Original Article

Titin mutation in circulatory tumor DNA is associated with efficacy to immune checkpoint blockade in advanced non-small cell lung cancer

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Background: Only a fraction of patients with advanced non-small cell lung cancer (NSCLC) respond well to immune checkpoint blockade (ICB) therapy. Here, we investigated whether Titin (*TTN*) mutation, which has been demonstrated to be a predictive biomarker in tissue-based analysis, can identify patients with a greater likelihood in response to ICB based on circulatory tumor DNA (ctDNA) sequencing.

Methods: In this retrospective analysis, 92 patients with advanced NSCLC from two independent cohorts who received ICB treatment were included. A probe panel covering all exons of TTN was developed and validated to detect *TTN* mutation in ctDNA. Baseline plasma samples were collected and subjected to ctDNA sequencing with the *TTN* probe panel.

Results: Of the 92 patients, 28.3% harbored *TTN* mutation in their baseline ctDNA. Progression-free survival was significantly improved in patients with the mutated *TTN* (212 days and 334.5 days for cohort 1 and 2) compared to those without the mutation (113 days and 147 days for cohort 1 and 2). Objective response to ICB treatment (40% for TTN^{mut} and 15.8% for TTN^{wt} in cohort 1; 50% for TTN^{mut} and 23.4% for TTN^{wt} in cohort 2) was common in patients with mutated *TTN*. Stratified analysis showed a generally predictive potential of *TTN* mutation in patients with advanced NSCLC.

Conclusions: The presence of mutated *TTN* in pre-treatment peripheral blood was associated with favorable objective response and survival with ICB administration. Therefore, circulatory *TTN* mutation may be applicable for guiding ICB immunotherapy in patients with NSCLC.

Keywords: Titin; biomarker; non-small cell lung cancer (NSCLC); ctDNA sequencing

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Introduction

Immune checkpoint blockade (ICB) has substantially improved the clinical outcomes of advanced non-small-cell lung cancer (NSCLC) (1,2). However, only a minority of patients satisfactorily respond to ICB treatment. Currently, all clinically approved companion diagnostic tests for ICB immunotherapy, including immunohistochemistry of programmed cell death ligand-1 (PD-L1) expression (3), tumor mutational burden (TMB) (4), and microsatellite instability-high (MSI-H) (5), are based on formalin-

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fixed paraffin-embedded (FFPE) tumor tissue specimens. Despite the availability of these assays, the identification of responders to ICB immunotherapy is not always possible, partially due to the unavailability of archived tissue, refusal of re-biopsy, or lack of sufficient tumor cells in the specimen. Therefore, it is imperative to develop novel predictive biomarkers to expand the number of patients who may benefit from receiving ICB immunotherapy.

Circulatory tumor DNA (ctDNA) has been considered as a promising biomarker in guiding decision-making in numerous tumor types (6-8), and the utility of ctDNA sequencing is under active investigation in the field of ICB immunotherapy (9-11). Accumulating evidence supports that the estimation of TMB from blood ctDNA (bTMB) has application potential in cases where archival tissues are lacking or insufficient for routine tissue-based TMB assessment (12,13). However, the evaluation of bTMB requires sufficient input genomic DNA, which is not always available. Moreover, a cutoff for delineating high or low bTMB and an optimal sequencing pipeline to determine bTMB remain incompletely defined and need further investigation.

Recently, non-synonymous mutations in Titin (encoded by *TTN*), which is responsible for the passive elasticity of muscle tissue, have been related to higher TMB and improved ICB treatment efficacy (14). However, the usability of *TTN* mutation in ctDNA in selecting responders has not been investigated. Here, the predictive significance of *TTN* mutation was investigated in two independent cohorts of patients with NSCLC to determine whether *TTN* mutation in ctDNA can serve as a biomarker for sensitivity to ICB immunotherapies. We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/tlcr-20-1118).

Methods

Study design and patients

This retrospective study enrolled 29 and 63 patients who were diagnosed with advanced non-small cell lung cancer (NSCLC) and were treated with ICB immunotherapy at Xinqiao Hospital (Army Medical University, Chongqing) and Shanghai Pulmonary Hospital & Thoracic Cancer Institute (Tongji University School of Medicine, Shanghai), respectively. Patients were enrolled from Jul 2017 to Feb 2019. Both males and females were enrolled. Patients older than 80 years were excluded from the study. Clinical response to ICB treatment was evaluated with RESCIST 1.1 criteria using radiological assessments. The median follow-up was 12 months (ranging from 3–21-month for all patients). Plasma from 5 mL of peripheral blood was obtained immediately before the first infusion of ICB treatment for all patients. Meanwhile, we performed *TTN* validation sequencing using peripheral blood and matched FFPE biopsies in additional 24 NSCLC cases. In these 24 NSCLC cases, matched samples from 6 patients were used for investigating the consistency between panel-based and whole-exome sequencing (WES); the remaining 18 cases were used for comparing blood-based and tissue-based sequencing of *TTN* gene.

Sample preparation and DNA isolation

Plasma was isolated from the blood via centrifugation at 1,600 ×g for 10 min and then at 16,000 ×g for 10 min. Cell-free (cf) DNA was extracted from 3 to 5 mL of plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions. Tumor FFPE DNA was extracted from 5 to 10 sections (5–10 μm thick) using the QIAamp DNA FFPE Tissue Kit (Qiagen), and genomic DNA (gDNA) was extracted from white blood cells (WBCs) using the Blood Genomic DNA Mini Kit (CWBiotech).

Target capture and sequencing

A custom-designed panel with DNA probes covering the entire coding sequence of *TTN* was developed on the BOKE TargetCap™ platform. For each sample, 20–100 ng cfDNA or 200–500 ng FFPE DNA and 500 ng gDNA was used for library preparation and quantification according to KAPA HyperPrep protocols (R). Pools of four libraries were hybridized to the capture panel at 65 ℃ for 16 h. Washing, recovery, and amplification were conducted sequentially according to the manufacturer's standard procedures (BOKE). Next, the libraries were purified using AMPure XP (Beckman) and quantified using the Qubit™ dsDNA HS Assay Kit (Thermo Fisher). xGen Exome Research Panel v1.0 (Integrated DNA Technologies) was used to generate WES data for tumor tissue DNA, according to the manufacturer's standard procedures. The libraries were sequenced on an Illumina Novoseq6000 instrument in PE150 mode (Illumina).

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Quality control and variant calling

Adapter sequences were trimmed from the raw reads using Trimmomatic (15). The reads were then aligned with the human reference genome (hg19) using BWA (16). Duplicated reads were removed using Picard (http://broadinstitute.github.io/picard/). Mapped reads were realigned to the genome using the Genome Analysis Tool Kit (17). Somatic and germline mutations were observed using Mutect2 and GATK's HaplotypeCaller (3.7) with a paired workflow and GATK, respectively. Variants were annotated using ANNOVAR (18) and an in-house developed code. An in-house script was used to verify the identity concordance of paired samples. Somatic copy number alterations were detected using GATK (17).

Germline variant filtering

Germline variants determined by GATK in WBC samples were first filtered using a threshold of minimum coverage of 50× and an allele frequency of >30%. Variants outside coding regions and synonymous mutations annotated with ANNOVAR (18) were filtered out. Further, variants with a minor allele frequency of >0.1% in the ExAC database were considered less functional and ignored in downstream analysis.

