Integrative and Comparative Genomic Analysis and the Immune Microenvironment Features of Lung Cancer Patients with Tuberculosis

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Research

Keywords: Tuberculosis, lung cancer, tumour microenvironment, PD-L1, C1QB

Posted Date: July 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-667647/v1

Abstract

Background: Limited information was known because of the low incidence of co-existence with lung cancer and tuberculosis (TB), it remains special challenging populations for clinical management of cancer immunotherapy. Thus, to investigate the difference on tumour immune microenvironment and genomics between patients with LC alone and LC patients with TB is urgently needed.

Methods: Tumour specimens were collected from 87 patients who had LC, with or without TB, at two medical centres. Immunohistochemistry was used to evaluate PD-L1 expression and CD3+/CD4+/CD8+ T-cell infiltration. Whole-exome sequencing was performed using samples from 19 patients with LC&TB and 21 patients with LC.

Results: Relative to patients with LC alone, patients with LC&TB had lower PD-L1 expression and CD4+/CD8+ T-cell infiltration (all $P< 0.001$). A tumour microenvironment with no PD-L1 expression and CD8- T-cell infiltration was most common in the LC&TB patients. Genomic alterations analysis revealed an increased mutation frequency among patients with LC and active TB, obsolete/cured TB, or no TB in terms of the TP53 (23.08% vs. 66.67% vs. 76.19%, $P = 0.01$), while a decreased trend of the number of single-nucleotide variants/insertions/deletions ($P< 0.001$), tumour mutation burden ($P< 0.001$), and number of neoantigens ($P< 0.001$). Patients with LC&TB had a higher frequency of a specific mutation signature (32.99% vs. 11.23%), as well as potential driver mutations involving the complement C1qB chain (*C1QB*) mutations.

Conclusion: The present study revealed significant differences in the tumour microenvironment and genomic alterations between patients with LC&TB and patients with LC alone.

Highlight

- This study compared lung cancer specimens according to the presence or absence of tuberculosis.
- Lung cancer with tuberculosis had lower PD-L1 expression and less CD3+/CD4+/CD8+ T-cell infiltration.
- Genomic alterations were significantly different according to the presence or absence of tuberculosis.
- Patients with lung cancer and tuberculosis had a unique potential driver gene (*C1QB*), may influence macrophage and dendritic cell infiltration.

Background

Lung cancer (LC) remains the major cause of cancer-related death, with an estimated 1.59 million deaths in 2016, which accounts for approximately 20% of all cancer-related deaths worldwide (1). Epidemiological research regarding LC has revealed that the major risk factors are smoking, air pollution,

occupational factors, and tuberculosis (TB), which will provide the basis for future interventions to improve LC management (2–4).

Mycobacterium tuberculosis (MTB) is the pathogen that causes TB, which is the deadliest infectious disease in the world today (5, 6). Infection by MTB affects approximately one-third of the global population, and insufficient treatment or compromised immune defences lead to the development of TB in 5–10% of infected patients (5, 6). Most MTB infections have no symptoms, although TB-induced inflammation typically leads to genetic changes over time, which can drive the development of LC (3). Thus, patients with TB have a significantly increased risk of developing LC and related mortality (3). The increased risk of LC is related to the immunosuppressive state caused by MTB infection (7–9), and co-existence of LC and TB (LC&TB) has been reported in numerous cases and case-control studies (3, 10, 11).

Immunotherapy has become a mainstream treatment option for various cancers, including LC and melanoma (12–17), in addition to surgery, radiotherapy, and chemotherapy. Immune checkpoint inhibitors are a type of immunotherapy that activate T-cell responses in the tumour microenvironment and can enhance the immune system's response to the tumour.

Patients with LC&TB are rare and thus often overlooked in clinical trials, with only limited global data regarding immunotherapy in this patient population (18). Thus, it is unclear whether immune checkpoint inhibitors including anti-PD-1/PD-L1 therapy are contraindicated in patients with LC&TB. Furthermore, the T-cell response plays a key role in the development and progression of TB (19), and different immune microenvironments of LC patients with and without TB might explain their potentially different treatment responses. Therefore, the present study aimed to evaluate the tumour microenvironment characteristics of LC patients with and without TB, including their PD-L expression and tumour-infiltrating lymphocytes (TILs). Furthermore, we evaluated differences in somatic mutations, copy number variations, tumour mutation burden (TMB), HLA expression, and neoantigen numbers according to the presence or absence of TB.

Materials And Methods

2.1 Patient population

The study was approved by the ethics committees of Zhejiang Cancer Hospital and Affiliated Hangzhou Chest Hospital. This study evaluated data from 87 patients with stage I–IV LC with TB, who were treated between 2013 and 2019 at two Chinese medical centres (Zhejiang Cancer Hospital and Affiliated Hangzhou Chest Hospital). The eligibility criteria were as follows: LC confirmed via pathological or cytological evaluation, complete clinical and follow-up data, availability of ≥ 5 blank sections from tissue specimens, and no history of malignancy within the previous 5 years. The presence of active TB (ATB) was confirmed based on 3 sputum samples for mycobacterium culture, nucleic acid amplification test and/or lung biopsy (if feasible) (20), while the obsolete TB (OTB) was confirmed based on the patient’s

medical records, the T-cell spot test (TSPOT) or interferon gamma release assay (IGRA), and imaging findings. Cured TB (CTB) was defined as "history of active TB and have been cured when cancer diagnosed". Of note, latent TB was not included in our study. The control group contained stage I-IV LC patients from Zhejiang Cancer Hospital. The study was approved by the ethics committee of Zhejiang Cancer Hospital and Affiliated Hangzhou Chest Hospital.

For genomic analysis, 21 LC&TB patients, treated with surgery, with more than 15 white paraffin tablets was selected. During 2013–2019, specimens from 192 LC cases had been collected in the hospitals' biobank and we performed propensity score analysis to select 21 paired samples from patients with LC alone. These samples, and normal control tissues, were subjected to whole-exome sequencing (WES) and analysis of copy numbers and somatic mutations. These cancer-specific genomic alterations were then evaluated for associations with the patients' clinical characteristics. The study flowchart is shown in Fig. 1.

2.2 DNA extraction, exome sequencing, and data processing

After omitting 2 samples from the LC&TB group because of poor quality, DNA was extracted from the remaining 40 formalin-fixed paraffin-embedded (FFPE) samples using the QIAamp FFPE DNA Kit (Qiagen, Frankfurt, Germany). The DNA was then fragmented using a Covaris M220 focused ultrasonicator (Covaris, Woburn, USA) and subjected to sequencing library construction. The DNA for WES was captured using the VariantBaits™ Human All Exon Kit (LC-Bio) according to the manufacturer's recommended protocol. Sequencing was performed using the Illumina NovaSeq™ 6000 system to generate 150-bp paired-end reads, and FASTQ software (version 0.20.0) (21) was used to remove low-quality reads and clean the data. The supplementary materials (available online) show the methods for somatic mutation calling, identification of somatic copy number alterations, identification of potential driver genes, extraction of mutation signatures, and prediction of HLA types and neoantigens.

