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Original Research

Prediction of unfavourable response to checkpoint blockade in lung cancer patients through an integrated tumour-immune expression score

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ABSTRACT

Background: Treatment by immune checkpoint blockade (ICB) provides a remarkable survival benefit for multiple cancer types. However, disease aggravation occurs in a proportion of patients after the first couple of treatment cycles.

Methods: RNA sequencing data was retrospectively collected. 6 tumour-immune related features were extracted and combined to build a lung cancer-specific predictive model to distinguish responses as progression disease (PD) or non-PD. This model was trained by 3 public pan-cancer datasets and a lung cancer cohort from our institute, and generated a lung cancer-specific integrated gene expression score, which we call LITES. It was finally tested in another lung cancer dataset.

Results: LITES is a promising predictor for checkpoint blockade (area under the curve [AUC]=0.86), superior to traditional biomarkers. It is independent of PD-L1 expression and tumour mutation burden. The sensitivity and specificity of LITES was 85.7% and 70.6%, respectively. Progression free survival (PFS) was longer in high-score group than in low-score group (median PFS: 6.0 vs. 2.4 months, hazard ratio=0.45, P=0.01). The mean AUC of 6 features was 0.70 (range=0.61-0.75), lower than in LITES, indicating that the combination of features had synergistic effects. Among the genes identified in the features, patients with high expression of *NRAS* and *PDPK1* tended to have a PD response (P=0.001 and 0.01, respectively). Our model also functioned well for patients with advanced melanoma and was specific for ICB therapy.

Conclusions: LITES is a promising biomarker for predicting an impaired response in lung cancer patients and for clarifying the biological mechanism underlying ICB therapy.

Introduction

Immune checkpoint blockade (ICB) has demonstrated unprecedented clinical efficacy in the treatment of multiple cancer types. Several anti-programmed death (PD)-1/PD-ligand (L)1 inhibitors have been approved for the treatment of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). When the treatment is effective, patients can achieve a durable clinical response and prolonged overall survival (OS) [1–5]. The 5- year survival rates were 42.9% for stage III NSCLC patients [6] and 16-30% for advanced-stage NSCLC patients, respectively [7,8]. However, the response rate is low. A proportion of patients do not respond to checkpoint inhibitors and may even develop progressive disease (PD), such as an enlarged tumour or distant metastasis, after the first cycles of treatment [9]. Additionally, new patterns of progression, such as hyper progressive disease (HPD), limit the clinical application of ICB therapy [10].

Abbreviations: AUC, area under the receiver operating characteristic curve; GLCI, Guangdong Lung Cancer Institute; HR, hazard ratio; HPD, hyper progressive disease; HLA, human leukocyte antigen; ICB, immune checkpoint blockade; IPS, immunephenoscore; LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer; OS, overall survival; PD-1, programmed death-1; PD-L1, programmed death -ligand 1; PD, progression disease; PFS, progression free survival; SCLC, small cell lung cancer; TIDE, tumour immune dysfunction and exclusion; TME, tumour microenvironment; TMB, tumour mutation load.

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Although the US Food and Drug Administration has approved the assessment of PD-L1 expression and tumour mutation load (TMB), these traditional biomarkers are not perfect for predicting the benefit from checkpoint blockade [11,12]. The tumour-immune interactions in the microenvironment are diverse and complex, and it is not enough to consider only the protein expression of PD-1/PD-L1 pathway or the number of nonsynonymous mutation [13]. Liquid biopsy and dynamic change of circulating tumor DNA has become a new direction to explore predictive markers [14,15]. In addition to tumour cell biomarkers, immune cell subtypes and immune regulatory factors should be considered when developing a multifactorial biomarker to improve the predictive efficacy of ICB therapy. Some studies have shifted their focus toward integrated biomarkers such as PD-L1 expression plus immune cell composition, or TMB combined with copy number alterations [16–19]. With the continuous advances in sequencing technology, recent studies have demonstrated that gene expression profiles via RNA sequencing display tremendous potential in the prediction for ICB treatment in multiple cancer types, by considering the complex tumour-immune interaction of the tumour microenvironment (TME) [20–23].

Different TME subtypes cause the heterogeneity of the response to immune checkpoint inhibitors [24,25]. Patients with an inflamed phenotype of the TME, such as high PD-L1 expression and CD8+ T cell infiltration, have the potential to achieve a durable clinical response, ultimately leading to longer OS [26]. In contrast, activation of regulatory T cells, overexpression of other compensatory immune checkpoints and a high frequency of myeloid-derived suppressor cells may constitute an unfavourable TME subtype [27,28]. After several cycles of ICB treatment, such patients could develop severe disease progression, which may even lead to a rapid increase in tumour volume, such as in patients with driver gene mutations [29,30]. Such patients are not only confronted with aggravated disease and high medical costs but may also suffer from immune-related adverse reactions, which may affect later anti-tumour treatment.

As a result, there is an urgent need to identify patients that may not benefit clinically from ICB therapy. A more effective approach could help find a "PD signature''. In the present study, we considered three key aspects of the TME: immune regulation, tumourigenesis, and tumour-immune interaction. We used transcriptional profiles of tumour tissue to establish a predictive biomarker for progression disease of checkpoint inhibitors, compared this with traditional biomarkers, and attempted to identify the mechanism underlying ICB treatment.

Material and methods

Participants and sample collection

We collected archived fresh-frozen tumour tissue from 75 patients at Guangdong Lung Cancer Institute (GLCI) at baseline of ICB therapy, and subjected it to RNA expression profiling via RNA sequencing. 75 patients were randomly assigned to training (n=30) and testing cohorts (n=45) by a stratified fashion to keep the same label ratio. Because some patients have insufficient tumor tissue, only 55 of the 75 patients have enough tissue for NGS testing and 45 patients for PD-L1 immunohistochemistry staining. The clinical-pathological characteristics and checkpoint inhibitors of all 75 patients were summarized in Table 1. Tumour response and time to progression in individual patients was defined using the Response Evaluation Criteria in Solid Tumours, version 1.1. The cut-off date for last follow-up was September 2019.

We included three high-quality public datasets in the training cohort (Supplementary Table 1). The patients in these datasets had been diagnosed as advanced stage and received checkpoint blockade treatment, including for melanoma, bladder and gastric cancers [24,31,32]. Their RNA sequencing data are available online.