Somatic variant filtering

Somatic mutations detected in cfDNA samples were filtered according to the following parameters: (I) ≥4 allele reads support the mutation; (II) >0.5% allele frequency; (III) <4 supporting reads in the WBC control; (IV) mutation frequency is 5 times higher than in the WBC control; (V) mutations do not occur more than two times in the PoN; (VI) no significant strand bias (GATK parameter FS >60 for single nucleotide polymorphisms and FS >200 for indels). Similar filtering rules were applied for somatic mutations from FFPE samples, except that allele frequency had to be >5% and mutation frequency had to be 8 times higher than in the WBC control. Mutations within non-coding regions and synonymous mutations were removed for downstream analysis.

Statistics

The number of mutations in *TTN* was defined as the sum of missense, nonsense, and indels in the coding region of *TTN*. The correlation between plasma-based panel-assessed *TTN* mutation and FFPE-based WES-assessed TMB was determined via the Pearson correlation coefficient. Discrete variables (partial response, stable disease, or disease progression) were summarized as percentages. Kaplan-Meier curves and the log-rank test were used for survival analysis. The hazard ratio (HR) and 95% confidence interval (CI) were estimated using a Cox proportional hazards regression model. $P<0.05$ was considered statistically significant. Basic statistical analysis was performed using Excel in Office 365 or GraphPad Prism software, version 5.0 (GraphPad Software Inc.).

Availability of data and materials

All raw sequencing data are accessible upon request to the corresponding authors. The clinical characteristic and survival outcomes can be found in the supplementary tables.

Study approval

The trial was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional review board of XinQiao Hospital, Army Medical University, Chongqing (Identifier: 2017YD104-01) and Shanghai Pulmonary Hospital & Thoracic Cancer Institute, Tongji University School of Medicine, Shanghai (Identifier: 82072568). Individual consent for this retrospective analysis was waived.

Results

Development of a sequencing panel for detecting TTN mutation in ctDNA

We developed a customized panel of oligonucleotide probes targeting the *TTN* gene (Table S1) to determine the mutation status of *TTN* in ctDNA via high-throughput sequencing. This customized panel was designed to cover the whole coding sequencing (CDS) of *TTN*. By sequencing tumor tissues from six NSCLC patients, technical validation of the sequencing panel confirmed a sufficient depth of coverage of the sequencing pipeline (*Figure 1A*, Figure S1), supporting the reliability of the assay to identify mutated sites in the *TTN* exons. Subsequently, we investigated the effectiveness of the *TTN* panel by comparing the results with parallel WES results based on tumor tissues from six treatment-naïve patients with NSCLC. The concordance

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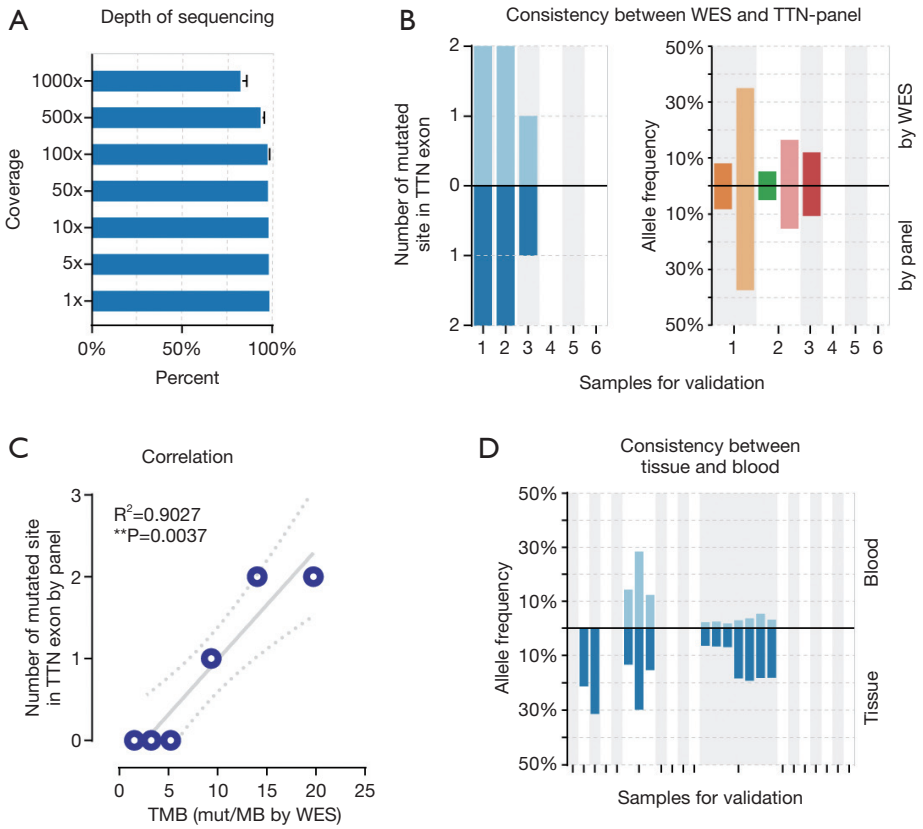

Figure 1 Validation of the sequencing strategy for detecting ctDNA *TTN* mutation. (A) Sequencing depth for the panel of sequencing probes designed to detect *TTN* mutation in ctDNA. Data represent the mean percentage and standard error of the mean. (B) Consistency between panel-based and WES-based assays using tumor tissues. Left panel, number of detected mutations in the *TTN* exons. Right panel, allele frequencies for each mutation detected. Upper quadrants, panel-based sequencing; lower quadrants, WES. (C) Correlation between panel-based number of mutation sites and WES-based TMB. Solid line, regression fitting line; dashed curves, 95% CI. R^2 and P values for Pearson correlation are shown. (D) Consistency of panel-based *TTN* mutation between tumor tissue and blood samples. Grey/blank lanes, samples for validation of the sequencing results. Blue bars, mutated site in the *TTN* exons. Upper quadrants, blood-based sequencing; lower quadrants, tumor tissue-based sequencing.

between the panel-based and WES-based *TTN* mutations in tumor tissue are summarized in *Figure 1B*. The mutation status was consistent between panel-based and WES-based sequencing results, measured either by the total number of mutated sites (*Figure 1B*, left panel) or by the allele frequency of each mutated site (*Figure 1B*, right panel) in the *TTN* coding region. Furthermore, a positive correlation was found between the panel-based total number of mutated sites and WES-based TMB (*Figure 1C*, R^2=0.9027, **P=0.0037), which has been previously established in an analysis based on The Cancer Genome Atlas (TCGA) (14). Finally, the performance of *TTN* panel-based sequencing of ctDNA was examined using tumor tissue samples and peripheral blood samples from an additional 18 patients with NSCLC (*Figure 1D*). In brief, for these 18 cases, all ten mutation sites detected via panel-based ctDNA sequencing were successfully recapitulated using tumor tissue-based sequencing. In contrast, 14 of 16 ctDNA *TTN* mutation-negative patients (87.5%) were verified via tumor tissue-based sequencing, suggesting a reasonable false negativity rate of our pipeline in detecting *TTN* mutation in ctDNA.

