The landscape of tumor mutational burden and its clinical significance in patients with lung cancer: a Multi-omics study with a meta-analysis

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Abstract

Background: Current research on tumor mutational burden (TMB) has focused on tumor immunotherapy responsiveness, but the role of TMB in non-immunotherapy patients is unclear. The purpose of this study is to explore the effect of TMB on lung cancer patients in order to clarify and expand the clinical significance of TMB in lung cancer.

Methods: We download mutation data of lung cancer cases from The Cancer Genome Atlas (TCGA) database to analyze TMB and its composition, and study the relationship between TMB and clinicopathological characteristics of lung cancer patients. We then systematically retrieved and analyzed studies on the relationship between TMB and survival outcomes. The hazard ratio (HR) and its 95% confidence interval (CI) were used as an effective size to assess the survival outcomes. The subgroup analyses based on the pathological type, treatment method, TMB detection method and detection materials were also performed to explore the factors that might affect the interpretation of TMB results.

Results: TMB in lung squamous cell carcinoma is lower than those in lung adenocarcinoma. In lung adenocarcinoma, patients with EGFR mutation have lower TMB than patients with EGFR wild-type. The summary analysis found that TMB is a better prognostic factor in small cell lung cancer, and more evident in small cell lung cancer receiving immunotherapy. TMB is a neutral or poor prognostic indicator in non-small cell lung cancer, but a better prognostic factor in non-small cell lung cancer receiving immunotherapy. In patients with lung adenocarcinoma, including those with EGFR mutation and receiving EGFR-targeted therapies, high TMB means worse survival. TMB detected by blood specimens is inconsistent and unstable compared to TMB detected by tissue. The clinical significance of TMB from blood specimens needs further study on extensive sample data.

Conclusions: The pooled results indicated that TMB is a good prognostic factor in lung cancer patients receiving immunotherapy. But high TMB is connected with worse survival in non-small cell lung cancer without receiving immunotherapy, especially in lung adenocarcinoma. For lung adenocarcinoma patients with both EGFR mutation and high TMB, how to make a choice between EGFR-targeted therapy and immunotherapy is still a problem that requires further research.

1. Background

Lung cancer, including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), is the most common malignant tumor and the leading cause of tumor-related death in the world [1]. NSCLC, accounting for approximately 85% of all lung cancer incidences, is mainly composed of lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and lung large cell carcinoma [2]. SCLC, accounting for around 10–15% of all lung cancers, is characterized by neuroendocrine and has a high degree of malignancy [3]. Different pathological types of lung cancer have different biological characteristics and various treatment options [4–6]. The clinical application of targeted therapy has led to a dramatic change in the treatment of patients with LUAD [7, 8]. Unfortunately, targeted therapy for oncogenic driver mutations in EGFR or ALK fusions that works on LUAD are generally ineffective against LUSC and SCLC [5, 9]. Immunotherapy based on immune checkpoint inhibitors (ICIs) provides definite efficacy for patients with chemotherapy-resistant lung cancer, and is gradually moving towards first-line treatment for lung cancer [10]. But there are also some patients who have no response to ICI therapy, especially those containing oncogenic driver mutation [11].

With the development of next-generation sequencing technology, whole-exome sequencing (WES) and targeted next-generation sequencing (targeted NGS) have been used in clinical decision-making [12]. The changes in tumor driver genes such as EGFR, ALK, MET, BRAF, ROS1, HER2 can be detected based on NGS, and those results are used to guide the application of targeted drugs [13]. Tumor mutational burden (TMB), as a product of the era of NGS, is also gradually widely used in clinical practice [14]. Under this circumstance, how to precisely understand the meaning of TMB and use it to manage lung cancer is of great practical significance.

In addition to PD-L1 expression, microsatellite instability (MSI), and mismatch repair deficiency (MMR), TMB also has emerged as a promising biomarker for response to ICIs therapy in clinical trials [15]. The hypothesis that tumors with more neoantigens may respond better to immunotherapy is based on the positive relationship between tumor-specific neoantigens and increased immunogenicity [16]. Most current researches focus on the relationship between TMB and the efficacy of immunotherapy in various cancers [17–19]. However, the role of TMB in lung cancer patients without receiving immunotherapy has not been fully elucidated. TMB is counted by the sum of non-synonymous mutations that also contain the oncogenic driver mutations, but the additional mutations may constitute potential resistance pathways to targeted therapies [11]. In order to expand the interpretation of the potential clinical significance of TMB, we analyzed the composition of TMB in lung cancer, and then reviewed the role of TMB in lung cancer patients with different characteristics.

2. Methods

2.1 Multi-omics study

2.1.1. Data acquisition and preprocessing

First, we download the somatic mutation data of patients with LUAD and LUSC respectively from “simple nucleotide variation” category in The Cancer Genome Atlas (TCGA) database (<http://portal.gdc.cancer.gov/>). The “Masked Somatic Mutation” data processed by VarScan2 software was used for further TMB analysis. Then, the transcriptome data with HTSeq-FPKM format of patients with LUAD and LUSC were download from “transcriptome profiling” in TCGA database. Moreover, the clinical information of LUAD and LUSC patients, including age, gender, TNM stages, and survival status were all obtained from the TCGA database. Next step, all transcriptome data and clinical data were extracted from the corresponding single files and merged.