Table 1
Clinical and pathological characteristics of lung cancer patients in training and testing cohorts.

75 lung cancer patients in training and testing cohorts			
Age, mean ±standard deviation	59.2±7.6	**Performance status score, n (%)**	
Sex, n (%)		**0-1**	66 (88.0%)
Male	60 (80.0%)	**2-3**	9 (12.0%)
Female	15 (20.0%)	**Line of checkpoint inhibitors, n (%)**	
Smoking history, n (%)		**1st**	23 (30.7%)
Never	29 (38.7%)	**2nd**	31 (41.3%)
Former/Current	46 (61.3%)	**3th**	10 (13.3%)
Pathology, n (%)		**4th and beyond**	11 (14.7%)
Adenocarcinoma	52 (69.3%)	**Checkpoint inhibitors, n (%)**	
Squamous carcinoma	13 (17.3%)	**PD-1/L1 monotherapy**	57 (76.0%)
Lymphoepithelioma-like carcinoma	5 (6.7%)	**Chemo combination**	12 (16.0%)
Small cell lung cancer	3 (4.0%)	**Double Checkpoint inhibitors combination**	6 (8.0%)
others	2 (2.7%)	**Treatment response**	
Stage, n (%)		**PR**	22 (29.3%)
IIIB	5 (6.7%)	**SD**	25 (33.3%)
IVA	29 (38.7%)	**PD**	28 (37.3%)
IVB	41 (54.7%)		
With brain metastasis			
Yes	17 (22.7%)		
No	58 (77.3%)		

DNA and RNA extraction, library preparation, and sequencing

For the 75 tumour samples from GLCI, we co-extracted the genomic DNA and RNA using a kit (All Prep-DNA/RNA-Micro Kit; Qiagen) following standard protocols. Genomic DNA from whole blood was extracted using a kit (Blood DNA kit; AmoyDx) according to standard protocols and used the results to exclude germline mutations and calculate the TMB of the tumour tissue. DNA libraries were established after end-repairing, A-tailing, adaptor ligation and polymerase chain reaction with indexed primers, and then captured the libraries using a panel (Pan-cancer gene panel; AmoyDx; 448 cancer-relevant genes). Complementary DNA libraries were constructed using the additional steps of first- and second-strand synthesis, and USER Enzyme processing. Exome capture was conducted by hybridising the complementary DNA libraries with an RNA exome panel. All libraries were sequenced on a platform (NovaSeq 6000; Illumina) with 2 × 150bp pair-end reads.

Gene expression estimation

We mapped RNA sequencing pair-end reads to *Homo sapiens* genome assembly GRCh37 (hg19) using STAR [33] software (version 020201) with transcriptome annotation (Genecode version 20) and performed gene quantification using RSEM software (version 1.2.28) [34]. Considering the different kinds of library preparation, we counted coding region reads to calculate transcripts per million at the gene-level.

Calculation of other predictive biomarkers as previously reported

PD-L1 expression was assessed via immunohistochemistry analysis by staining on a platform (BOND-MAX; Leica) using PD-L1 monoclonal antibody (E1L3N; AmoyDx), which is comparable to the approved antibody (22C3; Dako). The PD-L1 tumour proportion score (TPS) was evaluated by at least two pathologists independently.

TMB was assessed using a targeted 448-gene next-generation sequencing panel and calculated as the total number of somatic mutations divided by the panel size (1.16 MB). The following were excluded from the calculation: germline mutations, low frequency mutations (<5%), and mutation hotspots.

To infer patients' human leukocyte antigen (HLA) loss of heterozygosity (LOH), we used FACETS software, an allele-specific copy number analysis tool that can annotate the genome for LOH based on targeted panel sequencing platforms [35,36]. The HLA LOH score was binary, with a value of 1 if the patient had LOH. Neoantigen recognition was predicted using the procedure described by Luksza et al., processed by NeoPredPipe software [37,38].

The IFN-γ gene expression score (IFNG) is the mean value of 18-gene signature, as previously reported.[20] Tumour Immune Dysfunction and Exclusion (TIDE) is a set of signatures related to T cell dysfunction and exclusion for predicting ICB response [39]. Immunophenoscore (IPS) defines an arbitrary 0–10 scale summing the weighted, averaged Z-scores of genes in the four categories of antigen processing, immune modulators, effector cells, and suppressor cells [40].

Feature selection and LITES model construction

To avoid the 'large p, small n' problem, all potential gene expression biomarkers of ICB reported in the literature were selected as candidate features for our model, which we called LITES. We integrated three categories of features to predict the clinical benefit of patients from ICB, including immune regulation, tumorigenesis, and tumour-immune interaction, which can be further divided into six subclasses (Fig. 1, Supplementary Table 2). We used the z-score-normalized expression of candidate genes in each pathway or gene set separately. In view of tumour heterogeneity (including the immune microenvironment, pathological mechanisms, etc.), gene expression features were also separated into pan-cancer and lung cancer-specific subgroups, with the upper limits of these set to 2 and 5, respectively, according to our grid search results (Supplementary Table 3, Supplementary Fig. 1A).

Feature selection in each subclass and critical hyper-parameter optimization were conducted with the training cohort (n=147). The performance of LITES was validated through Leave-One-Out Cross-Validation. In the training stage, the above process was repeated for each individual sample, and the area under the receiver operating characteristic curve (AUC) was used to evaluate the performance of our prediction model (AUC=0.825, Supplementary Fig. 1B). Next, the final model was built according to all samples in the training cohort. Supplementary Table 4 lists the genes selected for LITES. For a binary response prediction of ICB treatment, a logistic regression algorithm tuned by 3-fold internal cross-validation was applied to each tumour dataset and candidate gene set. The final predicted score is calculated by two steps: 1) The median rank ranking of each gene set is used as the predicted score. 2) The median rank ranking of the predicted scores of the six gene sets is used as the final score.