2.3 Immunohistochemistry

Immunohistochemistry was used to evaluate the expression of PD-L1 and the presence of tumour-infiltrating $CD3^+$ T-cells, $CD4^+$ T-cells, and $CD8^+$ T-cells. We prepared FFPE tissue sections (4-μm) on positively charged glass slides, which were stained using primary antibodies targeting CD3 (SP162, 1:150; Abcam, Cambridge, UK), CD4 (B468A1, 1:200; Santa Cruz, TX, USA), CD8 (144B, 1:100; Abcam), PD-L1 (VENTANA PD-L1 [SP263] assay; Roche, AZ, USA), and ALK (VENTANA ALK [D5F3] assay; Roche, AZ, USA). The immunohistochemistry was performed using the BenchMark XT platform and the VENTANA OptiView DAB IHC detection kit. The tumour expression of PD-L1 was evaluated using the tumour proportion score (TPS), which is based on the proportion of tumour cells with positive staining, with a positive result considered present at a PD-L1 TPS of ≥ 1% (22). The proportions of CD3/CD4/CD8 staining on lymphocytes were evaluated as the proportions of positive cells among all nucleated cells in the stromal compartments, with a positive result considered present at a proportion of ≥ 25% (22). All

immunohistochemistry results were judged by two pathologists who were blinded to the patients' clinical characteristics, and disagreements were resolved via discussion. The expressions of PD-L1 and T-cell markers were used to create different subtype groups, and differences between the LC and LC&TB groups were displayed using stacked bar graphs.

2.4 Statistical analysis

All data were expressed as mean ± standard deviation or number (percentage), unless otherwise indicated. Categorical variables were compared using Fisher's exact test. The Kruskal-Wallis test was used to compare clinicopathological factors, gene alterations, and TMB. Paired groups were compared using the Wilcoxon test, and comparisons of more than two groups were performed using the Kruskal-Wallis chi-squared test. Linear correlations were evaluated using Pearson's correlation coefficient. Most statistical analyses were performed in the R statistical environment (version 3.5.1 or later) and the "survival" package was used to generate and compare the Kaplan-Meier survival curves. The survival event was defined as patients died with lung cancer. GraphPad Prism software (version 8.4; GraphPad Software, San Diego, CA, USA) was used to create the stacked bar graphs. Multivariable survival analyses were performed using the Cox proportional hazards model and propensity score matching analysis was performed using SPSS software (version 24; IBM Corp., Armonk, NY, USA). Differences were considered statistically significant at two-sided *P*-values of < 0.05.

Results

3.1 Clinicopathological characteristics

The study included 59 patients with LC&TB (33 patients with ATB and 26 patients with O/CTB) and 28 patients with LC alone. The groups' clinicopathological characteristics are summarized in Table 1 and **Supplementary Table S1** (available online). There were no significant inter-group differences in terms of sex, smoking status, histology, pathological T status, lymph node metastasis, distant metastasis, clinical stage, differentiation degree, venous/lymphatic/perineural invasion, adjuvant radiotherapy, adjuvant chemotherapy, or family history of cancer. Most patients had non-small-cell lung cancer (NSCLC, adenocarcinoma or squamous cell carcinoma), although the LC&TB group included 1 patient with adenosquamous carcinoma, 1 patient with large-cell carcinoma, and 1 patient with small-cell carcinoma. No significant differences in *EGFR* mutation status were observed between the LC&TB and LC groups. All tested samples were negative for *ALK* mutations, although related testing was not performed for 20% of the patients because of insufficient samples.

Table 1
The clinicopathological characteristics of entire patient cohort (divided into two groups).

Patient Characteristics	LC&TB group (N = 59)	LC group (N = 28)	*P* value
Sex			0.295
Male	48 (81.4%)	20 (71.4%)	
Female	11 (18.6%)	8 (28.6%)	
Median Age (Range)	62 (51–79) years	62 (37–75) years	
Smoking status			0.995
Non-smoker	19 (32.2%)	9 (32.1%)	
Smoker	40 (67.8%)	19 (67.9%)	
Histology			0.688
adenocarcinoma	28 (47.5%)	12 (42.9%)	
Non-adenocarcinoma	31 (52.5%)	16 (57.1%)	
Pathologic T stage			0.132
T1 + T2	40 (69.0%)	22 (84.6%)	
T3 + T4	18 (31.0%	4 (15.4%)	
Lymph node metastasis			0.319
N0	24 (42.1%)	15 (53.6%)	
N1-3	33 (57.9%)	13 (46.4%)	
Distant metastasis			0.931
M0	44 (75.9%)	21 (75.0%)	
M1	14 (24.1%)	7 (25.0%)	
Clinical stage			0.093
I + II	22 (37.9%)	16 (57.1%)	
III + IV	36 (62.1%)	12 (42.9%)	
Differentiation degree			0.865
Poor or undifferentiated	21 (48.8%)	6 (46.2%)	
Well-intermediate	22 (51.2%)	7 (53.8%)	
EGFR			0.127

Patient Characteristics	LC&TB group (N = 59)	LC group (N = 28)	*P* value
Positive	1 (5.3%)	6 (21.4%)	
Negative	18 (94.7%)	22 (78.6%)	
PD-L1			**< 0.001**
Positive	13 (23.2%)	18 (64.3%)	
Negative	43 (76.8%)	10 (35.7%)	
CD3			**0.001**
Positive	7 (14.6%)	11 (52.4%)	
Negative	41 (85.4%)	10 (47.6%)	
CD4			**< 0.001**
Positive	3 (6.3%)	14 (66.7%)	
Negative	45 (93.8%)	7 (33.3%)	
CD8			**< 0.001**
Positive	3 (6.3%)	15 (53.6%)	
Negative	45 (93.8%)	13 (46.4%)	
Venous/lymphatic/Perineural invasion			0.632
No	18 (75.0%)	17 (81.0%)	
Yes	6 (25.0%)	4 (19.0%)	
Adjuvant radiotherapy			0.593
No	21 (50.0%)	12 (57.1%)	
Yes	21 (50.0%)	9 (42.9%)	
Adjuvant chemotherapy			0.109
No	49 (83.1%)	19 (67.9%)	
Yes	10 (16.9%)	9 (32.1%)	
Family history of cancer			0.242
No	52 (88.1%)	22 (78.6%)	
Yes	7 (11.9%)	6 (21.4%)	

3.2 Comparing the tumour microenvironment between patients with LC&TB and LC

The LC&TB group included 10 patients with both tumour and TB samples, which were evaluated for differences in terms of PD-L1 expression and TILs (**Supplementary Table S2**). No significant differences were observed, although the TB lesions tended to have greater $CD3^+$ and $CD4^+$ T-cell infiltration (vs. the tumours), especially in patients with ATB. However, a comparison of specimens from the LC and LC&TB groups revealed significant differences in TILs and PD-L1 expression. First, PD-L1 expression was remarkably less common in the LC&TB group (13 patients, 23.2%) than in the LC group (18 patients, 64.3%). Moreover, the LC&TB group had clearly lower infiltration of $CD3^+$, $CD4^+$, and $CD8^+$ T-cells, relative to the LC group (all $P < 0.001$, Table 1, **Supplementary Figures S1–4**). Moreover, the predominant phenotype in the LC&TB group involved no PD-L1 expression and $CD8^-$ T-cell infiltration ($P < 0.001$), while the predominant phenotype in the LC group involved PD-L1 expression and $CD8^+$ T-cell infiltration (Fig. 2A-C).