2.1.2. The landscape of TMB for each patient and survival analysis

In this study, TMB was defined as the total number of variants, which include detected base deletions, insertions or substitutions. The “maftools” package running in R software is used to analyze the genomic mutation profiles and create visualizations using the “Masked Somatic Mutation” data [20]. A Perl script was used to extract the genomic mutations of patients with LUAD or LUSC, and calculate the TMB value for each patient. TMB values were then merged with corresponding clinical data and genetic mutation data, respectively. The LUAD or LUSC patients were divided into high TMB and low TMB groups by median TMB value. Kaplan-Meier analysis with log-rank test was used to evaluate the difference in overall survival (OS) between the two groups. In addition, we assessed the relationship between TMB and several clinicopathological characteristics.

2.1.3. Statistical Analysis

The Wilcoxon rank-sum test was applied for comparison of continuous variables, and the chi-square test was used for comparison of categorical variables. The Kaplan-Meier analysis with log-rank test was performed by the “survival” package of the R software.

All the statistical analyses were conducted in R software (version 3.6.0). All the tests were two-sided, and a P-value less than 0.05 was considered to be significant.

2.2. Systematic Review And Meta-analysis

2.2.1. Literature search strategy

The systematic review and meta-analysis were performed based on the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [21]. The electronic databases, including PubMed, PubMed Central (PMC), EMBASE, and Ovid, were used to search related pieces of literature from inception to January 11, 2020. Search terms included the following: “lung cancer or lung carcinoma” and “TMB or tumor mutation burden” and “survival or prognosis”. All references lists of identified articles were also manually searched for potentially relevant reports.

2.2.2. Inclusion And Exclusion Criteria

The eligibility criteria for included studies were as follow: 1) RCT or cohort studies to study the role of TMB as a biomarker for lung cancer prognosis, and 2) studies comparatively analyze the survival results of lung cancer patients with high TMB and those with low TMB. The exclusion criteria were as follow: 1) review articles without original data, 2) studies focused on the TMB detection technology, 3) studies without TMB-related survival data, and 4) studies in non-lung cancer. For multiple articles reporting a duplicate population, only the most complete or recent one was included. Two reviewers independently performed the selection process of eligible studies, and differences were resolved through discussion.

2.2.3. Data Extraction And Quality Assessment

Two reviewers independently collected the following data using a predesigned form: first author, publication time, study period, country, sample size (low- and high- TMB), age (low- and high- TMB), gender (low- and high- TMB), tumor histology (low- and high- TMB), tumor stage (low- and high- TMB), driver mutation, treatment, sample source, TMB detection method, cutoff value, follow-up time, and primary endpoint. Disputes in this process were handled through discussion. The hazard ratio (HR) and 95% confidence intervals (CI) of the high TMB group compared to the low TMB group for OS and progression-free survival (PFS) were primarily collected. Disease-free survival (DFS) was considered as PFS for further analysis due to the similar clinical significance of DFS and PFS. For studies in which the HR and 95% CI were not provided explicitly, we used Tierney’s methods to extract survival data from the Kaplan-Meier curve or the original data [22]. If the above item could not be obtained in the original study, this item was marked as “not acquired (NA)”. Two reviewers independently assessed the quality of included cohort studies based on the Newcastle-Ottawa Scale (NOS) [23]. The NOS evaluated the quality of a survey with a scale ranged from 0 to 9. Studies with a score of 6 or high were deemed as high-quality studies.

2.2.4. Statistical Analysis

The HR and its 95% CI were used as effect size (ES) to assess the role of TMB in the survival of lung cancer patients. Heterogeneity across the studies was evaluated using I-squared statistics [24]. A random-effect model was utilized if I^2 greater than 50%; Otherwise, a fixed-effect model was chosen. To detect the different prognostic effects of TMB in different pathological types of lung cancer, various treatments, different methods of TMB detection and diverse sampling, we performed the subgroup analyses based on different scenarios. To confirm the robustness of the pooled results, sensitivity analyses by eliminating a single study at a time were adopted. Potential publication bias was assessed by Begg’s funnel plot and Egger’s test. The statistical analyses were conducted using the software STATA version 12.0 (Stata Inc, TX, USA). All the tests were two-sided, and a P value less than 0.05 was considered to be significant.