TTN mutation in ctDNA predicts responsiveness to ICB treatment in patients with NSCLC

To investigate whether *TTN* mutation in ctDNA allows identifying patients with a great likelihood to benefit from ICB immunotherapy, two independent cohorts of patients

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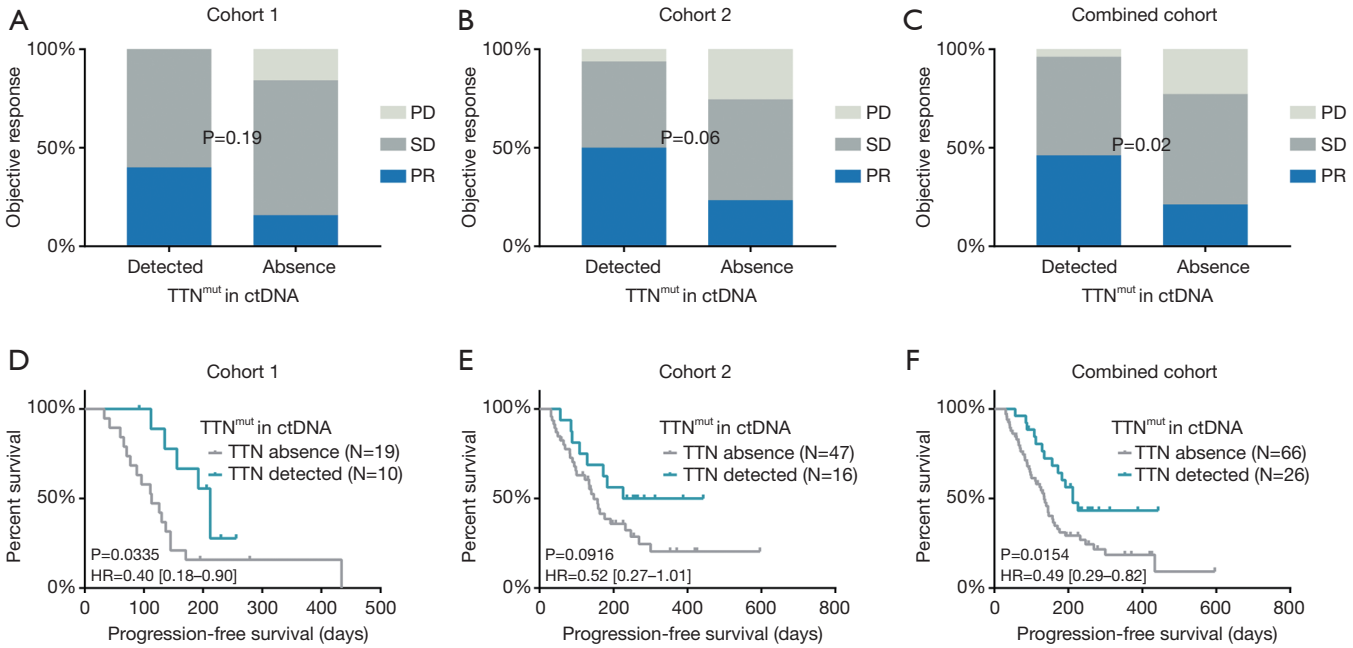

Figure 2 Usability of ctDNA *TTN* mutation to predict the clinical efficacy of ICB treatment for patients with NSCLC. Comparison of the best objective response between patients with and without detected *TTN* mutation for cohort 1 (A), cohort 2 (B), and combined cohorts (C). CR, complete response; PR, partial response; SD, stable disease. (D,E,F) Survival analysis based on *TTN* mutation status for each cohort. Kaplan–Meier survival plots are shown. P values of log-rank tests and HRs with 95% CIs are shown.

who were diagnosed with advanced NSCLC and treated with ICB immunotherapy were enrolled in this retrospective analysis (29 patients in cohort 1 and 63 patients in cohort 2 (Tables S2,S3). The overall positivity rate of *TTN* mutation in ctDNA was 28.26% (26/92) for all the patients enrolled (10/29 or 34.5% for cohort 1 and 16/63 or 25.4% for cohort 2), which was comparable to the reported frequency of *TTN* mutation occurrence (14) (2,928/9,654, 30.33%) in TCGA database.

Compared to *TTN* mutation-negative patients (absence of detectable *TTN* mutation by panel-based ctDNA sequencing), *TTN* mutation-positive patients (with detectable *TTN* mutation) demonstrated a superior objective response to ICB treatment (*Figure 2A,B,C*). The objective response rates for the *TTN* mutation-positive and negative patients were 40.0% (4/10) and 15.8% (3/19) for cohort 1, 50.0% (8/16) and 23.4% (11/47) for cohort 2, and 46.2% (12/26) and 21.2% (14/66) for the combined cohorts, respectively. In addition, *TTN* mutation-positive patients were associated with a durable progression-free survival (PFS) compared to *TTN* mutation-negative patients (*Figure 2D,E,F*). The median PFS was 212 days for positive patients and 113 days for the negative patients (*Figure 2D*, *P=0.0335; HR =0.40; 95% CI, 0.18–0.90) in cohort 1. In cohort 2, the median PFS was 334.5 and 147 days for patients with and without detectable *TTN* mutation, respectively (*Figure 2E*, P=0.0916; HR =0.52; 95%, 0.27–1.01). Overall, the median PFS for the positive and negative patients was 212 and 135 days, respectively, (*Figure 2F*, *P=0.0154; HR =0.49; 95% CI, 0.29–0.82) in the combined cohort. When designating patients achieving CR/PR or SD for more than 6 months as responders, we found that the proportion of responders were significantly higher in *TTN* mutant patients in cohorts 1, 2 and the combined cohort (Figure S2, *P=0.021 for cohort 1, *P=0.041 for cohort 2 and *P=0.002 for combined cohort). Taken together, these observations suggest that the ctDNA *TTN* mutation may be a promising biomarker to identify potential responders to ICB immunotherapy.

In the univariate Cox proportional hazards regression model, only *TTN* mutation status was significantly associated with PFS (Table S4, HR =0.40; 95% CI, 0.18–0.90; *P=0.03) in cohort 1. In cohort 2, the pathologic type (squamous *vs.* non-squamous carcinoma), treatment regimen (single agents or combinational regimen), and *TTN* mutation status tended to be significantly associated (with

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P≤0.15) with survival outcomes (Table S4, pathological type, HR =0.51, 95% CI, 0.25-1.05, P=0.12; regimen of treatment, HR =1.71, 95% CI, 0.87-3.38, P=0.09; *TTN* mutation, HR =0.52, 95% CI, 0.27-1.02, P=0.09). By examining the combined analysis, the regimen and TTN mutation status remained significantly associated with PFS with ICB therapy (Table S4, regimen of treatment, HR =1.64, 95% CI, 0.97–2.58, *P=0.05; *TTN* mutation, HR =0.49, 95% CI, 0.29–0.82, *P=0.02). Variables with P value less than 0.15 were included in the multivariate Cox proportional hazards regression model. The mutation status showed the strongest association with PFS in each cohort (cohort 1, HR =0.39, 95% CI, 0.15–1.00, *P=0.05; cohort 2, HR =0.56, 95% CI, 0.25–1.23, P=0.11; combined cohort, HR =0.47, 95% CI, 0.26–0.85, *P=0.01).

Predictive significance of TTN mutation in subgroups

We further explored the clinical relevance of the *TTN* mutation in different subgroups of patients (*Figure 3*). This stratified analysis showed that patients with positive *TTN* mutation tended to benefit more from the improved benefits of ICB treatment in most stratified subgroups. The predictive significance seemed more informative in female patients, patients with non-squamous NSCLC, or patients who received a combinational regimen (*Figure 3C*). Although the effects for most stratifications were not statistically significant, probably due to the small population size, the numerical trends of better survival for *TTN* mutation-positive patients were observed in nearly all the subgroups of each cohort.

Discussion

In the past decades, tumor-associated neoantigens, which arise from non-synonymous somatic mutations, have been recognized as the main targets of cytotoxic T lymphocytes in the tumor microenvironment (19,20). These tumor cell-recognizing T cells can be potentiated by the administration of ICB agents, either by reinvigorating their exhausted cytolytic activity (21,22) or by depleting immunoregulatory cell types (23). The quantity and quality of the neoantigen repertoire significantly contribute to clinical response to ICB treatment in patients with NSCLC (24). There is currently no consensus on how to extract biologically meaningful neoantigens from massive genomic data (25). Due to this shortcoming, TMB is still the best alternative measure of neoantigens. However, accurate estimation of the TMB remains a technical challenge and is not always possible in clinical practice. In the present study, we developed a non-invasive, cost-effective, and easy-to-accomplish method to detect *TTN* mutation in peripheral blood samples. Using this method, we demonstrated that the detectable *TTN* mutation in ctDNA was associated with significant benefit from ICB treatment in patients with advanced NSCLC.

