Original Article

Comprehensive analysis of T cell receptor repertoire in patients with *KRAS* mutant non-small cell lung cancer

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Background: Kirsten rat sarcoma viral oncogene homolog (*KRAS*) is one of the most frequently mutated oncogenes in non-small cell lung cancer (NSCLC). The administration of immunotherapy has demonstrated significant efficacy in prolonging the overall survival of patients with *KRAS* mutation in recent years. However, the efficacy of immunotherapy in *KRAS* mutant NSCLC is variable. Analysis of T cell receptor (TCR) repertoire may contribute to a better understanding of the mechanisms behind such differential outcomes.

Methods: A total of 47 patients with *KRAS* mutant NSCLC were enrolled in this study. Deep sequencing of the TCR β chain complementarity-determining regions in tumor tissue and paired peripheral blood

specimens was conducted. Comprehensive analysis of TCR repertoire metrics was performed with different *KRAS* mutation subtypes and concomitant mutations. Moreover, the associations between TCR repertoire metrics and tumor mutation burden (TMB), as well as programmed death-ligand 1 were explored, respectively.

Results: TCR repertoire metrics, including Shannon index, Clonality, and Morisita index (MOI), showed no significant differences among different *KRAS* mutation subtypes. The similar results were observed between patients with tumor protein p53 (*TP53*) mutation and those with wild-type *TP53*. In contrast, although no significant differences were found in Shannon index and Clonality, patients with KRAS/serine/threonine kinase 11 (*STK11*) comutation showed a significantly higher MOI compared to their *STK11* wild-type counterparts (P=0.012). In addition, TCR repertoire metrics were neither associated with TMB nor programmed death-ligand 1 expression in *KRAS* mutant NSCLC.

Conclusions: This retrospective study comprehensively described the TCR repertoire in *KRAS* mutant NSCLC. A higher MOI represented more overlap of the TCR repertoire between tumor tissue and paired peripheral blood, indicating distinctive immunological features in NSCLC with *KRAS*/*STK11* comutation.

Keywords: T cell receptor repertoire (TCR repertoire); Kirsten rat sarcoma viral oncogene homolog (*KRAS*); mutations; immunotherapy; non-small cell lung cancer (NSCLC)

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Introduction

Lung cancer is the most common cause of cancer-related death, and the discovery of driver mutations has led to a dramatic paradigm shift in its treatment strategy (1). The Kirsten rat sarcoma viral oncogene homolog (*KRAS*) serves as one of the most frequent driver mutations identified in non-small cell lung cancer (NSCLC), and previous studies have found considerable differences in the frequency of *KRAS* mutation between Eastern and Western patients (2,3). Mechanistically, *KRAS* mutation could lead to aberrant activation of the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways, and result in aggressive tumor growth and metastasis (4).

Patients with *KRAS* mutant NSCLC generally have shortened survival time when treated with conventional antitumor therapies compared to patients with wild-type *KRAS* (5). Despite various therapeutic attempts, *KRAS*-mutant NSCLC responds poorly to cytotoxic chemotherapy, and no clear efficacy differences are seen between various chemotherapy regimens. Therefore, numerous novel potential therapeutic agents and treatment strategies have been developed to prolong the survival of patients with *KRAS*-mutant NSCLC. Targeting *KRAS* mutations directly is a great challenge and has long been a focus of research in NSCLC. Unlike the epidermal growth factor receptor (*EGFR*), *KRAS* is considered undruggable due to its small size, relatively smooth surface, and high affinity towards abundant guanosine triphosphate (6). With the recent development of *KRAS*-targeted inhibitors such as sotorasib and adagrasib, there is light on the horizon for one specific subtype, *KRAS* G12C mutation (7-9). However, all patients included in these studies were receiving *KRAS* G12C inhibitors as second-line or later treatment, and the majority had previously received immunotherapy during the course of treatment (7). Furthermore, effective *KRAS* targeted therapies have not been developed for patients with NSCLC harboring the *KRAS* non-G12C mutation subtypes.

Recently, the advent of new treatment modalities by using immune checkpoint inhibitors (ICIs) with or without chemotherapy in the front-line setting has completely revolutionized the therapeutic landscape in advanced NSCLC (10,11). Many studies have suggested *KRAS* mutation could contribute to immune escape by inducing the upregulation of programmed cell death-ligand 1 (PD-L1) expression, indicating the prospect of applying ICIs to *KRAS* mutant NSCLC (12,13). A recent study has shown NSCLC patients harboring *KRAS* mutation had a better prognosis and long-term survival upon immunotherapy than those with wild-type *KRAS* (14).

However, this conclusion may not apply to all *KRAS* mutant NSCLC. Patients with *KRAS* mutation actually represent a quite heterogeneous population, and different subtypes may trigger distinct downstream signaling pathways and lead to differential responses to antitumor therapies (15). The presence of concomitant mutations such as tumor protein p53 (*TP53*) and serine/threonine kinase 11 (*STK11*) has also been found to be associated with the therapeutic efficacy of immunotherapy (16,17). Emerging evidence has suggested tumor gene mutations can modulate the overall tumor immune landscape, which may account for the diverse efficacy of ICIs among patients with different *KRAS* mutant NSCLC (18-20). However, the specific immunological features of different *KRAS* mutation subtypes and concomitant mutations in NSCLC remain unclear.