3. Results

3.1. Multi-omics study

3.1.1. Genome-wide mutation spectrum in LUAD and LUSC

The gene mutation data, transcriptome data, and clinical information of 585 LUAD patients and 504 LUSC patients were obtained from the TCGA cohorts. Of the 585 LUAD patients, 569 has simple nucleotide variation data, 515 has transcriptome data, and 522 the clinical data. Of the 504 LUSC cases, 497 had simple nucleotide variation data, 501 had transcriptome data, and 504 had clinical data. TMB values were extracted from 561 LUAD cases and 491 LUSC cases. We combined TMB values with clinical data and expression profile data, and cases with incomplete data were discarded. Genome-wide mutation profiling was visualized via “maftools” package. Overall mutation types in patients with LUAD and LUSC were shown in Figs. 1 and 2. In both types of lung cancer, missense mutations were the most common type of mutation (Fig. 1A and 2A). Compared with insertion and deletion, single-nuclear polymorphisms (SNPs) accounted for the vast majority (Fig. 1B and 2B), of which C>A and C>T were the most common types (Fig. 1C and 2C). Waterfall plots revealed the top 80 mutant genes with the highest mutation frequency in patients with LUAD (Fig. 1D) and LUSC (Fig. 2D). Various colors in the waterfall chart represented different types of mutations. TP53 (47%), TTN (41%), MUC16 (40%), RYR2 (34%), CSMD3 (34%), LRP1B (29%), ZFHX4 (27%), USH2A (27%), KAS (25%), and XIPR2 (22%) were the top 10 mutated genes in LUAD. EGFR mutation frequency in LUAD is 12%, ranking 53rd. The top ten mutations in LUSC were TP53 (77%), TTN (68%), CSMD3 (40%), MUC16 (36%), RYR2 (35%), LRP1B (30%), USH2A (30%), SYNE1 (29%), ZFHX4 (26%), and KMT2D (22%). The total number of mutated bases in each patient is shown in Fig. 3. The median TMB was 140 mutations in LUAD (Fig. 3A) and 182 mutations in LUSC (Fig. 3B).

3.1.2. Relationship Between Tmb And Clinicopathological Characteristics

The clinicopathological characteristics of LUAD and LUSC patients were summarized in Table 1. In the LUAD cohort, the age of the low TMB group was higher than that of the high TMB group (P = 0.003). There were more women in the low TMB group and more men in the high TMB group (P = 0.014). In the LUSC cohort, the age of the low TMB group was also higher than that of the high TMB group, but the statistical significance was not significant (P = 0.061). The proportion of stage I patients is higher in the low TMB group, and the ratio of stage III-IV patients in the high TMB group is higher (P = 0.004). Kaplan-Meier analysis with log-rank test showed that TMB did not significantly affect OS in LUAD (Fig. 4A, P = 0.983) and LUSC (Fig. 4B, P = 0.602).

Table 1
Clinicopathological characteristics of LUAD and LUSC patients from TCGA cohort

	LUAD			LUSC		
	Low-TMB	High-TMB	P value	Low-TMB	High-TMB	P value
Age, year	Median 67.0, range (41–86)	Median 64.5, range (33–88)	0.003	Median 69.0, range (39–90)	Median 67.0, range (40–83)	0.061
Gender						
Female	150 (59.1%)	123 (48.2%)	0.014	58 (23.9%)	69 (27.8%)	0.317
Male	104 (40.9%)	132 (51.8%)		185 (76.1%)	179 (72.2%)	
Stage						
I	137 (53.9%)	139 (54.5%)	0.998	131 (53.9%)	108 (43.5%)	0.004
II	63 (24.8%)	60 (23.5%)		81 (33.3%)	78 (31.5%)	
III	41 (16.1%)	42 (16.5%)		29 (11.9%)	56 (22.6%)	
IV	12 (4.7%)	13 (5.1%)		1 (0.4%)	6 (2.4%)	
Unknown	1 (0.4%)	1 (0.4%)		1 (0.4%)	0 (0%)	
Abbreviation: LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TMB, tumor mutational burden. The patients were divided into Low- and high- TMB groups by the median of TMB value as the threshold.						

To explore the possible efficacy of ICI immunotherapy in EGFR mutation-driven lung cancer patients, we examined the relationship between EGFR mutation status and two predictive indicators commonly used in immunotherapy, TMB value and PD-L1 expression. Patients with EGFR mutations in LUAD had significantly lower TMB values than those with EGFR wild type (Fig. 1D, the red line indicates the main population of EGFR mutations; Fig. 5A, P = 5.225e-07). However, there was no significant difference in TMB values between the EGFR mutation group and wild type (WT) group in LUSC (Fig. 5B, P = 0.678). There was also no obvious correlation between EGFR mutation and PD-L1 mRNA expression in LUAD (Fig. 6A, P = 0.299) and LUSC (Fig. 6B, P = 0.779).

3.2. Systematic Review And Meta-analysis

3.2.1. Search results and study characteristics

The literature selection process was shown in Fig. 7. After removing duplicates, a total of 24229 records were retrieved by the search strategy above. By viewing titles and abstracts, 24115 irrelevant articles were excluded. The remaining 114 articles were read in full-text. Finally, 30 articles were included in this analysis.

Thirty eligible articles included 37 independent cohort studies. The main characteristics of the 37 studies were shown in Table 2. Thirty-one studies report the role of TMB in NSCLC and six studies indicate the role in SCLC. Based on the NOS score, these 37 studies have a quality of 6 to 9, with an average score of 7.2.