The cut-off point of gene expression score in LITES model

The cut-off point of gene expression score in LITES model was determined by minimizing the value of $\sqrt{(1-sensitivity)^2+(1-specificity)^2}$, which is similar to the method of

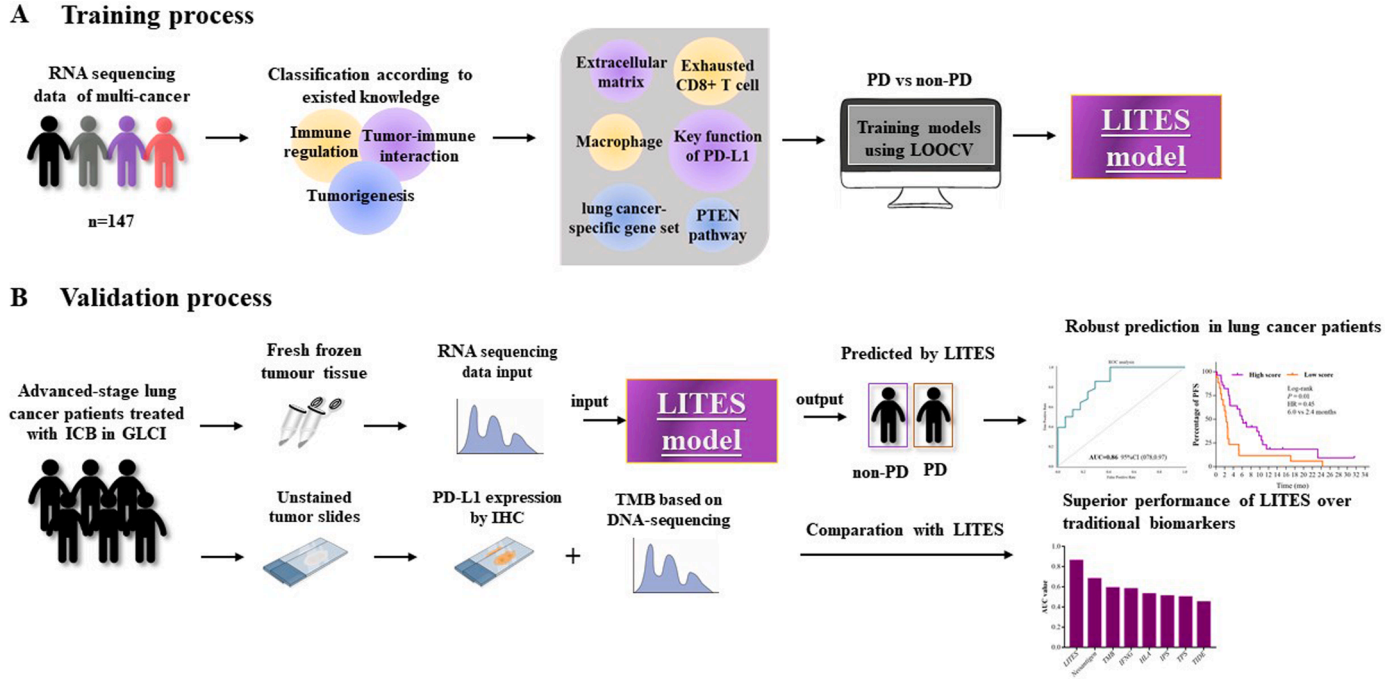

Fig. 1. Flow chart of the establishment of the predictive model, LITES.
A) The model training process. We considered 3 key aspects of the tumour microenvironment and combined 6 tumour-immune-related features in the mode. We built the integrated tumour-immune expression score of LITES using the method of Leave-One-Out Cross-Validation (LOOCV) and the RNA sequencing data of multiple types of cancer.
B) The model validation process. To build the lung cancer dataset, we retrospectively collected fresh tumour tissue and unstained tumour slides. LITES gave promising prediction of the clinical response to checkpoint blockade treatment and achieved better performance than single biomarkers, such as programmed death-ligand (PD-L1) expression and tumour mutation burden.
AUC: area under the curve; GLCI: Guangdong Lung Cancer Institute; HR: hazard ratio; ICB: immune checkpoint blockade; IHC: immunohistochemistry; PD-L1: programmed death-ligand 1; PD: progressive disease; PTEN: phosphatase and tensin homolog; ROC: receiver operating characteristic.

Yuden index. Patients with high score was considered as non-PD responder to ICB therapy and those with low score as PD responder. The cut-off point was adjusted to zero, which is convenient for bar plot drawing.

LITES model with melanoma test datasets

To investigate the predictive performance of LITES in melanoma patients who received ICB therapy, we replaced the lung cancer-specific gene set in the model with a melanoma-related candidate gene set, considering the difference in tumour biology. We then used RNA sequencing data from three public datasets and the lung cancer dataset in the present paper as melanoma-specific training dataset [24]. Finally, we adopted the same LITES hyperparameters to construct a melanoma-specific ICB predictor.

The gene-set enrichment analysis

The gene expression that correlated with LITES with significance at the 0.05 level was selected to perform the GSEA analysis (Supplementary Table 5). Using the R software package clusterProfiler,[25] we identified the top 30 biologically functional Gene Ontology terms with P value < 0.05 that corrected using the Benjamini-HochTberg method.

Statistical analysis

In the present study, we obtained the P-values of differential gene expression and distribution via the one-sided Mann-Whitney U-test. Correlations between LITES features and their P-values were assessed via the Pearson correlation coefficients. We performed Kaplan–Meier analysis to compare the progression free survival (PFS) and overall survival (OS) of patients in high- and low-score groups using a two-sided log-rank test.

Data sharing

The processed DNA mutation calling and RNA expression data can be obtained from the corresponding author.

Results

LITES is a better predictive biomarker for unfavourable response to ICB therapy

LITES achieved an AUC value of 0.86 in the validation cohort of lung cancer patients (Fig. 2A). According to the best cut-off value of gene expression score calculated by LITES, patients were divided into two groups. Patients with a high (low) score were predicted to have a non-PD (PD) response. Of the 29 predicted non-PD patients, 24 responded to anti-PD-1/L1 therapy with a partial response or stable disease, and of the 16 predicted PD patients, 12 responded with PD, reflecting a sensitivity and specificity of 85.7% and 70.6%, respectively (Fig. 2B). The AUC value of LITES was higher than those of PD-L1 TPS, TMB and other predictive biomarkers, demonstrating a substantial advantage with respect to predictive accuracy (Fig. 2C). Moreover, we performed Kaplan-Meier analysis to examine the predictive value of LITES in survival improvements. Patients with a high score exhibited a much longer PFS than those with a low score in training and validation cohorts (median PFS = 19.7 vs. 3.0 months, hazard ratio [HR] = 0.28, P = 0.003; median PFS = 6.0 vs. 2.4 months, HR = 0.43, P = 0.01; Fig. 2D and 2F). The high-score group also had longer OS in these two cohorts (median OS = 27.5 vs. 7.0 months, HR = 0.26, P = 0.01; median OS = 15.9 vs. 6.0 months, HR = 0.42, P = 0.02; Fig. 2E and 2G).