In addition to MSI-H (5), KRAS/TP53 (26), POLE (27), or STK11 (28), high TMB (≥10 mutations/MB) has been approved as a biomarker for predicting responsiveness to ICB immunotherapy in patients with unresectable or metastatic disease. In the KEYNOTE-158 trial, the objective response rate for TMB-high patients was 29%, including 4% of patients with complete response and 25% of patients with partial response. As for response duration, 57% of patients exhibited a response duration of more than one year, and half of the patients experienced response of more than 2 years. These data supported that high TMB is a practical predictive biomarker. However, there are two major issues with the clinical application of high TMB. First, high TMB was determined using an FDA-approved companion diagnostic test, FoundationOneCDx, which counts non-synonymous mutations in tumor tissues, whereas the implementation of TMB assessment is limited by the availability of nucleic acid materials or the heterogeneity of the tumor microenvironment (29). Second, as a continuous variable, there is no absolute cutoff value to define high TMB, making it difficult for clinicians to make decisions in some cases. The cutoff of 10 mutations/MB to define high TMB was selected rather arbitrarily. Based on this definition, only 15.89% of patients (120 of 755 patients with evaluable TMB) were classified as TMB-high, and inevitably, a considerable portion of the patients must have missed the opportunity to receive ICB immunotherapy. Therefore, it is necessary to improve the strategy for selecting optimal candidates who will benefit from ICB therapy.

ctDNA sequencing is actively and widely used in immuno-oncology (10) and has also been introduced in routine clinical practice. An advantage of the technique is that dynamic monitoring of treatment can be easily achieved using ctDNA sequencing without the limitation of tissue availability (30,31). Using ctDNA-assessed bTMB as a surrogate measurement of tissue TMB has been long investigated in the stratification of patients suitable for ICB therapy (32). However, the protocol and sequencing panel used in bTMB assessment is not fully standardized

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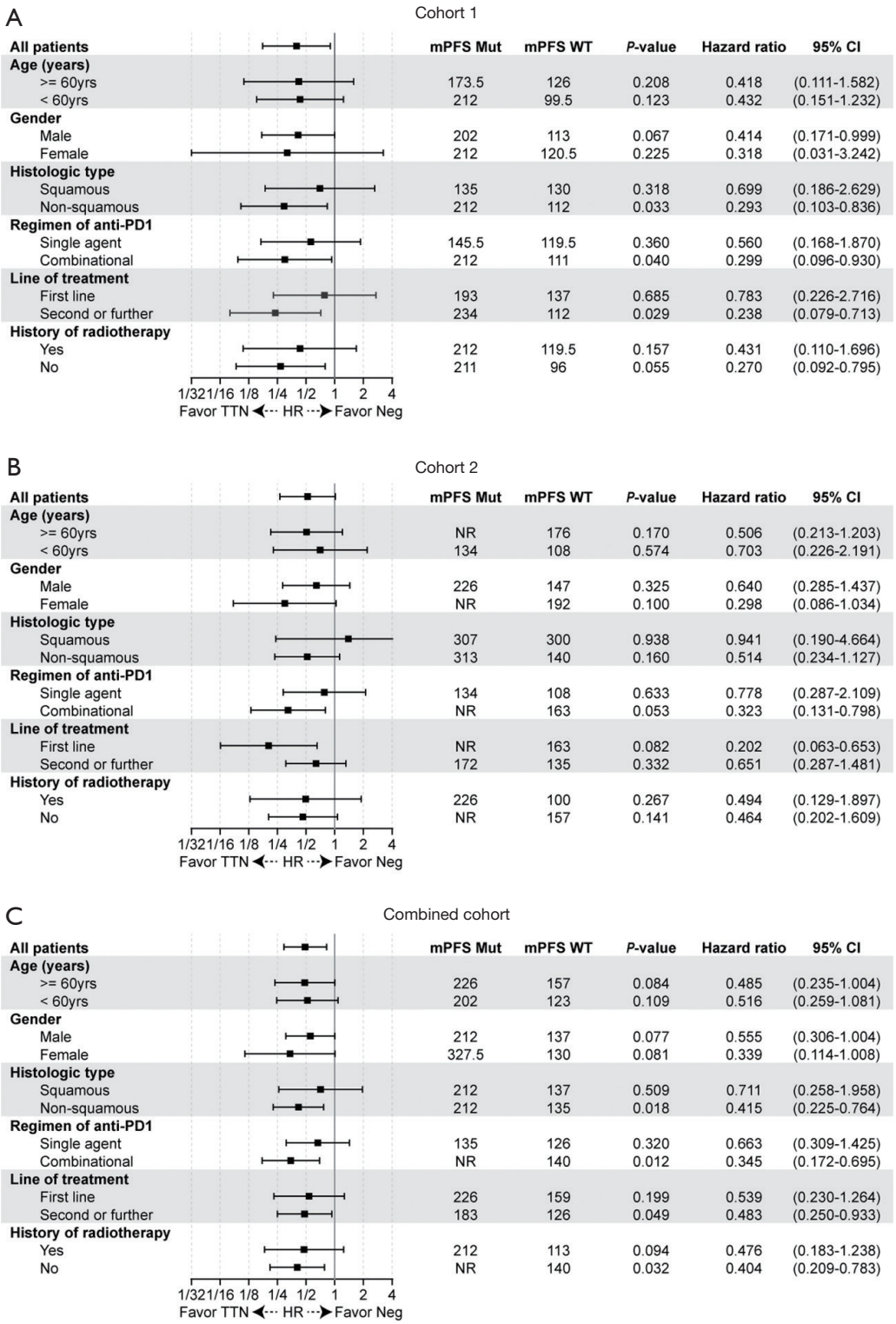

Figure 3 Stratified analysis of the predictive significance of *TTN* mutation. Forest plot showing the association between ctDNA *TTN* mutation and PFS for patients receiving ICB treatment, stratified by age, gender, pathologic type, regimen of agents, line of treatment, and the history of radiotherapy in cohort 1 (A), cohort 2 (B), and combined cohorts (C). HRs within each subgroup are shown on a log2 scale. Bars represent the 95% CI.

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yet, and its robustness as a predictive biomarker needs to be validated in prospective clinical trials. Most importantly, in contrast to tumor tissue-based TMB estimation, the ctDNA-based TMB estimation cannot effectively avoid interference from germline mutations; thus, it leads to a bias in calculations, especially in patients with a relatively low mutation count. Further, the cutoff for defining the TMB as "TMB-high" needs to be determined. Thus, a ctDNA-based assay with binary readout may provide an alternative solution to overcome the adverse impacts of unreliable tissue supply and technical defects.

Titin is a structural protein found in striated muscles (33). In our previous study, by performing a genome-scale screening, we found that the length of the coding regions for each gene was positively associated with mutation frequencies of the gene in solid tumors (14). Coincidentally, *TTN* is the gene with the longest coding sequence in the human genome. Moreover, the highest number of mutations was found in the coding regions of *TTN*, suggesting a high possibility of accumulating somatic mutations in genes with longer coding regions. Furthermore, we demonstrated that tumor-tissue *TTN* mutation was associated with high TMB, high objective response rate, and durable survival in seven publicly accessible cohorts. These pilot analyses led us to investigate whether *TTN* mutation in ctDNA could predict the clinical efficacy of ICB therapy in patients with advanced NSCLC.