3.3 Survival

Relative to the LC group, the LC&TB group had shorter median overall survival (OS: 34.1 months vs, not reached, $P = 0.041$, Fig. 2D). Univariate and multivariable survival analyses were performed for the LC&TB group, although OS was not significantly associated with $CD3^+/CD4^+/CD8^+$ T-cell infiltration or PD-L1 expression (Fig. 2E–H). However, OS was significantly associated with pathological T status ($P = 0.049$), lymph node metastasis ($P = 0.007$), and clinical stage ($P = 0.006$) (Fig. 2I, **Supplementary Table S3**). Cox regression analysis revealed that OS was independently predicted by clinical stage (hazard ratio: 5.70, 95% confidence interval: 1.26–25.92, $P = 0.024$).

3.4 Genomic alterations

To reduce the effects of selection bias, propensity score matching analysis was performed to create 21 pairs of patients with LC&TB or LC alone, who were matched according to sex, age, smoking status, clinical stage, and pathological type (adenocarcinoma or non-adenocarcinoma) (Fig. 1). The samples from these patients were subjected to WES, although 2 samples from the LC&TB group were excluded because of poor DNA quality. The paired patients' clinicopathological characteristics are shown in Table 2. Figure 3 shows the somatic mutations, clinical features, potential driver genes, 30 most commonly mutated genes, and genes that are related to the efficacy of immune checkpoint inhibitor treatment.

Table 2
The clinicopathological characteristics of entire patient cohort (divided into two groups).

Patient Characteristics	LC with TB(N = 19)	LC (N = 21)	*P* value
Sex			0.906
Male	13 (68.4%)	14 (66.7%)	
Female	6 (31.6%)	7 (33.3%)	
Median Age (Range)	54 (36–75)	61 (37–74)	
Smoking status			0.583
Non-smoker	6 (31.6%)	5 (23.8%)	
Smoker	13 (68.4%)	16 (76.2%)	
Histology			0.105
adenocarcinoma	13 (68.4%)	9 (42.9%)	
Non-adenocarcinoma	6 (31.6%)	12 (57.1%)	
Pathologic T stage			0.874
T1 + T2	15 (78.9%)	17 (81.0%)	
T3 + T4	4 (21.1%)	4 (19.0%)	
Lymph node metastasis			0.385
N0	15 (78.9%)	14 (66.7%)	
N1-3	4 (21.1%)	7 (33.3%)	
Distant metastasis			0.287
M0	18 (94.7%)	21 (100.0%)	
M1	1 (5.3%)	0 (0%)	
Clinical stage			0.583
I + II	13 (68.4%)	16 (76.2%)	
III	6 (31.6%)	5 (23.8%)	
Differentiation			0.588
Poor or undifferentiated	9 (56.3%)	6 (46.2%)	
Well-intermediate	7 (43.8%)	7 (53.8%)	

Patient Characteristics	LC with TB(N = 19)	LC (N = 21)	*P* value
Venous/lymphatic/Perineural invasion			0.807
No	14 (77.8%)	17 (81.0%)	
Yes	4 (22.2%)	4 (19.0%)	
Adjuvant radiotherapy			0.573
No	8 (53.3%)	6 (42.9%)	
Yes	7 (46.7%)	8 (57.1%)	
Adjuvant chemotherapy			0.583
No	15 (78.9%)	15 (71.4%)	
Yes	4 (21.1%)	6 (28.6%)	
Family history of cancer			0.916
No	17 (89.5%)	19 (90.5%)	
Yes	2 (10.5%)	2 (9.5%)	
EGFR			0.101
Positive	1 (5.3%)	5 (23.8%)	
Negative	18 (94.7%)	16 (76.2%)	
PD-L1			**0.005**
Positive	2 (10.5%)	11 (52.4%)	
Negative	17 (89.5%)	10 (47.6%)	
CD3			0.163
Positive	4 (28.6%)	11 (52.4%)	
Negative	10 (71.4%)	10 (47.6%)	
CD4			**0.009**
Positive	3 (21.4%)	14(66.7%)	
Negative	11 (78.6%)	7 (33.3%)	
CD8			**0.045**
Positive	1 (7.1%)	8 (38.1%)	
Negative	13 (92.7%)	13 (61.9%)	

Patient Characteristics	LC with TB(N = 19)	LC (N = 21)	*P* value
TP53			**0.012**
Positive	7 (36.8%)	16 (76.2%)	
Negative	12 (63.2%)	5 (23.8%)	
C1QB			
Positive	4 (21.1%)	0 (0%)	**0.042**
Negative	15 (63.2%)	21 (100%)	
CDKN2A			
Positive	2 (10.5%)	2 (9.5%)	0.916
Negative	17 (89.5%)	19 (90.5%)	
POU3F3			0.141
Positive	4 (21.1%)	1 (4.8%)	
Negative	15 (78.9%)	20 (95.2%)	
Median TMB (Range)	17.4 (7.5–30.6) muts/MB	10.8 (2.0-25.1) muts/MB	

The most common alterations in the LC&TB group were mutations in *ZNF208* (74%), *FLG* (68%), *MUC17* (68%), *ZNF729* (63%), and *HRNR* (58%) (**Supplementary Figure S2A**). The most common alterations in the LC group were mutations in *TP53* (76%), *TTN* (67%), *RYR2* (43%), *KMT2D* (38%), and *MUC4* (38%). The differential analysis results for somatic mutations in the LC&TB and LC groups are listed in **Supplementary Table S4**. There were no significant differences between the LC&TB and LC groups in terms of somatic mutations in *EGFR*, *PIK3CA*, *KRAS*, and *BRAF*, as well as *CDKN2A* copy number variations (CNVs) (all $P > 0.05$), (**Supplementary Figure S6)**. However, a significant difference in the average number of single-nucleotide variants/insertions/deletions was observed between the LC and LC&TB groups ($P = 0.002$), as well as between the LC&ATB (n = 1,131.08), LC&O/CTB (n = 1,143.83), and LC (n = 633.95) groups ($P < 0.001$) (Fig. 4A). The most common base substitution was C > T in all three groups, with frequencies of 34.4% in the LC&ATB group, 35.41% in the LC&O/CTB group, and 35.27% in the LC group, although the differences were not statistically significant (all $P > 0.05$) (Fig. 4B). The average number of CNVs per sample were 41.84 in the LC&ATB group, 38.67 in the LC&O/CTB group, and 36.67 in the LC group, although the differences were not statistically significant (Fig. 4C). A heatmap of the gain/loss CNVs is shown in **Supplementary Figure S7A** and a heatmap of the 50 most commonly affected genes in the LC&ATB and LC groups is shown in **Supplementary Figure S7B**. There were also no significant differences in the numbers of CNVs in the *CD274* and *CDKN2A* genes (**Supplementary Figure S6H**). The results of the GO and KEGG pathway analyses are shown in **Supplementary Figure S7C–D**, which revealed that the top functional clusters in the LC&TB group involved cancer-related pathways.