Table 2
Characteristics of studies included in the r

Author	Year	Period	Country	Sample size		Age		Gender		Tumor histology		Stage	
				TMB low	TMB high	TMB low	TMB high	TMB low	TMB high	TMB low	TMB high	TMB low	TMB high
Alborelli I [16]	2020	2013–2018	Switzerland	51	25	Median 65, range (49–79)	Median 67, range (31–90)	M 30, F 21	M 16, F 8	LUAD 47, LUSC 4	LUAD 23, LUSC 2	I–III 17, IV 32	I–III 8, IV 17
Ricciuti B [45]	2019	2014–2018	USA	26	26	Median 67.5, range (43–83)	Median 63.5, range (47–84)	M 15, F 11	M 10, F 16	SCLC	SCLC	Extensive 19, Limited 7	Extensive 15, Limited 11
Ricciuti B [45]	2019	2014–2018	USA	38	23	NA	NA	NA	NA	SCLC	SCLC	Extensive	Extensive
Samstein RM [30]	2019	2013–2017	USA	280	70	range 31–90	range 31–90	M 136, F 144	M 34, F 36	NSCLC	NSCLC	Advanced	Advanced
Samstein RM [30]	2019	2013–2017	USA	498	125	NA	NA	NA	NA	NSCLC	NSCLC	Advanced	Advanced
Offin M [11]	2019	2010–2017	USA	102	51	NA	NA	M 30, F 72	M 23, F 28	NSCLC	NSCLC	Metastatic	Metastatic
Jiao XD [25]	2019	2009–2014	USA	111	178	NA	NA	NA	NA	LUAD	LUAD	Advanced	Advanced
Zhou M [46]	2019	2014–2017	China	26	27	≤60 25, ≥60 28	≤60 25, ≥60 28	M 49, F 4	M 49, F 4	SCLC	SCLC	Limited 24, Extensive 29	Limited 24, Extensive 29
Chen Y [26]	2019	2009–2016	China	118	18	≤60 55, ≥60 63	≤60 9, ≥60 9	M 71, F 47	M 15, F 3	LUAD	LUAD	IIIB 46, IV 72	IIIB 8, IV 10
Chen Y [26]	2019	2009–2016	China	37	14	≤60 17, ≥60 20	≤60 7, ≥60 7	M 28, F 9	M 12, F 2	LUSC	LUSC	IIIB 14, IV 23	IIIB 7, IV 7
Heeke S [47]	2019	2016–2018	France	21	15	Median 59.2, range (44.5–82.2)	Median 60.2, range (37.6–83.1)	M 16, F 5	M 15, F 0	LUAD 10, LUSC 9, LCNC 1, PSC 1	LUAD 11, LUSC 3, PSC 1	I 0, II 3, III 9, IV 9	I 2, II 2, III 2, IV 9
Jiang T [48]	2019	2012–2015	China	94	95	≤65 52, ≥65 42	≤65 53, ≥65 42	M 88, F 6	M 91, F 4	LUSC	LUSC	I 47, II 29, III 18	I 45, II 25, III 25
Ready N [49]	2019	2016	USA and Canada	50	48	65.5 (42–85)	65.5 (42–85)	M 54, F 44	M 54, F 44	LUSC 34, non-LUSC 64	LUSC 34, non-LUSC 64	IIIB 20, IV 78	IIIB 20, IV 78
Yu H [50]	2019	NA	USA	78	77	Median 71.73, range (43–86.18)	Median 70.77, range (44.81–92.22)	M 53, F 25	M 102, F 28	LUSC	LUSC	I–II	I–II
Wang C [51]	2019	NA	China	68	33	Median 59.24, range (25–82)	Median 59.24, range (25–82)	M 62, F 39	M 62, F 39	LUAD	LUAD	I 19, II 18, III 56, IV 8	I 19, II 18, III 56, IV 8
Chae YK [38]	2019	2015–2016	USA	90	46	Mean 65.7	Mean 71.5	M 36, F 54	M 17, F 29	LUAD 70, LUSC 13, PNE 5	LUAD 29, LUSC 14, PNE 1	I–III 16, IV 74	I–III 9, IV 37
Chae YK [40]	2019	2013–2016	USA	54	18	Median 64.5, range (37–88)	Median 64.5, range (37–88)	M 33, F 49	M 33, F 49	LUAD 64, LUSC 11	LUAD 64, LUSC 11	I–III 11, IV 43	I–III 4, IV 14
Singal G [52]	2019	2011–2018	USA	1116	161	Median 66.0, IQR (58.0–73.0)	Median 66.0, IQR (58.0–73.0)	M 1955, F 2109	M 1955, F 2109	non-LUSC 3153, LUSC 726	non-LUSC 3153, LUSC 726	I 388, II 309, III 846, IV 2304	I 388, II 309, III 846, IV 2304

Abbreviations: TMB, tumor mutational burden; M, male; F, female; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NA, not acquired; ICI, immune checkpoint inhibition; WES, whole exome sequencing; mut, mutation; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; PSC, pulmonary sarcomatoid carcinoma; DFS, disease-free survival; IQR, interquartile range.