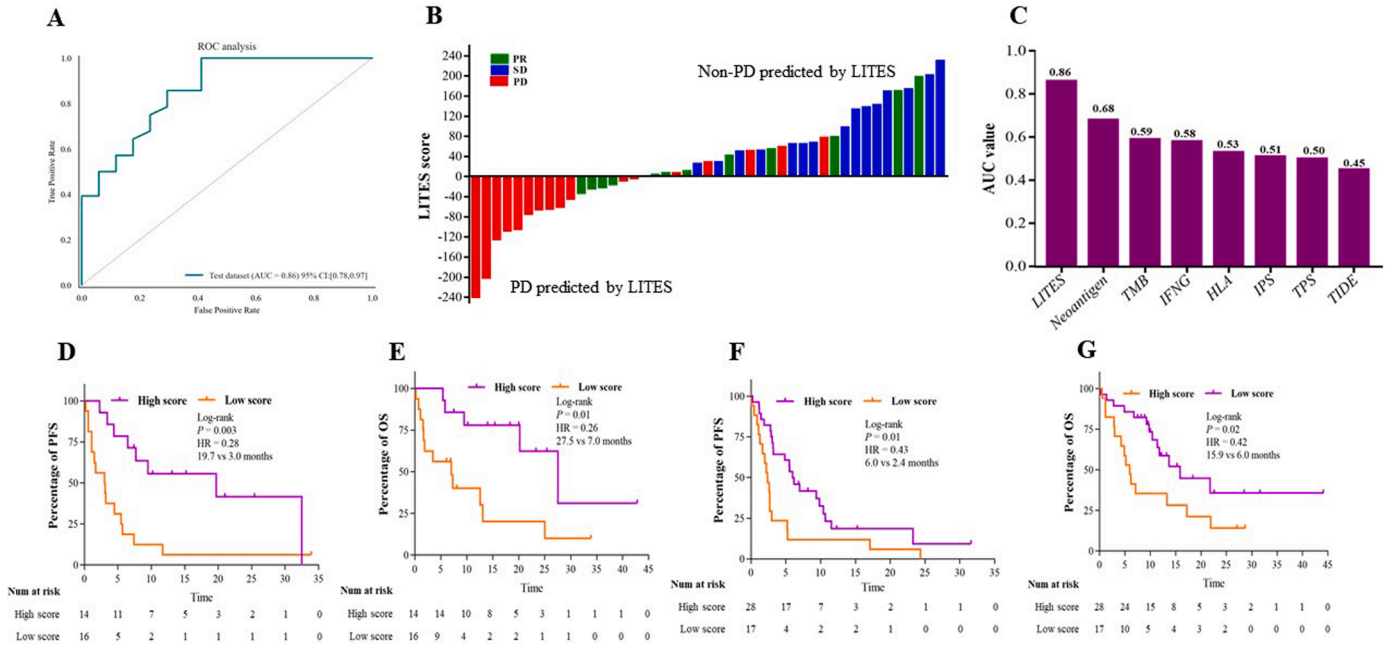

Fig. 2. Classification performance of the LITES predictor on an independent lung cancer validation set.
A) Receiver operating characteristic (ROC) curve for the performance of LITES in prediction of checkpoint blockade response in the independent lung cancer validation dataset with a 95% confidence interval (CI).
B) Waterfall plot of LITES scores in patients with different clinical responses from to immune checkpoint blockade.
C) Comparison of area under the curve (AUC) values between LITES and conventional biomarkers.
D) Kaplan–Meier plots of progression free survival (PFS) in the lung cancer training cohort.
E) Kaplan–Meier plots of overall survival (OS) in the lung cancer training cohort.
F) Kaplan–Meier plots of progression free survival (PFS) in the lung cancer validation cohort.
G) Kaplan–Meier plots of overall survival (OS) in the lung cancer validation cohort.
HR: hazard ratio; PD: progressive disease; PR: partial response; SD: stable disease.

We summarized the detailed information of the 75 lung cancer patients from GLCI, including both the training (n = 30) and testing cohorts (n = 45), and divided them into two groups based on the LITES prediction results: non-PD and PD (Fig. 3). PFS and OS was higher in the non-PD group than in the PD group. PD-L1 expression and TMB appeared to be evenly distributed between the two groups and had no relationship with the response to anti-PD-1/L1 therapy. The non-PD group had more smokers than the PD group and the treatment regimens were similar between the groups.

We further investigated the association of the gene expression score calculated by LITES with PD-L1 expression and TMB. No difference in PD-L1 expression or TMB was observed between the high- and low-score groups (Supplementary Fig. 2A). Also, there was no correlation between LITES score and TMB or PD-L1 expression (Supplementary Fig. 2B). These results indicate that LITES is a predictive biomarker for ICB therapy, independent of PD-L1 expression and TMB. These two biomarkers contain complementary information for tumor microenvironment. Perhaps when we combined the LITES model and these 2 metrics, it would give a better prediction for checkpoint blockade. Because some patients had no results on these two markers, it is impossible for further analysis of the predictive value of the combination with LITES model in our study. But it should be furtherly investigated.

Predictive synergy observed when combining multiple features

We next examined the predictive performance of the 6 biological features and investigated their interrelationships. The mean AUC of the features was 0.70 (range = 0.61-0.75) and when all 6 features constituted the LITES model, the AUC increased to 0.86, suggesting that the combination of these features yielded synergistic effects for the prediction of response to ICB therapy (Fig. 4A). Furthermore, we found that these features had significant discriminatory power between the non-PD and PD subgroups. Patients responding to ICB with PD has a lower score than those responding as a partial response or stable disease (Fig. 4B). Cox regression analysis revealed a favourable clinical benefit regarding PFS in patients with a high score in 3 of the 6 features: exhausted CD8+ T cell, macrophage and extracellular matrix related features (Fig. 4C). Subsequently, we identified the interrelationships among the features. The immune checkpoints-related feature was highly associated with that of exhausted CD8+ T cell ($r = 0.54$, $P < 0.001$), while the phosphatase and tensin homolog pathway was closely associated with that of the lung cancer-specific signatures ($r = 0.42$, $P < 0.001$); both of these relationships were expected due to their functional similarities (Fig. 4D). These results provide strong evidence for the necessity of combining multiple tumour-immune-related features for prediction of the clinical benefit from ICB therapy.