Our study suggests the value of ctDNA *TTN* mutation in predicting clinical response in patients with advanced NSCLC. The binary readout (positive/negative) of our assay overcomes the difficulty in identifying a reasonable threshold for selecting patients eligible for ICB immunotherapy. The clinical significance of *TTN* mutation was confirmed in two independent cohorts of patients receiving ICBs. However, there were three major limitations to our study. (I) Due to limited tissue availability and the high cost of sequencing, the *TTN* panel for ctDNA sequencing was validated in the paired tumor biopsy and peripheral blood samples of only 18 patients. Hence, the consistency between tumor-based and ctDNA-based *TTN* mutation needs to be confirmed in a larger cohort. (II) The correlation between ctDNA-based TMB and the number of mutations in TTN CDS should be validated in a larger cohort. (III) The association between improved survival, objective response, and detectable *TTN* mutation in ctDNA was validated in the two cohorts in a retrospective manner. The performance of PD-L1 expression, MSS or MSI-H status or other established ICB-predictive biomarkers should be compared to the performance of ctDNA TTN mutation in identifying responders to ICB treatment. (IV) *TTN* is not routinely included in the commercially available sequencing panels. Therefore, publicly accessible data could not be used to test the performance of our biomarker in mixed solid tumor types.

Conclusions

In conclusion, we used ctDNA sequencing to demonstrate that the *TTN* mutation has the potential to serve as an easy-to-apply predictive biomarker for ICB immunotherapy in patients with advanced NSCLC. However, prospective clinical trials with multiple solid tumor types are required to validate the universal application and robustness of this biomarker.

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Footnote

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Data Sharing Statement: Available at http://dx.doi.org/10.21037/tlcr-20-1118

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr-20-1118). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The trial was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional review board of Xinqiao Hospital, Army Medical University, Chongqing (Identifier: 2017YD104-

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01) and Shanghai Pulmonary Hospital & Thoracic Cancer Institute, Tongji University School of Medicine, Shanghai (Identifier: 82072568). Individual consent for this retrospective analysis was waived.

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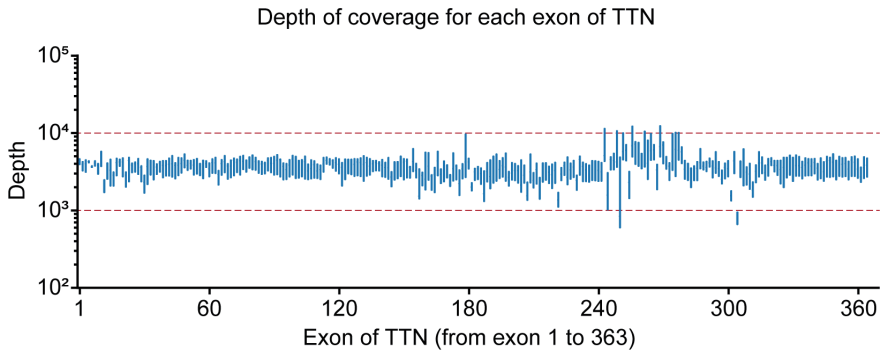

Figure S1 Range of coverage of individual exons of the TTN gene. The blue lines represent the range of coverage of 363 exons from six TTN-sequenced tumor tissues.

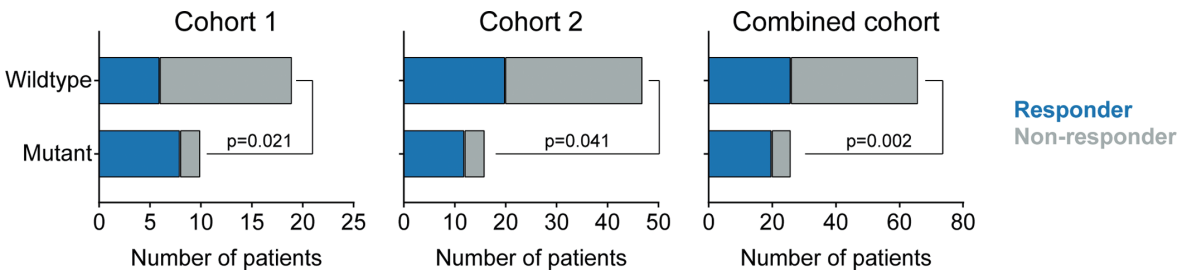

Figure S2 Comparison between patients with mutant or wild type TTN in ctDNA. Upper bar, patients with wild-type TTN; lower bar, patients with mutant TTN. Blue bar, responders; grey bar, non-responders. Comparison is based on Chi-square test.

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Table B.26 Exon Counts for TTN transcripts

Gene	Transcript	Exon	Total length	Protein length
TTN	ENST00000342992	363	106199	35213
TTN	ENST00000359218	362	105948	35213
TTN	ENST00000342175	361	105948	35213
TTN	ENST00000359550	360	105948	35213
TTN	ENST00000359976	359	105948	35213
TTN	ENST00000360870	358	105948	35213
TTN	ENST00000342992	358	106199	35213
TTN	ENST00000359218	357	105948	35213
TTN	ENST00000342175	356	105948	35213
TTN	ENST00000359550	355	105948	35213
TTN	ENST00000359976	354	105948	35213
TTN	ENST00000360870	353	105948	35213
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TTN	ENST00000342175	351	105948	35213
TTN	ENST00000359550	350	105948	35213
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TTN	ENST00000360870	348	105948	35213
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TTN	ENST00000359218	62	105948	35213
TTN	ENST00000342175	61	105948	35213
TTN	ENST00000359550	60	105948	35213
TTN	ENST00000359976	59	105948	35213
TTN	ENST00000360870	58	105948	35213
TTN	ENST00000342992	58	106199	35213
TTN	ENST00000359218	57	105948	35213
TTN	ENST00000342175	56	105948	35213
TTN	ENST00000359550	55	105948	35213
TTN	ENST00000359976	54	105948	35213
TTN	ENST00000360870	53	105948	35213
TTN	ENST00000342992	53	106199	35213
TTN	ENST00000359218	52	105948	35213
TTN	ENST00000342175	51	105948	35213
TTN	ENST00000359550	50	105948	35213
TTN	ENST00000359976	49	105948	35213
TTN	ENST00000360870	48	105948	35213
TTN	ENST00000342992	48	106199	35213
TTN	ENST00000359218	47	105948	35213
TTN	ENST00000342175	46	105948	35213
TTN	ENST00000359550	45	105948	35213
TTN	ENST00000359976	44	105948	35213
TTN	ENST00000360870	43	105948	35213
TTN	ENST00000342992	43	106199	35213
TTN	ENST00000359218	42	105948	35213
TTN	ENST00000342175	41	105948	35213
TTN	ENST00000359550	40	105948	35213
TTN	ENST00000359976	39	105948	35213
TTN	ENST00000360870	38	105948	35213
TTN	ENST00000342992	38	106199	35213
TTN	ENST00000359218	37	105948	35213
TTN	ENST00000342175	36	105948	35213
TTN	ENST00000359550	35	105948	35213
TTN	ENST00000359976	34	105948	35213
TTN	ENST00000360870	33	105948	35213
TTN	ENST00000342992	33	106199	35213
TTN	ENST00000359218	32	105948	35213
TTN	ENST00000342175	31	105948	35213
TTN	ENST00000359550	30	105948	35213
TTN	ENST00000359976	29	105948	35213
TTN	ENST00000360870	28	105948	35213
TTN	ENST00000342992	28	106199	35213
TTN	ENST00000359218	27	105948	35213
TTN	ENST00000342175	26	105948	35213
TTN	ENST00000359550	25	105948	35213
TTN	ENST00000359976	24	105948	35213
TTN	ENST00000360870	23	105948	35213
TTN	ENST00000342992	23	106199	35213
TTN	ENST00000359218	22	105948	35213
TTN	ENST00000342175	21	105948	35213
TTN	ENST00000359550	20	105948	35213
TTN	ENST00000359976	19	105948	35213
TTN	ENST00000360870	18	105948	35213
TTN	ENST00000342992	18	106199	35213
TTN	ENST00000359218	17	105948	35213
TTN	ENST00000342175	16	105948	35213
TTN	ENST00000359550	15	105948	35213
TTN	ENST00000359976	14	105948	35213
TTN	ENST00000360870	13	105948	35213
TTN	ENST00000342992	13	106199	35213
TTN	ENST00000359218	12	105948	35213
TTN	ENST00000342175	11	105948	35213
TTN	ENST00000359550	10	105948	35213
TTN	ENST00000359976	9	105948	35213
TTN	ENST00000360870	8	105948	35213
TTN	ENST00000342992	8	106199	35213
TTN	ENST00000359218	7	105948	35213
TTN	ENST00000342175	6	105948	35213
TTN	ENST00000359550	5	105948	35213
TTN	ENST00000359976	4	105948	35213
TTN	ENST00000360870	3	105948	35213
TTN	ENST00000342992	3	106199	35213
TTN	ENST00000359218	2	105948	35213
TTN	ENST00000342175	1	105948	35213
TTN	ENST00000359550	1	105948	35213
TTN	ENST00000359976	1	105948	35213
TTN	ENST00000360870	1	105948	35213