				Sample size		Age		Gender		Tumor histology		Stage	
Fang W [53]	2019	2015–2019	China	49	26	Median 54, range (28–73)		M 53, F 25		LUAD 47, LUSC 24, others 7		III 1, IV 77	
Wang Z [39]	2019	2016–2018	China	22	28	Median 56	Median 59	M 12, F 10	M 23, F 5	LUSC 7, nonLUSC 15	LUSC 12, nonLUSC 16	Advanced	Advanced
Chun YJ [54]	2019	2014–2016	Korea	88	104	Median 63, range (34–83)		M 87, F 114		LUAD	LUAD	I 113, II 44, III 42, IV 2	
Hellmann MD [31]	2018	2013–2018	USA	86	47	Median 63, range (29–83)		NA	NA	SCLC	SCLC	NA	NA
Hellmann MD [31]	2018	2013–2018	USA	52	26	Median 65, range (37–80)		NA	NA	SCLC	SCLC	NA	NA
Hellmann MD [31]	2018	2013–2018	USA	66	34	NA		NA	NA	SCLC	SCLC	NA	NA
Devarakonda S [55]	2018	NA	USA	616	292	Median 60, range (27–81)		M 662, F 246		LUAD 375, LUSC 414		I 114, II 676, III/IV 114	
Sorotsky H [27]	2018	NA	Canada	74		≤ 65 44, ≥ 64 30		M 22, F 52		NSCLC		I 38, II 14, III/IV 22	
Zheng S [56]	2018	1991–2014	USA	75	44	NA	NA	NA	NA	NSCLC	NSCLC	I	I
Owada-Ozaki Y [57]	2018	2013–2016	Japan	44	46	Median 70, range (40–87)		M 63, F 27		LUAD 63, LUSC 27		I 67, II 12, III 11	
Rizvi H [58]	2018	2011–2017	USA	119	121	Median 66, range (22–92)		M 118, F 122		LUAD 186, LUSC 34, Others 20		Advanced	Advanced
Rizvi H [58]	2018	2011–2017	USA	315	272	NA	NA	NA	NA	LUAD	LUAD	Advanced	Advanced
Hellmann MD [59]	2018	2014–2015	USA	38	37	Median 66, range (43–85)	Median 65, range (42–87)	M 17, F 21	M 20, F 17	LUSC 7, nonLUSC 31	LUSC9, nonLUSC 28	IIIB 6, IV 32	IIIB 3, IV 34
Miao D [60]	2018	NA	USA	28	28	Median 61, range (41–78)	Median 62, range (54–72)	M 11, F 17	M 13, F 15	LUAD 26, LUSC 2	LUAD 21, LUSC 5, Others 2	NA	NA
Kowanetz M [61]	2017	2015	USA	51	51	NA	NA	NA	NA	NSCLC	NSCLC	Advanced	Advanced
Kowanetz M [61]	2017	2015	USA	51	186	NA	NA	NA	NA	NSCLC	NSCLC	Advanced	Advanced
Wungki P [62]	2017	NA	USA	36		NA		NA		NSCLC	NSCLC	Advanced	Advanced
Xiao D [63]	2016	NA	China	288	47	≤ 65 228, ≥ 65 107		M 183, F 152		LUAD	LUAD	I 82, II 69, III 154, IV 29	
Rizvi NA [64]	2015	NA	USA	17	17	Median 60, range (41–80)	Median 63, range (56–78)	M 10, F 7	M 6, F 11	LUAD 14, LUSC 3	LUAD 15, LUSC 1, Others 1	IV	IV
Abbreviations: TMB, tumor mutational burden; M, male; F, female; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NA, not acquired; ICI, in sequencing; WES, whole exome sequencing; mut, mutation; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; PSC, pulmonary sarcomatoid carcinoma; disease-free survival; IQR, interquartile range.													

3.2.2. Meta-analysis Results

A total of 22 articles with 28 studies evaluated OS, and 22 articles with 25 studies evaluated PFS. The HRs and 95% CI were pooled using the random-effects models throughout the analysis. On the whole, high-TMB group lung cancer had better PFS compared to low-TMB group (Fig. 8B, P = 0.001). However, no

significant difference in OS was found between the high TMB group and the low TMB group (Fig. 8A, $P = 0.830$).

The above total lung cancer patients include different types of lung cancer, different treatment methods, different TMB detection methods, and different test samples. To explore the impact of these factors on results, we conducted a series of subgroup analyses. In a subgroup analysis based on whether to receive ICIs, we found that among patients receiving ICIs, the high TMB group had better OS (Fig. 9A, $P < 0.001$) and PFS (Fig. 10A, $P < 0.001$). However, in the non-ICI subgroup, the OS in the high TMB group was worse compared with the low TMB group (Fig. 9A, $P = 0.014$), and there was no significant difference in PFS between the two groups (Fig. 9A, $P = 0.464$). In the SCLC subgroup, the OS (Fig. 9B, $P = 0.052$) and PFS (Fig. 10B, $P < 0.001$) in the high TMB group are better than those in the low TMB group, but in NSCLC subgroup, there is no significant difference between the high and low TMB groups (Fig. 9B and Fig. 10B). NSCLC was further divided into LUAD and LUSC. The results showed that in the LUAD subgroup, the OS (Fig. 9C, $P < 0.001$) and PFS (Fig. 10C, $P = 0.051$) of the high TMB group were worse than the low TMB group. However, in the LUSC subgroup, there was no significant difference between the high and low TMB groups (Fig. 9C and Fig. 10C). Considering that immunotherapy has a greater impact on the clinical prognosis of TMB, we combined the tumor pathological type and ICIs for subgroup analysis. Results showed that in the NSCLC subgroup receiving ICIs, high TMB was associated with better OS (Fig. 9D, $P = 0.006$) and PFS (Fig. 10D, $P < 0.001$), with similar results in the SCLC subgroup (OS, Fig. 9D, $P = 0.065$; PFS, Fig. 10D, $P < 0.001$). Prognostic effect of TMB diminished in NSCLC and SCLC subgroups not receiving ICIs (Fig. 9D and Fig. 10D). High-TMB was a poor prognostic indicator in LUAD subgroup not receiving ICI (OS, Fig. 9D, $P < 0.001$; PFS, Fig. 10D, $P = 0.035$). However, in a LUAD subgroup not receiving ICI, TMB had no significant prognostic value (Fig. 9D and Fig. 10D).