LITES captured diverse aspects of tumour microenvironment

In the LITES model, we identified the gene members in the 6 functional feature groups (Supplementary Table 4). Among them, several key immune biomarkers, including CD8A (CD8+ T cell), CXCL9 (T cell migration and differentiation), HLA-DPB1 (tumour antigen cross-presentation), and CD276 (an immune costimulatory molecule of the B7 gene family), which has been reported to play a critical role in ICB response,[22,40] were included in LITES. Moreover, the oncogenes *NRAS* and *PDPK1* were incorporated in the feature of lung cancer-specific gene set. Patients with higher expression of such genes tended to have worse clinical responses to ICB (PD vs. non-PD, $P < 0.001$; $P = 0.01$; Fig. 5A).

To understand the composition of immune cells in the TME, we inferred the relative abundance of 64 immune and stromal cell types via xCell online software.[41] We found that CD8+ T cell abundance was highly correlated with the LITES score as expected, but the former was a weak predictor (AUC = 0.60, Fig. 5B). Additionally, comprehensive stroma score was moderately associated with LITES score, with an AUC value of 0.57 (Fig. 5C). We then evaluated the correlations between the LITES score and individual genes, and performed a functional

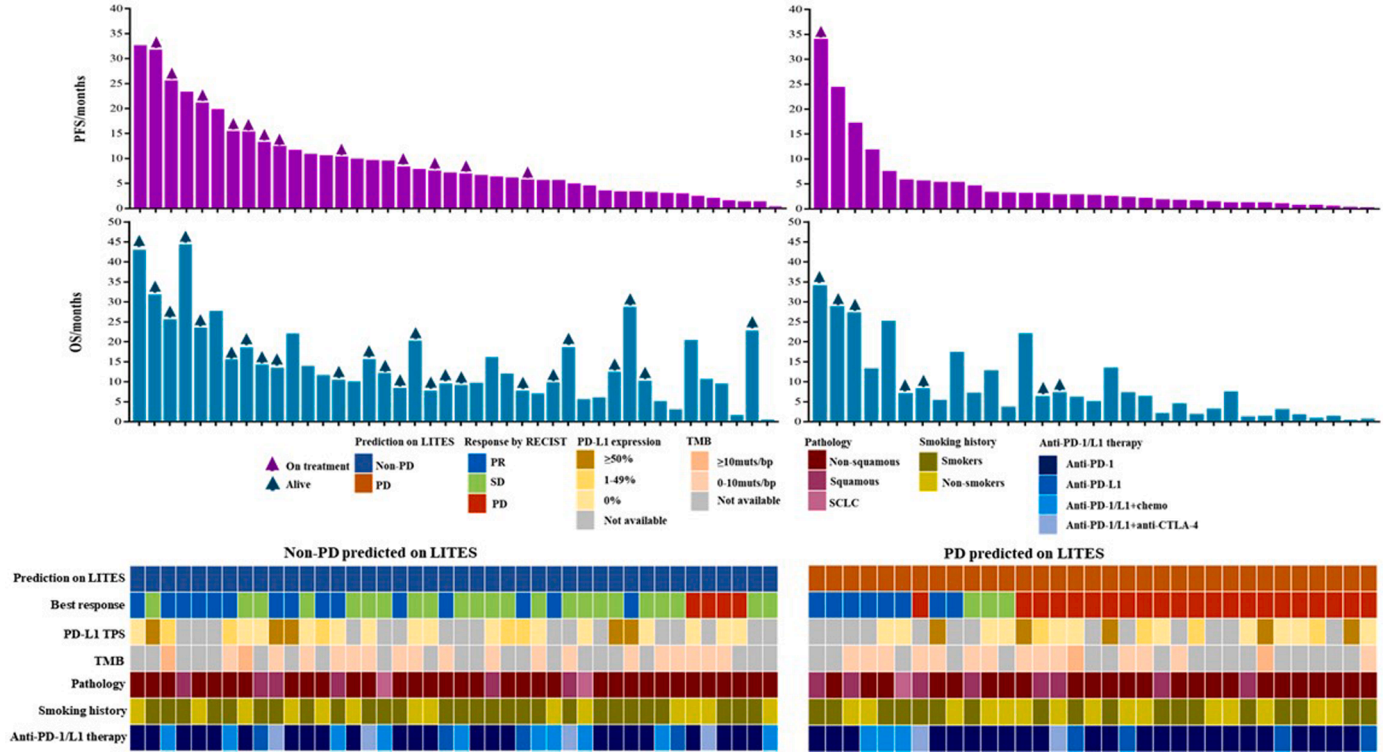

Fig. 3. Clinical and pathological information of patients and LITES model predictions.
CTLA-4: cytotoxic T-lymphocyte-associated protein-4; PD-L1: programmed death-ligand 1; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumours; SCLC: small cell lung cancer; SD: stable disease.