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Table B.25 (continued)

Type	Tag	Length	Value	Tag value
2A0	11000000	10000000	11000000	11000000
2A1	11000001	10000001	11000001	11000001
2A2	11000010	10000010	11000010	11000010
2A3	11000011	10000011	11000011	11000011
2A4	11000100	10000100	11000100	11000100
2A5	11000101	10000101	11000101	11000101
2A6	11000110	10000110	11000110	11000110
2A7	11000111	10000111	11000111	11000111
2A8	11001000	10001000	11001000	11001000
2A9	11001001	10001001	11001001	11001001
2AA	11001010	10001010	11001010	11001010
2AB	11001011	10001011	11001011	11001011
2AC	11001100	10001100	11001100	11001100
2AD	11001101	10001101	11001101	11001101
2AE	11001110	10001110	11001110	11001110
2AF	11001111	10001111	11001111	11001111
2B0	11010000	10010000	11010000	11010000
2B1	11010001	10010001	11010001	11010001
2B2	11010010	10010010	11010010	11010010
2B3	11010011	10010011	11010011	11010011
2B4	11010100	10010100	11010100	11010100
2B5	11010101	10010101	11010101	11010101
2B6	11010110	10010110	11010110	11010110
2B7	11010111	10010111	11010111	11010111
2B8	11011000	10011000	11011000	11011000
2B9	11011001	10011001	11011001	11011001
2BA	11011010	10011010	11011010	11011010
2BB	11011011	10011011	11011011	11011011
2BC	11011100	10011100	11011100	11011100
2BD	11011101	10011101	11011101	11011101
2BE	11011110	10011110	11011110	11011110
2BF	11011111	10011111	11011111	11011111
2C0	11100000	10100000	11100000	11100000
2C1	11100001	10100001	11100001	11100001
2C2	11100010	10100010	11100010	11100010
2C3	11100011	10100011	11100011	11100011
2C4	11100100	10100100	11100100	11100100
2C5	11100101	10100101	11100101	11100101
2C6	11100110	10100110	11100110	11100110
2C7	11100111	10100111	11100111	11100111
2C8	11101000	10101000	11101000	11101000
2C9	11101001	10101001	11101001	11101001
2CA	11101010	10101010	11101010	11101010
2CB	11101011	10101011	11101011	11101011
2CC	11101100	10101100	11101100	11101100
2CD	11101101	10101101	11101101	11101101
2CE	11101110	10101110	11101110	11101110
2CF	11101111	10101111	11101111	11101111
2D0	11110000	10110000	11110000	11110000
2D1	11110001	10110001	11110001	11110001
2D2	11110010	10110010	11110010	11110010
2D3	11110011	10110011	11110011	11110011
2D4	11110100	10110100	11110100	11110100
2D5	11110101	10110101	11110101	11110101
2D6	11110110	10110110	11110110	11110110
2D7	11110111	10110111	11110111	11110111
2D8	11111000	10111000	11111000	11111000
2D9	11111001	10111001	11111001	11111001
2DA	11111010	10111010	11111010	11111010
2DB	11111011	10111011	11111011	11111011
2DC	11111100	10111100	11111100	11111100
2DD	11111101	10111101	11111101	11111101
2DE	11111110	10111110	11111110	11111110
2DF	11111111	10111111	11111111	11111111
2E0	11000000	11000000	11000000	11000000
2E1	11000001	11000001	11000001	11000001
2E2	11000010	11000010	11000010	11000010
2E3	11000011	11000011	11000011	11000011
2E4	11000100	11000100	11000100	11000100
2E5	11000101	11000101	11000101	11000101
2E6	11000110	11000110	11000110	11000110
2E7	11000111	11000111	11000111	11000111
2E8	11001000	11001000	11001000	11001000
2E9	11001001	11001001	11001001	11001001
2EA	11001010	11001010	11001010	11001010
2EB	11001011	11001011	11001011	11001011
2EC	11001100	11001100	11001100	11001100
2ED	11001101	11001101	11001101	11001101
2EE	11001110	11001110	11001110	11001110
2EF	11001111	11001111	11001111	11001111
2F0	11010000	11010000	11010000	11010000
2F1	11010001	11010001	11010001	11010001
2F2	11010010	11010010	11010010	11010010
2F3	11010011	11010011	11010011	11010011
2F4	11010100	11010100	11010100	11010100
2F5	11010101	11010101	11010101	11010101
2F6	11010110	11010110	11010110	11010110
2F7	11010111	11010111	11010111	11010111
2F8	11011000	11011000	11011000	11011000
2F9	11011001	11011001	11011001	11011001
2FA	11011010	11011010	11011010	11011010
2FB	11011011	11011011	11011011	11011011
2FC	11011100	11011100	11011100	11011100
2FD	11011101	11011101	11011101	11011101
2FE	11011110	11011110	11011110	11011110
2FF	11011111	11011111	11011111	11011111
300	11100000	11100000	11100000	11100000
301	11100001	11100001	11100001	11100001
302	11100010	11100010	11100010	11100010
303	11100011	11100011	11100011	11100011
304	11100100	11100100	11100100	11100100
305	11100101	11100101	11100101	11100101
306	11100110	11100110	11100110	11100110
307	11100111	11100111	11100111	11100111
308	11101000	11101000	11101000	11101000
309	11101001	11101001	11101001	11101001
30A	11101010	11101010	11101010	11101010
30B	11101011	11101011	11101011	11101011
30C	11101100	11101100	11101100	11101100
30D	11101101	11101101	11101101	11101101
30E	11101110	11101110	11101110	11101110
30F	11101111	11101111	11101111	11101111
310	11110000	11110000	11110000	11110000
311	11110001	11110001	11110001	11110001
312	11110010	11110010	11110010	11110010
313	11110011	11110011	11110011	11110011
314	11110100	11110100	11110100	11110100
315	11110101	11110101	11110101	11110101
316	11110110	11110110	11110110	11110110
317	11110111	11110111	11110111	11110111
318	11111000	11111000	11111000	11111000
319	11111001	11111001	11111001	11111001
31A	11111010	11111010	11111010	11111010
31B	11111011	11111011	11111011	11111011
31C	11111100	11111100	11111100	11111100
31D	11111101	11111101	11111101	11111101
31E	11111110	11111110	11111110	11111110
31F	11111111	11111111	11111111	11111111