Mutation signatures were first described in 2013 (23) as groups of gene mutations that are related to malignant processes in tumour cells. We performed mutation signature analyses for each group, which revealed mutation signature 4 in the LC&TB and LC groups. Cluster analysis also revealed that the proportion of samples with mutation signature 1 was higher in the LC&TB group than in the LC group (32.99% vs. 11.23%) (**Supplementary Figure S8A–C**). Mutually exclusive and co-occurring gene pairs are shown in **Supplementary Figure S8D**.

3.5 TMB analysis, HLA analysis, and neoantigen prediction

The LC&ATB and LC&O/CTB groups were characterized by a high average TMB (18.65 mutations/Mb and 18.86 mutations/Mb), which was noticeably higher than the average TMB in the LC group (10.45 mutations/Mb) (Fig. 5A). Furthermore, a high TMB was significantly associated with a mutation signature that suggested exposure to cigarette smoke in the LC group ($P = 0.036$) and the LC&TB group ($P = 0.025$) (Fig. 5C). Interestingly, a lower TMB was observed in LC&TB patients with a low *TP53* mutation frequency ($P = 0.027$, Fig. 5D). The average numbers of neoantigens were 1,146.08 in the LC&ATB group, 888.33 in the LC&O/CTB group, and 395.29 in the LC group ($P < 0.001$, Fig. 5E), and the number of nonsynonymous mutations was correlated with the number of neoantigens ($r = 0.74$, $P < 0.01$, Fig. 5G). Expression of PD-L1 was not significantly correlated with the TMB among all patients with WES results ($r = 0.71$, $P = 0.258$, Fig. 5H). The three most common HLA types were identified in each group, and the results revealed heterozygous genotypes for all HLA class I subtypes (A, B, and C) in the LC&ATB, LC&O/CTB, and LC groups ($P > 0.05$, **Supplementary Table S5**).

We also found several potential driver genes in patients with LC&TB (**Supplementary Materials**).

Discussions

Preclinical studies have indicated that immune homeostasis in TB is regulated via immune checkpoint pathways, such as the PD-1/PD-L1 axis. For example, in animal models, the PD-1 pathway plays a key role in controlling excessive inflammation after MTB infection and regulating the resulting immune response (24). Furthermore, the PD-1/PD-L1 pathway suppresses the accumulation of $CD4^+$ T-cells and IFN-γ production, which is an essential part of the immune response to TB. However, treatment that targets PD-1/PD-L1 may cause CD4 + T-cells to overproduce IFN-γ, which can aggravate TB or cause TB recurrence (24–35). Another study has indicated that a TB antigen can inhibit the Th1 immune response and promote LC metastasis via the PD-1/PD-L1 signalling pathway (36). However, there are limited data regarding the immune microenvironment in patients with LC and previous/current TB, which highlights the need for additional information regarding PD-L1 expression and TILs in the tumour and surrounding microenvironment. Our findings revealed that, relative to the LC group, the LC&TB group had significantly decreased PD-L1 expression and less $CD3^+/CD4^+/CD8^+$ T-cell infiltration, which suggests that these patients have immunologically cold tumours in a noninflammatory microenvironment (Fig. 6A). To the best of our knowledge, this is the first comprehensive analysis of the tumour microenvironment landscape in LC&TB and LC.

Previous studies have suggested that PD-L1 expression, *TP53* mutation frequency, TMB (37), and HLA molecules may be biomarkers for predicting the response of LC to anti-PD-1/PD-L1 therapy (38–40), although these relationships remain controversial. In our study, the LC&TB group had a markedly higher TMB than the LC group, and the LC&TB group also had a significantly lower *TP53* mutation frequency. The present study revealed that the LC&TB and LC groups only had heterozygous HLA I genotype, and thus we did not perform any additional analyses. Interestingly, we also observed that the number of nonsynonymous mutations was correlated with the number of neoantigens, which is consistent with previous research (41). Moreover, the TMB was significantly associated with smoking history among patients with LC&TB and LC alone, which also agrees with previously reported results (42). These results suggest that there are significant differences in terms of genomic alterations and mutation signatures between the LC&TB and LC groups.

A few studies have attempted to identify potential driver genes in patients with LC who had a history of TB (43, 44). Adenocarcinoma was the main pathological type of LC among our patients with LC&TB, which is consistent with previously reported results (44). Although, patients with lung adenocarcinoma who have a history of lung scarring or TB are more likely to develop *EGFR* mutations, relative to patients with conventional lung cancer (44). Nevertheless, the present study failed to detect a significant difference between the LC&TB and LC groups in terms of the *EGFR* mutation frequencies.

Notably, there were potential gene mutations differences between ATB and O/CTB in present study. For instance, The *C1QB* gene encodes the B-chain polypeptide of complement subcomponent C1q. The complement pathway is an important part of the immune system, and complement-mediated bacteriolysis and cytolysis are important mechanisms in the response to infection by pathogenic microorganisms. We observed that it was mutated at a higher rate among patients with LC&TB (5/19 cases) than among patients with LC alone (0/21 cases). In addition, bioinformatics analysis predicted that *C1QB* c.274_311del would have a substantial effect on protein expression. Furthermore, the *C1QB* gene may influence macrophage and dendritic cell infiltration of NSCLC. Therefore, *C1QB* may be a driver gene that leads to the developing of LC in patients with lung scarring or TB. In addition, KRAS mutation differences are highly significant between ATB and O/CTB ($P = 0.003$ in **Supplementary Figure S6D**), but when combined as a single group, there is no difference between LT&TB and LC alone. However, due to the limited sample size of these two groups, it is difficult to draw a clear conclusion. Further studies with large sample size and mechanism investigation are urgently needed.

Because a noninflammatory microenvironment we have observed in LC&TB and the possibility of reactivation/exacerbation of TB by anti-PD-1/PD-L1 therapy, which suggests that there is a need for new approaches to treating patients with LC&TB. We propose three potential strategies for treating patients with LC&TB (Fig. 6B). First, chimeric antigen receptor T-cell immunotherapy strategies that directly target $CD8^+$ T-cells might help avoid overactivation of $CD4^+$ T-cells. Second, dendritic cell vaccines could be developed for patients with LC&TB. Third, it might be useful to consider the possibility of drugs that target mutated *C1QB*.