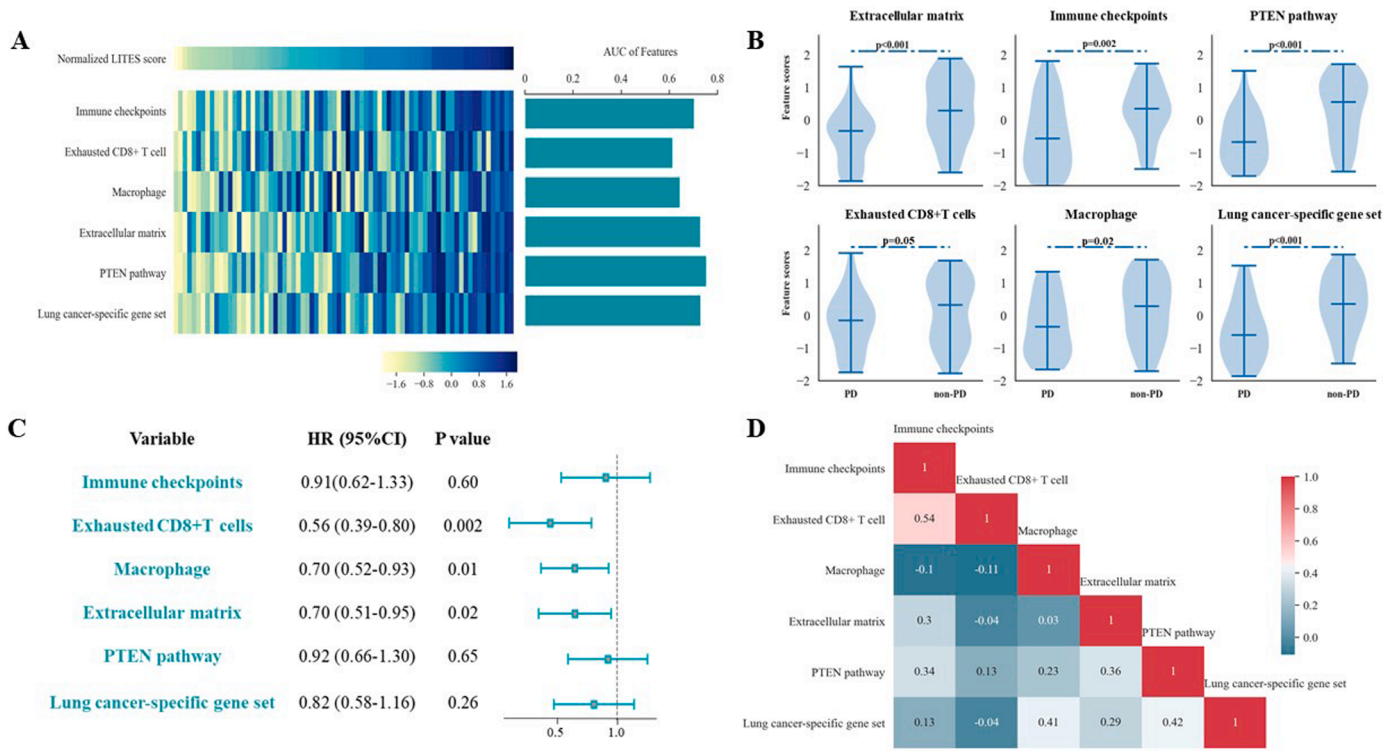

Fig. 4. Predictive power of individual features of ITES and their interrelationships.
A) Heatmap plot illustrating the predictive scores of each feature for each patient. Each row and column represent a feature and a patient, respectively. The bar plot shows the AUC value of each feature.
B) Violin plot showing the feature scores of the PD and non-PD groups.
C) Coefficients from Cox regression analysis to evaluate the performance of various features for predicting progression-free survival, where the error bars indicate 95% confidence intervals.
D) Heatmap plot specifying the correlation coefficient between each pair of features.
CI: confidence interval; HR: hazard ratio.

enrichment analysis of related genes. Positively correlated genes were mainly involved in the immune regulation pathway and extracellular structure, while negatively correlated genes were enriched in cell proliferation, cell division and DNA repair (Fig. 5D).

The validation in melanoma cohorts and prognostic specificity for ICB therapy

To investigate the predictive performance of LITES in melanoma patients that received ICB therapy, we replaced the lung cancer-specific gene set in the LITES model with the melanoma-related candidate gene set. We tested LITES in Chinese (n = 60) and Australian (n = 41) metastatic melanoma cohorts that had RNA sequencing data available. LITES achieved acceptable AUC values (0.78 and 0.70, respectively) with these datasets without tuning hyper-parameters (Fig. 6A, Methods). Melanoma patients with a high score had longer PFS and OS than those with a low score (Fig. 6B to 6D). The cancer-specific gene-set was important to the model development and could further improve the predictive efficacy of the model no matter in lung cancer cohort or in melanoma cohort.

To confirm LITES as a prognostic biomarker specific to ICB, we applied it to 128 and 934 NSCLC patients from the CTONG1308 study and The Cancer Genome Atlas, respectively. No patients in either cohort received any ICB therapy during their entire treatment course.[23] As expected, there was no difference in OS between patients with a high or low score (CTONG1308, P = 0.34; The Cancer Genome Atlas, P = 0.45; Fig. 6E and F).

Discussion

Researchers have recently been trying to establish new predictive models for checkpoint blockade to improve the unfavourable performance of traditional markers. They have used combinations of existing biomarkers, such as CD8+ T cells, PD-L1, TMB and microsatellite instability, to classify patients' TMEs into different subtypes [42,43]. With the help of machine learning, many studies have attempted to build a comprehensive signature with genetic variation and gene expression profiling. Several predictive models for pan-cancer patients have been published that was expected to screen patients who had the potential to benefit greatly from anti-PD-1/PD-L1 therapy [20,21,40]. Although tumour-specific prediction models have been reported for melanoma and NSCLC patients, these remain in their infancy [44].

With regard to the mechanism of PD-1/L1 pathway blockade [45], we used RNA sequencing data of multiple cancer types to build our predictive model. In our view, single biomarker cannot meet the need for improving response prediction for anti-PD-1/PD-L1 therapy. Therefore, in this study we considered 6 tumour and immune-related features, each of which represented one characteristic of the complex TME, and we finally built the integrated tumour-immune expression score. Due to the synergistic effect of these features, our model achieved superior performance in predicting response in patients treated with ICB therapy. LITES can distinguish whether patients will develop PD response after first cycles of ICB treatment with an AUC value of 0.86. Of the 16 patients predicted to have PD, 12 did not respond to anti-PD-1/L1 therapy, with a specificity of 85.7%. With superior performance over PD-L1 expression and TMB, LIES could identify an unfavourable response of patients using only RNA sequencing data of their tumour tissue.