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Table S2 Clinical characteristics of both cohorts

No	TTN	cohort	TumorType	Regimens	BOR	Responder(CR or PR or SD>6mos)	PFS	Progression?	Age	Gender	ECOG PS	Smoking	Line of treatment	TNM	History of radiotherapy
1	-	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	SD	No	60	Progression	61	Male	2	80	Second line or further	IV	Yes
2	-	Cohort 1	Lung adenocarcinoma	single IO agents	SD	No	145	Progression	53	Male	0	30	Second line or further	IIIB	Yes
3	Mut	Cohort 1	Lung squamous cell carcinoma	single IO agents	PR	Yes	112	Progression	38	Male	0	7	Second line or further	IV	Yes
4	-	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	PD	No	66	Progression	55	Male	1	30	Second line or further	IV	-
5	Mut	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	PR	Yes	230	Free	54	Male	1	Never	Second line or further	IIIA	-
6	Mut	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	212	Progression	69	Male	0	Never	Second line or further	IV	Yes
7	-	Cohort 1	Lung squamous cell carcinoma	single IO agents	SD	No	137	Progression	66	Male	1	Never	First line	IV	-
8	-	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	SD	No	77	Progression	54	Male	1	30	Second line or further	IV	-
9	-	Cohort 1	Lung adenocarcinoma	IO+Anti-angiogenic agent	PR	Yes	111	Progression	45	Female	0	Never	Second line or further	?	Yes
10	Mut	Cohort 1	Lung squamous cell carcinoma	single IO agents	SD	No	135	Progression	68	Male	0	40	First line	IV	Yes
11	-	Cohort 1	Lung adenocarcinoma	IO+Anti-angiogenic agent	SD	No	171	Progression	66	Male	1	45	Second line or further	IV	-
12	-	Cohort 1	Lung adenocarcinoma	single IO agents	SD	Yes	434	Progression	52	Male	0	Never	First line	IIIA	-
13	-	Cohort 1	Lung adenocarcinoma	single IO agents	SD	No	113	Progression	64	Male	0	40	Second line or further	IIIA	Yes
14	-	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	SD	No	88	Progression	40	Male	1	Never	First line	IV	-
15	Mut	Cohort 1	Lung squamous cell carcinoma	IO+Anti-angiogenic agent	SD	Yes	212	Progression	42	Female	0	Never	First line	IV	Yes
16	-	Cohort 1	Lung squamous cell carcinoma	IO+Chemotherapy	SD	Yes	195	Free	36	Male	0	20	Second line or further	IV	Yes
17	Mut	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	SD	No	92	Free	77	Male	0	20. former	Second line or further	IV	-
18	-	Cohort 1	Lung squamous cell carcinoma	single IO agents	PD	No	42	Progression	41	Male	0	5	First line	IV	-
19	-	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	PR	Yes	96	Progression	62	Male	1	2. former	Second line or further	IV	-
20	Mut	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	192	Progression	50	Male	0	Never	First line	IV	-
21	Mut	Cohort 1	Lung adenocarcinoma	single IO agents	PR	Yes	256	Free	52	Male	0	50	Second line or further	IV	Yes
22	Mut	Cohort 1	Lung adenocarcinoma	single IO agents	PR	Yes	156	Progression	56	Male	0	90	First line	IV	-
23	Mut	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	206	Free	50	Male	0	50	First line	IIIB	-
24	-	Cohort 1	Lung squamous cell carcinoma	single IO agents	SD	No	71	Progression	55	Male	0	20. former	Second line or further	IV	-
25	-	Cohort 1	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	279	Free	50	Male	0	Never	First line	IV	-
26	-	Cohort 1	Lung adenocarcinoma	single IO agents	SD	No	126	Progression	64	Male		45	Second line or further	IV	Yes
27	-	Cohort 1	Lung squamous cell carcinoma	IO+Anti-angiogenic agent	PR	Yes	145	Progression	63	Male	1	40	First line	IV	-
28	-	Cohort 1	Lung squamous cell carcinoma	single IO agents	PD	No	33	Progression	29	Male	0	Never	First line	IV	-
29	-	Cohort 1	Lung squamous cell carcinoma	IO+Chemotherapy	SD	No	130	Progression	48	Female	1	Never	Second line or further	IV	-
30	-	Cohort 2	Lung adenocarcinoma	single IO agents	PD	No	31	Progression	53	Male	1	20	First line	IV	-
31	-	Cohort 2	Large cell carcinoma	single IO agents	SD	No	100	Progression	67	Male	1	Never	Second line or further	V	Yes
32	Mut	Cohort 2	Lung squamous cell carcinoma	single IO agents	PR	Yes	108	Progression	50	Male	1	Never	Second line or further	IV	Yes
33	-	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	SD	No	98	Progression	52	Male	1	Never	First line	IV	-
34	-	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PR	Yes	596	Free	74	Male	1	Never	First line	IV	-
35	-	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PR	Yes	197	Free	37	Male	0	7.5	First line	IV	-
36	-	Cohort 2	Lung adenocarcinoma	single IO agents	SD	Yes	226	Free	66	Male	1	Never	Second line or further	IV	-
37	Mut	Cohort 2	Lung adenocarcinoma	single IO agents	PD	No	56	Progression	49	Male	1	10	Second line or further	IV	-
38	-	Cohort 2	Lung adenocarcinoma	single IO agents	PD	No	69	Progression	72	Female	1	Never	Second line or further	V	-
39	Mut	Cohort 2	Lung adenocarcinoma	single IO agents	PR	Yes	443	Free	65	Female	1	20	Second line or further	IV	-
40	-	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PR	Yes	138	Free	50	Male	1	30	First line	IIIC	Yes
41	Mut	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	253	Free	54	Female	1	Never	First line	V	-
42	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	117	Free	49	Female	1	Never	Second line or further	IV	-
43	-	Cohort 2	Lung adenocarcinoma	single IO agents	PR	Yes	426	Free	58	Male	1	Never	Second line or further	IV	Yes
44	Mut	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PR	Yes	388	Free	66	Male	1	30	First line	V	-
45	Mut	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PR	Yes	226	Progression	64	Male	1	30	First line	IV	Yes
46	-	Cohort 2	Large cell carcinoma	IO+Chemotherapy	SD	No	49	Free	71	Male	1	30	Second line or further	V	Yes
47	-	Cohort 2	Lung adenocarcinoma	single IO agents	PR	Yes	131	Free	68	Male	1	45	Second line or further	IV	Yes
48	-	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PD	No	39	Free	53	Male	1	30	First line	IIIb	-
49	-	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PD	No	56	Progression	50	Male	1	Never	Second line or further	IIIB	-
50	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	PR	Yes	89	Progression	47	Male	1	Never	First line	IV	-
51	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	255	Free	65	Male	1	Never	First line	IVB	-
52	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	42	Free	67	Male	1	Never	First line	V	-
53	Mut	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PR	Yes	265	Free	71	Male	1	Never	Second line or further	IIIC	Yes
54	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	159	Progression	53	Male	0	Never	First line	IV	-
55	-	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PD	No	45	Free	64	Male	1	Never	First line	IIIb	-
56	Mut	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	129	Progression	65	Male	1	40	Second line or further	V	-
57	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	371	Free	66	Male	1	Never	First line	IV	-
58	-	Cohort 2	Non-specific	single IO agents	SD	No	134	Progression	52	Male	1	Never	Second line or further	V	-
59	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	PD	No	49	Progression	47	Male	1	Never	First line	IV	-
60	-	Cohort 2	Lung squamous cell carcinoma	single IO agents	PR	Yes	157	Progression	74	Male	1	Never	First line	IV	-
61	Mut	Cohort 2	Lung adenocarcinoma	single IO agents	PR	Yes	312	Free	66	Male	1	Never	Second line or further	IV	-
62	-	Cohort 2	Lung adenocarcinoma	single IO agents	PD	No	34	Progression	72	Male	1	30	Second line or further	V	-
63	-	Cohort 2	Lung squamous cell carcinoma	single IO agents	SD	Yes	300	Progression	80	Male	1	Never	First line	IV	-
64	-	Cohort 2	Lung squamous cell carcinoma	single IO agents	PD	No	82	Progression	66	Male	1	Never	Second line or further	V	Yes
65	Mut	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	SD	Yes	283	Free	46	Male	1	Never	Second line or further	V	Yes
66	-	Cohort 2	Lung squamous cell carcinoma	IO+Chemotherapy	PR	Yes	420	Free	67	Male	1	Never	First line	IIIB	-
67	-	Cohort 2	Lung adenocarcinoma	single IO agents	PD	No	31	Progression	78	Female	1	Never	Second line or further	IV	Yes
68	Mut	Cohort 2	Lung squamous cell carcinoma	single IO agents	PR	Yes	172	Progression	67	Female	1	Never	Second line or further	IV	Yes
69	Mut	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	PR	Yes	260	Free	60	Female	1	Never	First line	IV	-
70	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	PR	Yes	192	Progression	61	Female	1	Never	First line	IV	-
71	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	140	Progression	55	Male	1	Never	Second line or further	IV	-
72	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	PD	No	83	Progression	50	Female	1	Never	Second line or further	IV	Yes
73	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	PD	No	41	Progression	65	Male	1	Never	Second line or further	IV	-
74	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	PD	No	65	Progression	68	Male	1	35	First line	IV	-
75	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	269	Progression	73	Female	1	Never	First line	V	-
76	Mut	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	236	Free	65	Male	1	Never	First line	V	Yes
77	Mut	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	183	Progression	72	Male	1	Never	Second line or further	IV	-
78	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	232	Progression	53	Female	1	Never	Second line or further	V	-
79	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	163	Progression	61	Male	1	Never	First line	IV	-
80	-	Cohort 2	Lung adenocarcinoma	single IO agents	SD	Yes	247	Progression	65	Female	2	Never	Second line or further	IV	-
81	Mut	Cohort 2	Lung adenocarcinoma	single IO agents	SD	No	88	Progression	46	Female	1	Never	Second line or further	IV	Yes
82	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	353	Free	76	Male	0	Never	Second line or further	IIB	-
83	-	Cohort 2	Non-specific	single IO agents	PR	Yes	123	Progression	27	Female	1	Never	First line	IV	-
84	-	Cohort 2	Lung adenocarcinoma	single IO agents	PR	Yes	147	Progression	55	Male	1	Never	Second line or further	V	-
85	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	94	Progression	29	Male	1	30	Second line or further	IV	-
86	Mut	Cohort 2	Lung adenocarcinoma	single IO agents	SD	No	85	Progression	65	Male	1	Never	Second line or further	IV	-
87	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	176	Progression	71	Male	1	Never	Second line or further	IIIB	-
88	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	135	Progression	66	Male	1	30	Second line or further	IV	-
89	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	44	Progression	70	Male	1	25	First line		-
90	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	59	Free	59	Female	1	Never	Second line or further	IV	-
91	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	Yes	206	Free	75	Male	1	Never	Second line or further	IV	-
92	-	Cohort 2	Lung adenocarcinoma	IO+Chemotherapy	SD	No	38	Progression	62	Male	1	Never	Second line or further	V	Yes

