caucasia population. Furthermore, the prognostic value of TMB and *TP53* together was not well demonstrated.

Our study indicated that in late stage Chinese LUAD patients, TMB was lower in patient with *EGFR*-mutant group than *EGFR* wild group. We verified that TMB was a negative prognostic biomarker of OS in *EGFR*-mutant LUAD patients. Furthermore, we first present a novel viewpoint that we should use TMB and *TP53* mutation together to judge the prognosis of *EGFR*-mutant LUAD patients more accurately.

However, OS data in our study was downloaded from TCGA database, several clinical details such as treatment regimens and biopsy time could not be acquired. On the other hand, the majority of *EGFR*-mutant Chinese patients was being treated with *EGFR*-TKI, so the predictive value

of TMB to the efficacy of *EGFR*-TKI could not be presented in this study. Further studies should be conducted to deeply understand the meaning of TMB as well as other co-mutations in *EGFR*-mutant LUAD patients.

Conclusions

In late stage LUAD patients, TMB was lower in patients with *EGFR*-mutant group than *EGFR* wild group. TMB was a negative prognostic biomarker of OS in *EGFR*-mutant LUAD patients, especially when *TP53* was mutated together. The prognostic value of TMB as well as other co-mutations in late stage *EGFR*-mutant LUAD patient needs to be further investigated.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Committee on Ethics of Changzheng Hospital, Shanghai (No. 2017SL016).

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