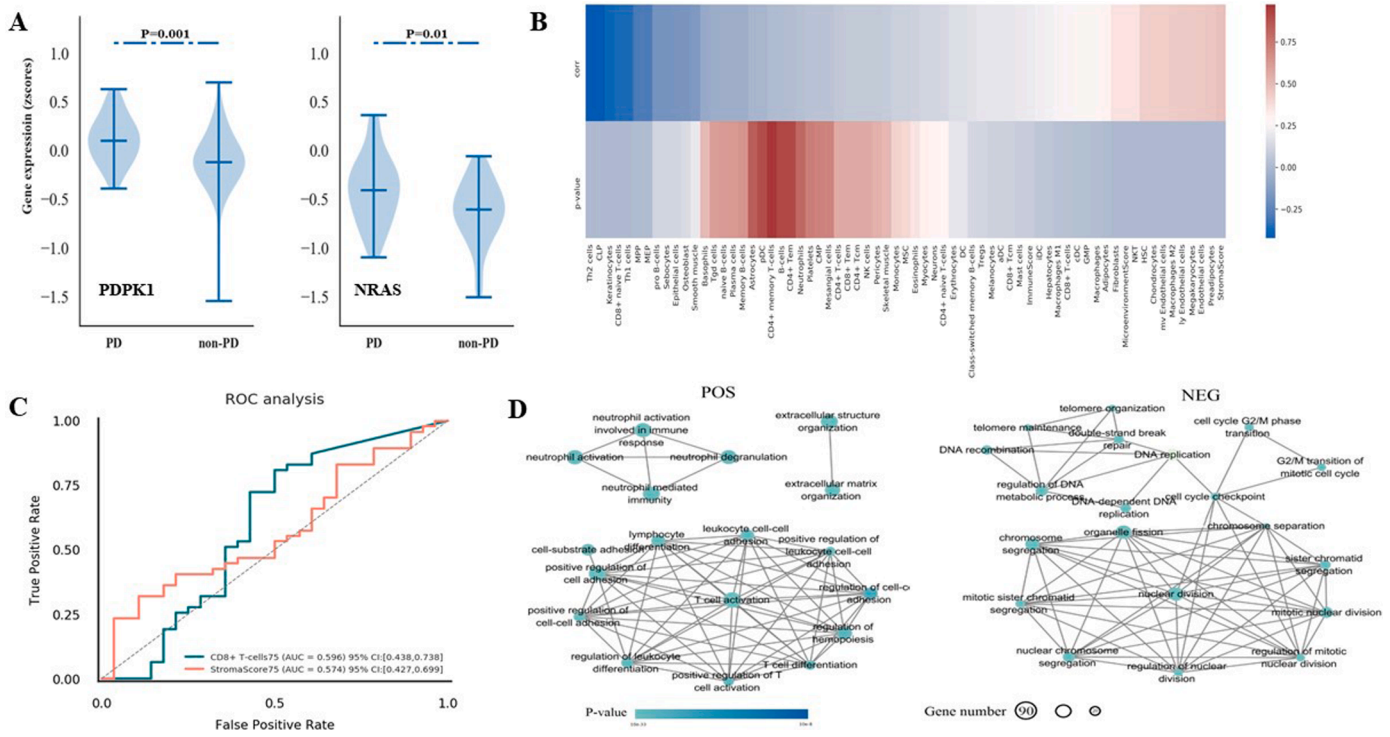

Fig. 5. Biological explanations of the LITES model.
A) Violin plot showing the *NRAS* and *PDPK1* gene expression score in PD and non-PD groups, respectively.
B) Correlation between LITES score and the abundance of cell types in the immune microenvironment.
C) Receiver operating characteristic (ROC) curves for CD8+ T cell abundance and stroma score in predicting checkpoint inhibitors response in lung cancer patients.
D) The enrichment map of Gene Ontology terms that significantly correlated with LITES score.
AUC: area under the curve; CI: confidence interval; NEG: negative; POS: positive.