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Table S3 Comparison of baseline characteristics

	Cohort 1 (N=29)	Cohort 2 (N=63)	Combined cohort (N=92)
Age, years	54 [29-77]	65 [27-80]	61 [27-80]
Sex			
Male	26 (89.7%)	48 (76.2%)	74 (80.5%)
Femail	3 (10.3%)	15 (23.8%)	18 (19.5%)
ECOG PS			
0-1	27 (93.1%)	62 (98.4%)	89 (96.7%)
>=2	1 (3.4%)	1 (1.6%)	2 (2.2%)
Unknown	1 (3.4%)	0 (0.0%)	1 (1.1%)
Smoking history			
Current or former smoker	18 (62.1%)	47 (74.6%)	65 (70.7%)
Never smoker	11 (37.9%)	16 (25.4%)	27 (29.3%)
Histology at diagnosis			
Adenocarcinoma	19 (65.5%)	42 (66.6%)	61 (66.3%)
Squamous cell carcinoma	10 (34.5%)	17 (25.8%)	27 (29.4%)
Others	0 (0.0%)	4 (6.3%)	4 (4.3%)
History of radiotherapy			
Yes	11 (37.9%)	16 (25.4%)	27 (29.3%)
No	18 (62.1%)	47 (74.6%)	65 (70.7%)
Regimen of anti-PD1 treatment			
Anti-PD1 alone	12 (41.4%)	22 (34.9%)	34 (37.0%)
Combinational	17 (58.6%)	41 (65.1%)	58 (63.0%)
Line of anti-PD1 treatment			
First	12 (41.4%)	27 (42.9%)	36 (39.1%)
Second or further	17 (58.6%)	36 (57.1%)	56 (60.9%)
RECIST best response			
PR	7 (24.1%)	19 (30.2%)	26 (28.3%)
SD	19 (65.5%)	31 (49.2%)	50 (54.3%)
PD	3 (10.3%)	13 (20.6%)	16 (17.4%)
TTN mutation in ctDNA			
Detected	10 (34.5%)	16 (25.4%)	26 (28.3%)
Absence	19 (65.5%)	47 (74.6%)	66 (71.7%)

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Table S4 Multivariable cox analysis

Parameter	Cohort 1				Cohort 2				Combined cohort			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	HR(95% CI)	P-value	HR(95% CI)	P-value	HR(95% CI)	P-value	HR(95% CI)	P-value	HR(95% CI)	P-value	HR(95% CI)	P-value
Age, >=60 vs <60	1.60(0.65-3.93)	0.25	-	-	0.63(0.34-1.25)	0.16	-	-	0.73(0.44-1.21)	0.21	-	-
Gender, male vs female	0.81(0.22-3.02)	0.73	-	-	0.97(0.47-2.00)	0.92	-	-	1.02(0.55-1.87)	0.96	-	-
Pathologic type, LUSC vs non-LUSC	1.70(0.68-4.24)	0.20	-	-	0.51(0.25-1.05)	0.12	0.61(0.25-1.48)	0.27	0.86(0.49-1.51)	0.62	-	-
Regimen, single vs combinational	1.41(0.61-3.27)	0.38	-	-	1.71(0.87-3.38)	0.09	1.70(0,89-3.22)	0.15	1.64(0.97-2.78)	0.05	1.53(0.91-2.58)	0.11
Line, first line vs further	0.76(0.33-1.71)	0.48	-	-	0.63(0.34-1.20)	0.17	-	-	0.68(0.42-1.14)	0.15	0.72(0.42-1.22)	0.23
History of radiotherapy, yes vs no	0.96(0.42-2.21)	0.92	-	-	1.04(0.49-2.22)	0.91	-	-	1.08(0.62-1.89)	0.78	-	-
TTN, mutation vs wildtype	0.40(0.18-0.90)	0.03	0.39(0.15-1.00)	0.05	0.52(0.27-1.02)	0.09	0.56(0.25-1.23)	0.11	0.49(0.29-0.82)	0.02	0.47(0.26-0.85)	0.01

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