In the subgroup analysis based on the detection method, we found that targeted NGS and WES did not significantly affect the indicator role of TMB in OS (Fig. 11A). However, different detection methods had a greater impact on the prognosis of TMB in PFS. In the targeted NGS subgroup, high TMB predicted higher PFS (Fig. 12A, $P < 0.001$). In the WES subgroup, TMB does not show an indicator effect (Fig. 12A). In the subgroup analysis of detection methods combined with immunotherapy, whether using the targeted NGS or WES methods, in the subgroup receiving ICI, high TMB tended to predict a better prognosis. Still in the subgroup not receiving ICI, high TMB tended to have a poor prognosis (Fig. 11B and Fig. 12B). Then we performed a subgroup analysis based on different test samples. In the subgroup tested using blood samples, the high TMB group had worse OS than the low TMB group (Fig. 11C, $P = 0.027$). In the subgroup detected using tumor tissue samples, the high TMB group had better PFS than the low TMB group (Fig. 12C, $P = 0.001$). Subgroup analysis was further performed by combining test samples and immunotherapy. The results showed that high-TMB had a better prognosis in the subgroup that used tissue detection and received ICIs (OS, Fig. 11D, $P < 0.001$; PFS, Fig. 12D, $P < 0.001$). However, in the subgroup that provided tissue testing but did not receive ICI, high TMB tended to have a worse prognosis (OS, Fig. 11D, $P = 0.045$).

3.2.3. Effect of TMB on EGFR-mutation or EGFR-TKIs treated lung cancer

Four articles reported the role of TMB in EGFR-mutant or EGFR-TKIs treated lung cancer [11, 25–27]. Machael Offin et al. studied EGFR exon19del or L858R mutant LUAD treated with EGFR-TKIs [11]. By using EGFR wild-type LUAD as a control group, they found that the TMB value of EGFR-mutated lung adenocarcinoma was lower than that of EGFR wild-type lung adenocarcinoma patients, and TMB was a poor prognostic factor in metastatic EGFR mutant LUAD treated with EGFR-TKIs [11]. Yanhui Chen et al. enrolled into two cohorts of LUAD and LUSC, and found that the TMB value in LUAD was lower than that in LUSC [26]. There were only two patients with EGFR mutations in the LUSC group, accounting for 4%. In the LUAD group, there were 58 patients with EGFR mutations, accounting for 43%. In EGFR wild-type LUAD, TMB did not correlate with survival outcomes ($P = 0.484$). Still, in mixed EGFR mutant and wild-type patients, TMB was closer to a worse prognostic factor ($P = 0.062$), which means that TMB tended to negatively related to survival in patients with EGFR mutant LUAD [26]. Through the study of EGFR-mutant advanced LUAD, Xiaodong Jiao et al. found that TMB was negatively correlated with OS in EGFR-mutant LUAD patients [25]. H. Sorotsky et al. studied early NSCLC with surgical resection of EGFR mutations and found that TMB is associated with shorter survival but has little to do with disease relapse [27].

3.2.4. Publication Bias And Sensitivity Analysis

According to Begg's and Egger's test, the publication bias of OS (Begg's $P = 0.767$; Egger's $P = 0.765$) and PFS (Begg's $P = 0.528$; Egger's $P = 0.591$) was not significant. By omitting each study one by one, no single study with a significant impact on the combined results was found, which means that the meta-analysis results were reliable.

4. Discussion

With the development of sequencing technology, TMB has become an essential clinical indicator [28, 29]. Currently, TMB is mainly studied as a predictive biomarker for ICIs treatment response [15, 30]. Recent studies have concluded that in patients with higher TMB, the survival benefit of patients receiving ICIs is better than that of patients receiving chemotherapy alone, but in patients with low TMB, the survival benefit of ICIs is not statistically significant [17, 18]. In this study, we did not group patients according to TMB value, but grouped according to lung cancer types, treatment methods, detection methods, test samples. We found that high TMB in the immunotherapy group was an excellent prognostic indicator, which corresponded to high TMB as a marker of immunotherapy responsiveness. But in NSCLC patients who receive chemo-radiotherapy or targeted therapy but not ICIs treatment, high TMB is associated with a worse prognosis, which suggests that TMB itself may be a poor prognostic indicator in NSCLC.