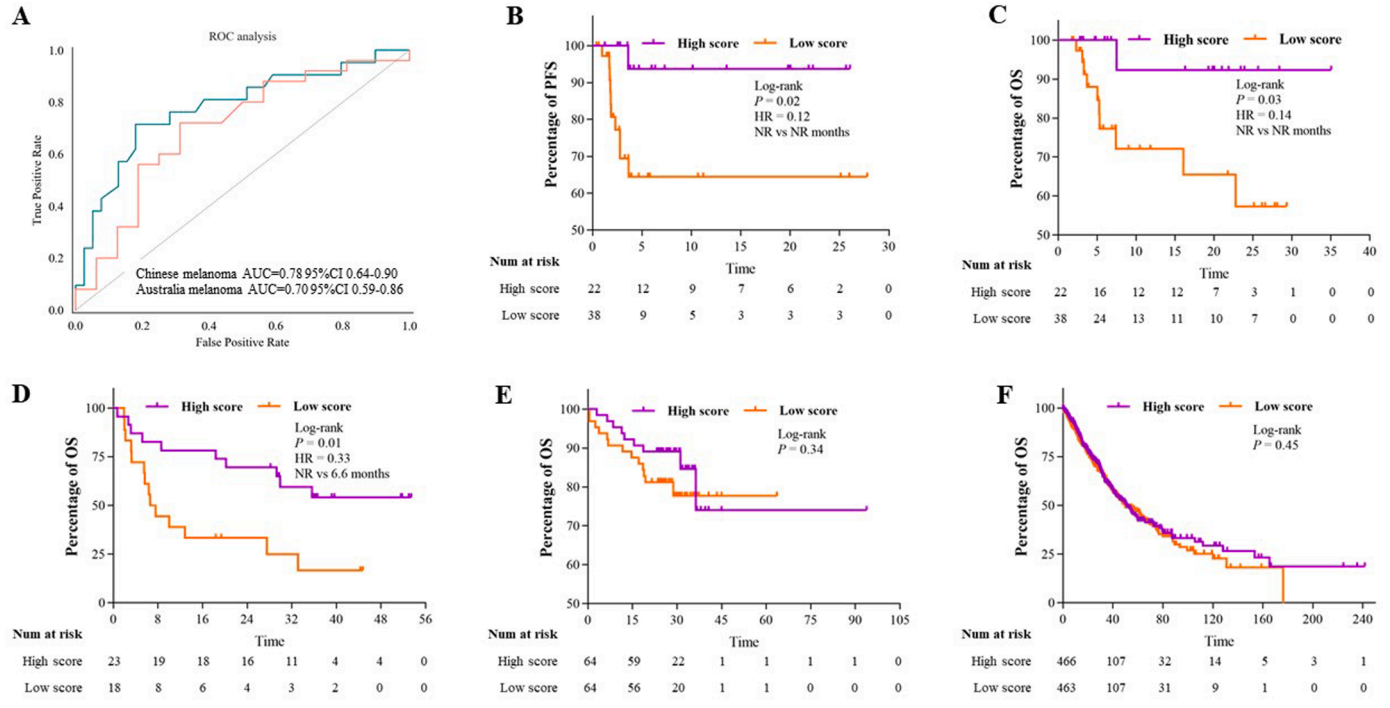

Fig. 6. LITES predictor performance on two melanoma cohorts and its association with clinical outcomes.
Receiver operating characteristic (ROC) curves for LITES in predicting response to checkpoint inhibitor with two external melanoma datasets (A). Kaplan–Meier plots of (B) progression-free (PFS) and (C) overall (OS) survival for the Chinese melanoma cohort, and OS for the (D) Australia melanoma, (E) CTONG 1308 study, and (F) The Cancer Genome Atlas database cohorts.
AUC: area under the curve; CI: confidence interval; HR: hazard ratio; NR: not reached.

Although we used pan-cancer sequencing data to establish LITES, to ensure its effectiveness with lung cancer, we ensured that 1of its 6 features was the lung cancer-specific gene set. In the validation cohort, the results yielded good performance in predicting an unfavourable clinical response. When the lung cancer gene set was replaced by the melanoma gene set, the predictive power of LITES in the melanoma validation cohort was also acceptable (AUC = 0.70-0.78). Although broader coverage led to some loss in the accuracy of prediction, the results demonstrated that the predictive power of the other 5 features was stable.

Increasing studies focused on the utility of circulating tumor DNA (ctDNA) as a dynamic biomarker for ICB therapy [46,47]. Particularly, it could be determined by the status of ctDNA if stage III patients after definitive chemoradiotherapy should receive immunotherapy [48]. Recently, Barzin et al. from the Memorial Sloan Kettering Cancer Center reported a predictive model for checkpoint blockade based on non-invasive liquid biopsy in NSCLC. The outcome was very promising (AUC = 0.93) and allowed a decision on whether to continue treatment depending on the result of a first blood drawing (HR = 0.14). Our model yielded good performance in predicting a PD response, which could prevent ICB treatment from even beginning. In clinical practice, testing with next-generation sequencing at the baseline of treatment is necessary for genetic variation profiling. Current technologies can support simultaneous DNA and RNA sequencing from a single sample of tumour tissue. The cost for patients to complete DNA and RNA sequencing and the requirements for specimens will be reduced. While obtaining the NGS results, patients could avoid disease aggravation and potential immune-related adverse reactions before ICB treatment based on the predicted result from our model.

By functional enrichment analysis, we found that the genes and pathways incorporated in LITES were related with many immunoregulatory factors in the TME, including DNA repair, immune regulation, and extracellular matrix. In other words, these factors also play critical roles in the tumour-immune reaction. Additionally, we found that patients with higher *NRAS* or *PDPK1* expression tended to have poor response to anti-PD-1/PD-L1 therapy. Previous studies have found that patients with certain genetic variations, such as *EGFR* and *STK11* mutations, have unfavourable or impaired responses to immunotherapy [49,50]. *NRAS* mutations could leave *NRAS* encoding protein in a state of continuous activation, leading to uncontrolled cell proliferation and tumour formation [51], and *PDPK1* mutation played a role in cancer cell invasion and dissemination [52]. Studies have shown the predictive role of *NRAS* mutation on ICB therapy in melanoma, but its predictive power was inconsistent among the studies [53–55]. More exploration is needed to clarify the roles of the *NRAS* and *PDPK1* oncogenes in immunotherapy, and their potential mechanism.

Note that our study has some limitations. First, the number of lung cancer patients included and validated in our study was limited; we plan to test the model in a prospective study. Second, our pool of features was dynamically selected based on prior biological knowledge; these could be stabilised and improved when additional data becomes available.

In conclusion, LITES is an ICB predictor that integrates several tumour-immune related features and can effectively screen lung cancer patients who may not get benefit from ICB treatment. Compared to existing single biomarkers, LITES considers cancer-specific biological mechanisms to improve its predictive performance. Although LITES was trained based on multiple datasets across different cancer types, trying to ensure its predictive efficacy and generalisability, it still need large sample size of lung cancer patients for further validation.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Guangdong Provincial People's Hospital (2013185H). All lung cancer patients in our institute provided written informed consent for the use of their tumour tissues.

Consent for publication

Obtained.

Availability of data and material

Public database with RNA sequencing data could be obtained online and detailed website was summarized in Supplementary Table 1. All other relevant data are available from the corresponding author of this study (Yi-Long Wu, syylwu@live.cn) upon reasonable request.

Declaration of Competing Interest

There are no interests to declare for Si-Yang Maggie Liu, Hao Sun, Jia-Ying Zhou, Jia-Tao Zhang, Kai Yin, Zhi-Hong Chen, Jian Su, Xu-Chao Zhang, Jin-Ji Yang, Qing Zhou and Hai-Yan Tu. Professor. Yi-Long Wu needs to declare personal financial interests: advisory services for AstraZeneca, Boehringer Ingelheim, Novartis, Takeda; personal fees from AstraZeneca, Beigen, Boehringer Ingelheim, BMS, Eli Lilly, MSD, Pfizer, Roche, Sanofi; grants from AstraZeneca, Boehringer Ingelheim, BMS, Hengrui, Roche.

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Author Contributions Statement

Conception and design: Yi-Long Wu and Si-Yang Maggie Liu; acquisition of the public data and tumour samples of patients in Guangdong Lung Cancer Institute: Si-Yang Maggie Liu, Jia-Ying Zhou, Jian Su and Zhi-Hong Chen; development of methodology: Si-Yang Maggie Liu and Hao Sun; analysis and interpretation of data: Si-Yang Maggie Liu, Hao Sun, Jia-Ying Zhou and Kai Yin; writing and revision of the manuscript: Si-Yang Maggie Liu, Hao Sun and Yi-Long Wu; review of the manuscript and put forward suggestions for amendment: all authors; final approval of the version to be submitted: all authors.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2021.101254.

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