Conclusions

In conclusion, we identified significant differences in the tumour microenvironment and genomic alterations when we compared patients with LC&TB and LC alone. In particular, patients with LC&TB had significantly lower PD-L1 expression and lower infiltration of immune cells. Moreover, patients with LC&TB had a lower *TP53* mutation frequency, as well as higher values for TMB, number of neoantigens, and frequency of mutation signature 1. Moreover, our findings suggest that *C1QB* may be a driver gene in patients with LC&TB, and further studies are needed to investigate the specific contributions of *C1QB* mutations in this setting.

Abbreviations

ATB: active tuberculosis; C1QB: completement C1q B chain; CNVs: copy number variations; FFPE: formalin-fixed paraffin embedded; LC&TB: lung cancer and tuberculosis; LC: lung cancer; MTB: Mycobacterium tuberculosis; NSCLC: non-small cell lung cancer; OS: overall survival; TB: tuberculosis; OTB: obsolete tuberculosis; ATB: active tuberculosis; CTB: Cured tuberculosis; O/CTB: obsolete/cured tuberculosis; IGRA: interferon gamma release assay; TIL: tumour-infiltrating lymphocytes; TMB: tumour mutation burden; TPS: tumour proportion score; WES: whole-exome sequencing.

Declarations

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by the Ethics Committee of the Zhejiang Cancer Hospital and Affiliated Hangzhou Chest Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Consent for publication

Not applicable.

Availability of data and material

Data supporting the results of this study are available from the corresponding author.

Competing interests

No potential conflicts of interest were reported by the authors.

Funding

This work was supported by grants from the National Natural Science Foundation of China [81802995 and81672315], CSCO foundation [Y-Q201802-074], the Zhejiang Province Public Welfare Funds [LGF19H280004].

Authors' contributions

YF and WMM designed and supervised the project. TXW CHB XH and YY performed the experiments described in the manuscript. CHB and TXW analyzed the data. XLX and TXW wrote the original manuscript. YF CCW and DC gave approval for the final version of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank the approve from the Zhejiang Cancer Hospital and Affiliated Hangzhou Chest Hospital in this study.

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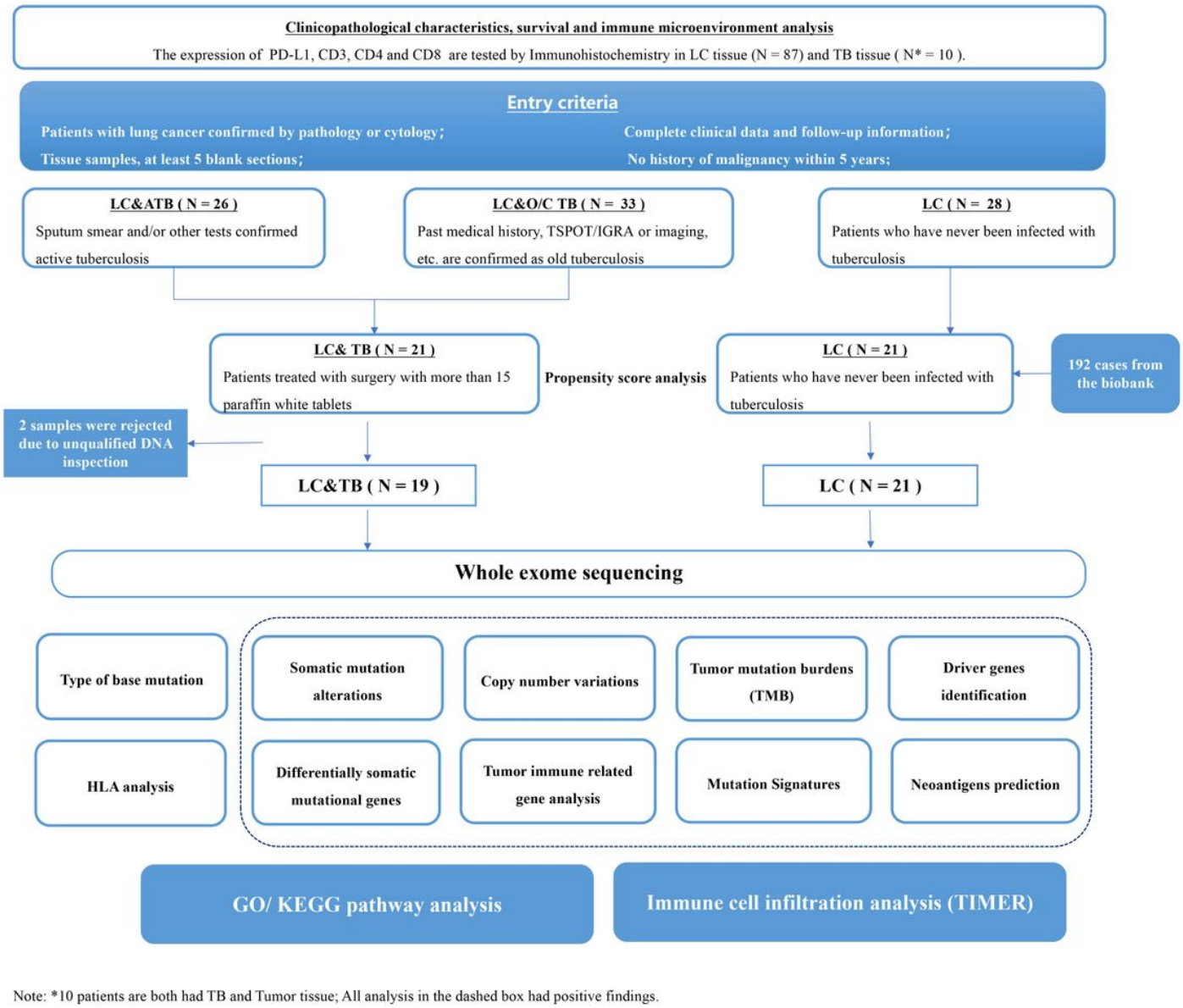
Figures



Note: *10 patients are both had TB and Tumor tissue; All analysis in the dashed box had positive findings.

Figure 1

A flowchart of the clinical and immune microenvironment analyses of 87 samples and the whole-exome sequencing analysis of 40 samples from patients with LC or LC&ATB.