According to the type of lung cancer, TMB is a good prognostic indicator in SCLC, but a poor prognosis indicator in NSCLC. Immunotherapy enhances the role of TMB in SCLC to indicate better prognosis, but reverses the role of TMB in NSCLC, making TMB from a poor prognostic indicator to a better prognostic indicator. The beneficial effect of high TMB on long-term survival of NSCLC and SCLC patients treated with ICIs validates the therapeutic value of immunotherapy for patients with high TMB [31, 32]. In LUAD, including EGFR mutated and EGFR-TKIs treated LUAD, high TMB is a poor prognostic indicator. Fortunately, it was found in this study that TMB values are usually lower in LUAD patients, especially those with EGFR mutations. However, for LUAD patients

with EGFR mutations and high TMB, whether targeted therapy is adequate and whether additional or alternative immunotherapy is needed should be further studied [33].

In terms of different detection methods, the detection results of WES and targeted NGS are consistent, and have no significant impact on the clinical effect evaluation of TMB. However, the TMB value detected by WES is higher than that detected by targeted NGS, which may be because the WES method contains more mutation sites. In clinical applications, targeted NGS is obviously more suitable for clinical work than WES because it saves testing resources and time [34]. At present, different types and quantities of gene panels are used for targeted NGS in different institutions, which results in various TMB benchmark values detected by various institutions. Exploring gene panels suitable for specific tumors in specific populations will help promote the better clinical application of TMB [35].

Compared with circulating tumor DNA (ctDNA) testing, tissue testing is still the current mainstream method [36]. Blood testing has the advantage of convenient material collection, but the testing technology needs further investigation [37]. In this study, a total of three articles with four studies [26, 38, 39] used blood samples, and the results were heterogeneous so that no credible conclusions could be drawn about the validity of the blood test results. In this meta-analysis, we observed that Chae YK et al. used tumor tissue and blood tissue to detect TMB in NSCLC, respectively, and obtained almost opposite conclusions [38, 40]. Chae YK et al. used tumor tissue to detect TMB in patients with NSCLC receiving ICIs and observed that high TMB predicting longer OS (HR = 0.10, 95%CI 0.01–0.76, P = 0.026) [40]. On the other hand, Chae YK et al. used blood samples to test TMB and found that high TMB was associated with poor OS (HR = 6.0, 95%CI 1.3–27.1, P < 0.01) and PFS (HR = 5.6, 95%CI 1.3–24.6, P < 0.01) among NSCLC patients receiving ICIs [38]. However, in the subsequent verification group, Chae YK et al. found that the ctDNA TMB had no significant effect on OS (HR = 2.8, 95%CI 0.56–14.4, P = 0.17) and PFS (HR = 1.1, 95%CI 0.31–4.2, P > 0.05) in NSCLC patients receiving ICIs [38]. The clinical evaluation of TMB using blood specimens from the same institution differs greatly from the results obtained using tumor tissues, and the results of blood specimens show some instability. The conclusion drawn from this blood specimen is also inconsistent with the mainstream view that the high TMB patients should have better survival benefits in NSCLC patients treated with ICIs [17, 18]. The study author Chae YK et al. also previously found a low correlation in ctDNA TMB and tissue TMB in paired patient samples [41]. In addition to the limitations of ctDNA detection technology, ctDNA TMB may have different clinical significance than tissue TMB. The relationship between ctDNA TMB and tissue TMB and its clinical significance remains to be further explored in larger sample studies.

Compared with the current mainstream researches [42], we focused on the prognostic role of TMB in different lung patient groups, supplementing the role of TMB in traditional chemoradiotherapy and targeted therapy populations. Despite our comprehensive analysis, there are some limitations be recognized. First, the studies included in our report were mainly observational cohort studies, which might provide weaker statistical power. Fortunately, the measurement of TMB values was objective, and most studies were grouped by the median, so the endpoint events were less affected by selective bias. Some of the included studies were secondary analyses of clinical trial results, which could also increase the validity of the results. Second, several HRs and its 95% CI were calculated by extracting data from the Kaplan-Meier curves, which may inevitably lead to statistical bias. Third, the baseline of TMB detected by different testing platforms was different, which might bring bias to combined results. Each study distinguished high and low TMB groups according to the relative value of TMB, which could avoid bias caused by different TMB baselines. The accepted standardization methods of cross-platform TMB results are still one of the critical directions for clinical application of TMB [43, 44].

Conclusion

In this study, we found that TMB is an excellent prognostic indicator in lung cancer patients receiving immunotherapy. Still, it is a poor prognostic indicator in patients with lung cancer receiving traditional chemo-radiotherapy or targeted therapy. EGFR mutation status in patients with lung adenocarcinoma is associated with lower TMB, but some patients have both EGFR mutation and high TMB status. How to choose immunotherapy or targeted therapy for lung adenocarcinoma patients with both EGFR mutation and high TMB needs to be further studied in well-designed randomized controlled clinical trials.