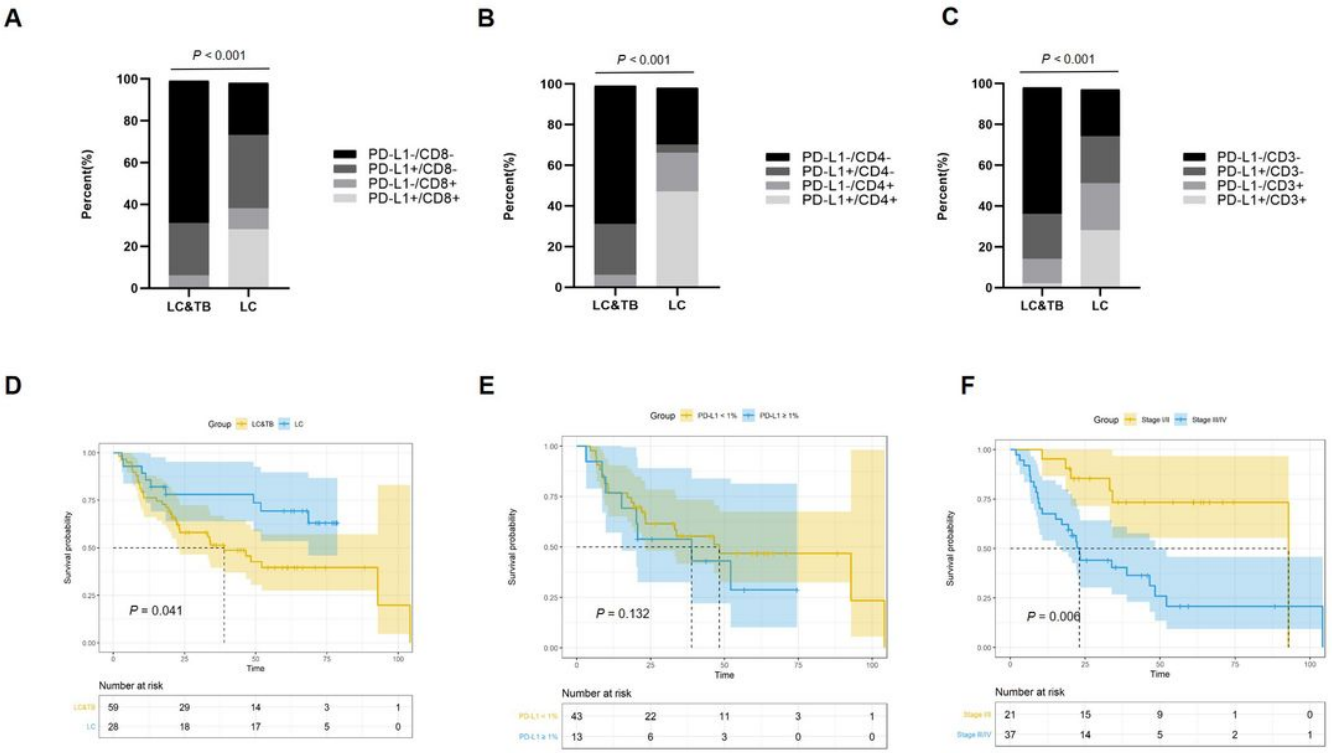


Figure 2

The correlations between tumour immune microenvironment factors and overall survival. Patients with LC alone or LC&TB were compared in terms of PD-L1 and CD8 expression (A), PD-L1 and CD4 expression (B), and PD-L1 and CD3 expression (C). (D) Kaplan-Meier curves for the LC and LC&TB groups revealed that the LC group had significantly better survival (P = 0.041). (E) Kaplan-Meier curves according to PD-L1 expression revealed no significant differences in overall survival among patients with LC&TB or LC (P = 0.132). (F) Kaplan-Meier curves according to disease stage revealed that stage III/IV disease was associated with significantly better survival than stage I/II disease) (P = 0.006).

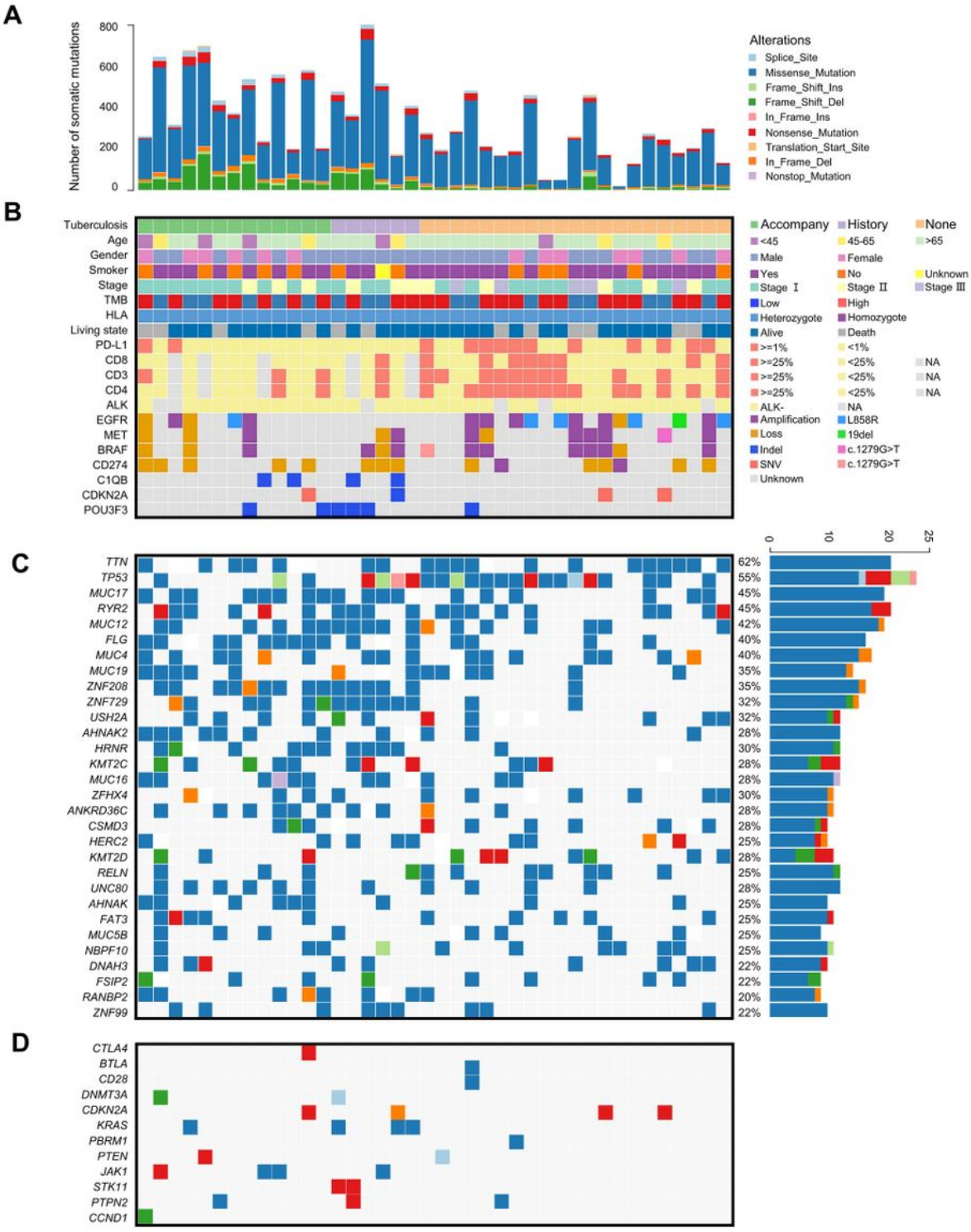


Figure 3

Summary of the clinical and molecular features associated with LC&TB or LC. (A) Bar plots showing the numbers of somatic mutations in each patient. (B) Pathology type, age, sex, smoking status, stage, TMB, HLA type, living status, marker expressions (PD-L1, CD3, CD4, and CD8), and somatic mutations for all patients. (C) Heatmap showing the distribution of significantly mutated genes in 40 patients with LC

(gene mutation frequencies are shown on the right). (D) Heatmap showing the distribution of mutations in immune-related genes from the 40 patients with LC.