Abbreviations

TMB, tumor mutational burden; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; ICI, immune checkpoint inhibitor; NGS, next-generation sequencing; WES, whole exome sequencing; MSI, microsatellite instability; MMR, mismatch repair deficiency; TKI, tyrosine kinase inhibitor; OS, overall survival; PFS, progression-free survival; DFS, disease-free survival; IQR, interquartile range; ES, effect size; HR, hazard ratio; CI, confidence interval; ctDNA, circulating tumor DNA.

Declarations

Ethics approval and consent to participate

Not applicable. This is a secondary analysis of gene sequencing data and literature.

Consent for publication

All authors agree to publish.

Availability of data and materials

Single nucleotide mutation, gene expression and patient follow-up data were obtained from The Cancer Genome Atlas (TCGA) database (<http://portal.gdc.cancer.gov/>).

Competing interests

The authors declare no conflict of interest.

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Authors' Contributions

YW and JH are involved in project design, improvement and finalization. LZ participates in the project design and undertakes the specific work of the research. PX, ZW, and LY participate in literature search, literature data extraction, and discussion of disputed data. WL participates in project improvement, dispute data discussion, article revision and finalization. All authors have read and approved the manuscript.

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Authors' Information

YW and JH are cooperating medical colleagues, working together to build an integrated diagnosis and treatment system for lung cancer patients. They have accumulated rich experience in neoadjuvant immunotherapy and neoadjuvant therapy for lung cancer patients. LZ, PX, ZW, and LY are all students of JH. They are engaged in the diagnosis and treatment of lung cancer patients and carry out basic research on lung cancer. WL is the administrator of JH's laboratory, mainly responsible for the laboratory's daily work and student guidance.

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Figures

Figure 1

The landscape of mutation in LUAD samples. (A) Missense mutation was the most mutation type; (B) SNPs showed higher frequency than deletions or insertions; (C) C>A occupies the most common type of SNV; (D) Landscape of mutation profiles in LUAD samples. The top 80 genes with mutation probability in each sample (TMB > 0, N = 533) was shown in the waterfall plot, where various colors meant various mutations types. The bar plot above the waterfall plot exhibited the number of mutation burden. The red lines below the waterfall plot indicated the enriched EGFR mutant population. LUAD, lung adenocarcinoma; Del, deletion; Ins, insertion; SNP, single-nucleotide polymorphism; SNV, single-nucleotide variant; TMB, tumor mutational burden; N, number.

Figure 2

The landscape of mutation in LUSC samples. (A) Missense mutation was the most mutation type; (B) SNPs showed higher frequency than deletions or insertions; (C) C>A occupies the most common type of SNV; (D) Landscape of mutation profiles in LUSC samples. The top 80 genes with mutation probability in each sample (TMB > 0, N = 485) was shown in the waterfall plot, where various colors meant various mutations types. The bar plot above the waterfall plot exhibited the number of mutation burden. LUSC, lung squamous cell carcinoma; Del, deletion; Ins, insertion; SNP, single-nucleotide polymorphism; SNV, single-nucleotide variant; TMB, tumor mutational burden; N, number.

Figure 3

Mutation load value for each patient in LUAD (A) and LUSC (B).

Figure 4

Kaplan-Meier survival curves with log-rank tests of high TMB and low TMB groups in LUAD (A) and LUSC (B).

Figure 5

Relationship between EGFR mutation status and TMB value in LUAD (A) and LUSC (B).

Figure 6

Relationship between EGFR mutation status and PD-L1 expression in LUAD (A) and LUSC (B).

Figure 7

Flow chart of searching the relevant studies included in this meta-analysis.

Figure 8

Meta-analyses of overall survival (A) and progression-free survival (B) of total lung cancer patients.

Figure 9

Subgroup analyses of overall survival in lung cancer patients based on immunotherapy and pathological type. (A) Subgroup analysis based on immunotherapy. (B) Subgroup analysis based on NSCLC and SCLC. (C) Subgroup analysis based on NSCLC (separating LUAD and LUSC) and SCLC. (D) Subgroup analysis by combined pathological type and immunotherapy.

Figure 10

Subgroup analyses of progression-free survival in lung cancer patients based on immunotherapy and pathological type. (A) Subgroup analysis based on immunotherapy. (B) Subgroup analysis based on NSCLC and SCLC. (C) Subgroup analysis based on NSCLC (separating LUAD and LUSC) and SCLC. (D) Subgroup analysis by combined pathological type and immunotherapy.

Figure 11

Subgroup analyses of overall survival in lung cancer patients based on test methods, sample sources, and immunotherapy. (A) Subgroup analysis based on test methods. (B) Subgroup analysis based on combined test methods and immunotherapy. (C) Subgroup analysis based on sample sources. (D) Subgroup analysis by combined sample sources and immunotherapy.

Figure 12

Subgroup analyses of progression-free survival in lung cancer patients based on test methods, sample sources, and immunotherapy. (A) Subgroup analysis based on test methods. (B) Subgroup analysis based on combined test methods and immunotherapy. (C) Subgroup analysis based on sample sources. (D) Subgroup analysis by combined sample sources and immunotherapy.

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