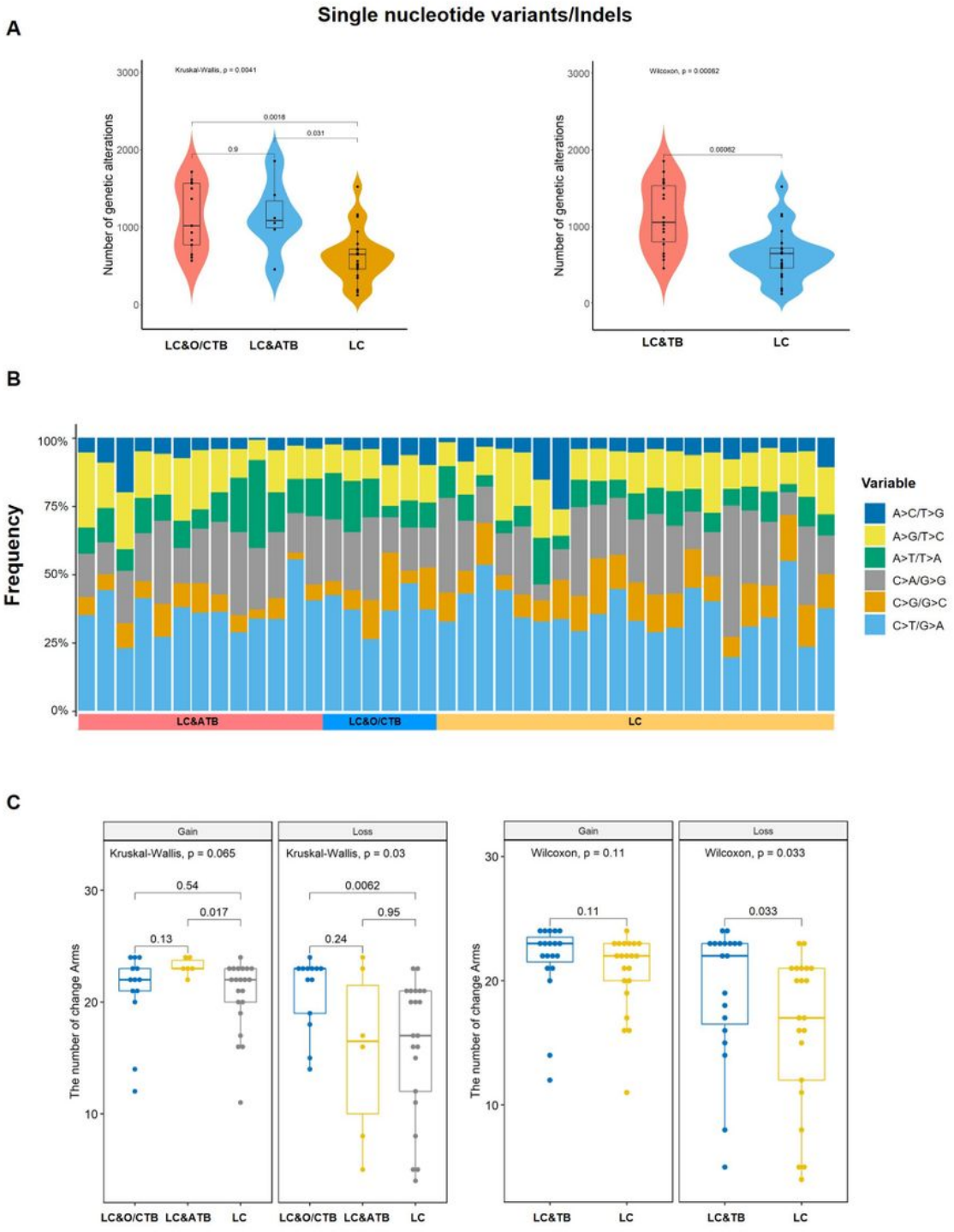


Figure 4

The number of genetic alterations, types of substitution mutations, and the number of gains/losses (CNVs) in patients with LC&O/CTB, LC&ATB, or LC. (A) Violin plots showing the numbers of genetic alterations among patients with LC&O/CTB, LC&ATB, or LC. (B) Frequencies of mutation substitution

types (classified as 6 substitution classes) in the genome among all samples. Vertical bars indicate individual patients and the vertical axis shows the frequency of each mutation category for a specific mutation type. Most patients with LC had predominantly C>T/G>A transitions. (C) Boxplots showing the number of gains/losses (CNVs) in each patient from the LC&O/CTB, LC&ATB, and LC groups. Significant inter-group differences in the number of losses per patient were detected using the two-sided Wilcoxon rank-sum test.

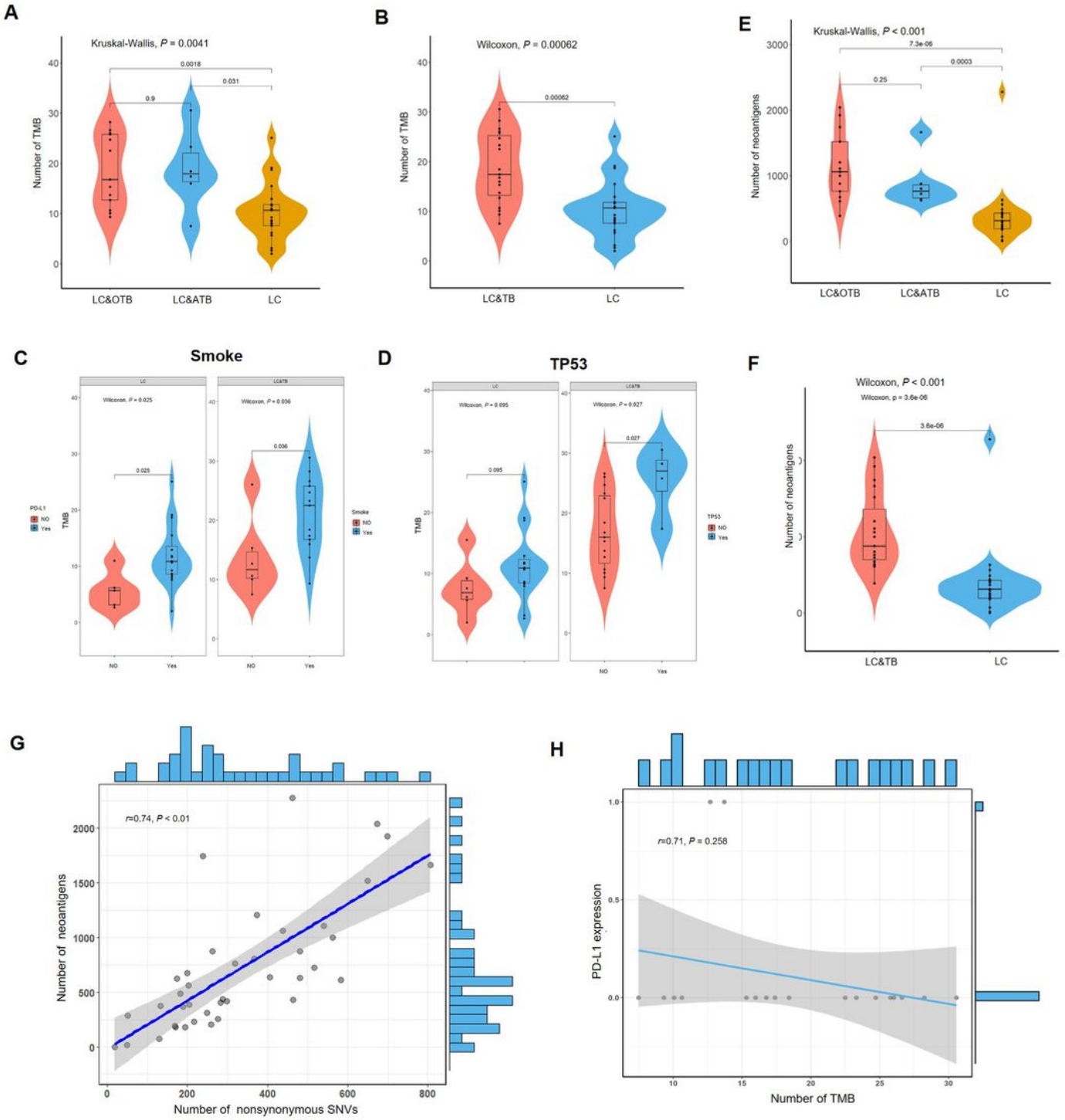


Figure 5

Tumour mutational burden and neoantigens in patients with LC&O/CTB, LC&ATB, or LC. (A) Violin plots showing the TMB in each patient from the LC&O/CTB, LC&ATB, and LC groups. Significant inter-group differences in TMB were detected using the Kruskal-Wallis test (P = 0.0041). (B) Violin plots showing the TMB in each patient from the LC&TB and LC groups. A significant inter-group difference in TMB was detected using the two-sided Wilcoxon rank-sum test (P = 0.00062). (C) Violin plots showing the differences in clinical characteristics between the LC&TB and LC groups. (D) The number of neoantigens was strongly correlated with the number of nonsynonymous mutations in the 40 patients (r = 0.74, P< 0.01). (E) The PD-L1 expression was not significantly correlated with TMB in the 40 patients (r = 0.71, P = 0.258). (F) Boxplots showing the number of neoantigens in each patient from the LC&O/CTB, LC&ATB, and LC groups. (G) Violin plots showing the number of neoantigens in each patient from the LC&TB and LC groups.

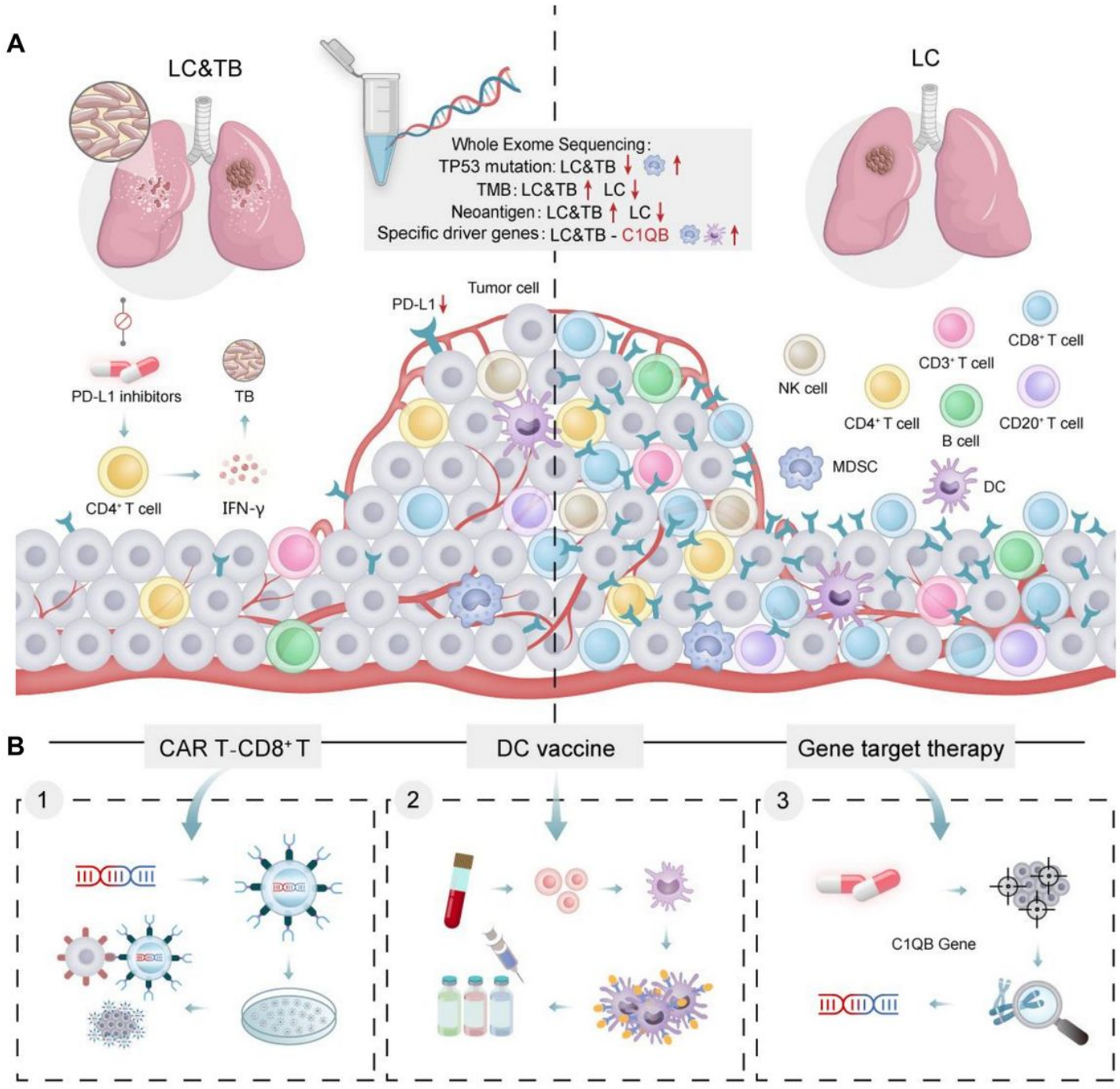


Figure 6

A summary of the study's conclusions (A) and potential future treatments (B).

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