T cells are the major component of adaptive immunity against tumor cells, and the T cell receptor (TCR) repertoire plays an important role in recognizing and interacting with various tumor antigens (21). The diversity of TCR is mainly determined by the highly variable complementarity determining region 3 (CDR3), and TCR sequencing provides the opportunity to comprehensively assess the activation status of T cells (22,23). Some studies have suggested several TCR repertoire metrics can be used to predict the efficacy of ICIs, and the dynamic changes in circulating TCR repertoire metrics before and after treatment are associated with the long-term prognosis of patients with NSCLC (24-26). Therefore, comprehensive analysis of the TCR repertoire would help us understand the heterogeneity of *KRAS* mutations and distinct immunological features in patients with *KRAS* mutant NSCLC. Meanwhile, TCR repertoire may give additional insight into the underlying mechanisms of differences in the therapeutic efficacy of immunotherapy. We present the following article in accordance with the MDAR reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-629/rc).

Methods

Patient cohorts and study design

A total of 47 patients with *KRAS* mutant NSCLC were enrolled in this retrospective study from February 2017 to October 2020 at the Department of Thoracic Surgery, Peking Union Medical College Hospital (Beijing, China). All patients were treated initially and had not received any antitumor therapy prior to sampling, and those with autoimmune disease were excluded from the study. This study was conducted following the Declaration of Helsinki (as revised in 2013) and approved by the ethics committee of Peking Union Medical College Hospital (No. S-K1670). Written informed consent for the acquisition of blood and tumor tissue was obtained from all participants. Tumor staging was evaluated based on the 8th edition of the American Joint Committee on Cancer (AJCC-8) TNM staging system for lung cancer.

Targeted next-generation sequencing

Genetic analysis was conducted as previously described (27). Briefly, the DNA from formalin-fixed, paraffin-embedded specimens (FFPE) and peripheral blood cell (PBC) was isolated using a DNeasy Blood & Tissue Kit and QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The libraries were sequenced on a Gene+ Seq-2000 sequencing system (GenePlus-Suzhou, Suzhou, China) or NextSeq CN 500 system (Illumina, San Diego, CA, USA) after hybridization to custom-designed biotinylated oligonucleotide probes (Roche NimbleGen, Madison, WI, USA) targeting 1,021 genes.

After removal of terminal adaptor sequences and low-quality reads, the remaining reads were mapped to the reference human genome (hg19) and aligned using the Burrows-Wheel Aligner (version 0.7.12-r1039) with default parameters. GATK (3.4-46-gbc02625) and MuTect2 (1.1.4) were used to call somatic single nucleotide variants (SNVs) and small insertions and deletions (InDels). Contra (2.0.8) was used to identify copy number variations, and structural variants (SVs) were detected using NCsv (in-house algorithm 0.2.3). All candidate variants were manually confirmed using the integrative genomics viewer browser, and were filtered to exclude clonal hematopoietic mutations, germline mutations in dbSNP, as well as variants that occur at a population frequency of >1% in the Exome Sequencing Project.

Tumor mutation burden (TMB) and PD-L1 expression evaluation

The TMB was defined as the number of somatic nonsynonymous mutations (single-nucleotide variants and small insertions/deletions) per mega-base in the coding region (with VAF ≥0.03 for tissue) (28). Tumor PD-L1 expression was assessed using immunohistochemistry with the PD-L1 IHC 22C3 pharmDx assay (Agilent

Technologies, Santa Clara, CA, USA). The percentage of tumor cells showing complete or partial membrane PD-L1 staining at any intensity was used to calculate the PD-L1 tumor proportion score (TPS).

TCR sequencing and assessment of the TCR repertoire

TCR sequencing was conducted as previously described and detailed below (29). The CDR3 region of the TCR β chain was inclusively and semi-quantitatively amplified by multiplex polymerase chain reaction (PCR), including PCR1 and PCR2. To amplify all possible V(D)J combinations, a set of 32 V forward and 13 J reverse primers were used to perform multiplex PCR1 assays, and PCR2 universal primers were used in the second round of PCR. Sequencing libraries were loaded onto an Illumina HiSeq X ten system, and 151-bp-length reads were obtained. The CDR3 region was identified according to the International ImMunoGeneTics (IMGT) collaboration, beginning with the second cysteine of the V region and ending with the conserved phenylalanine of the J region (30). The CDR3 sequences were identified and assigned using the MiXCR software package (31).

To comprehensively analyze the TCR repertoire of patients with *KRAS* mutation, we measured the Shannon index to assess the diversity and the Clonality value to estimate the clonality of the TCR repertoire. The Morisita index (MOI) is considered to be able to reflect overlap of the TCR repertoire between tumor tissue and paired PBC (32). The specific definitions of TCR repertoire metrics are listed below. As previously reported, Shannon index (Shannon's entropy) is defined as:

$$\text{Shannon index} = -\sum_{i=1}^{N} pi \ln pi \quad [1]$$

where pi is the proportion of sequence i relative to the total N sequences (33,34). A larger Shannon index reflects a more diverse distribution of the CDR3 sequences. TCR Clonality is further calculated as 1 – (Shannon index/ln K), where K is the number of productive unique sequences. Its value ranges from 0 to 1, where values near 0 indicate a very even distribution of the frequency of different clones (polyclonal) and those approaching 1 indicate a distinct asymmetric distribution in which a few activated clones present at high frequencies (monoclonal) (33,35). Taking into account both the specific T-cell rearrangements and their respective frequencies, MOI could be utilized to measure the degree of overlap and similarity in TCR repertoires between tumor tissue and paired PBC. It takes on the same range of values as Clonality, where 1 represents an identical TCR repertoire and 0 represents a completely different TCR repertoire (35,36).

Statistical analysis

Continuous variables were described using means and standard deviation, and categorical variables were reported with number and percentage. For two-group comparison, two-tailed student *t*-test or Mann-Whitney U test was performed, as appropriate. For multiple group comparisons, one-way analysis of variance (ANOVA) or Kruskal-Wallis test with Bonferroni correction was employed. Categorical variables were analyzed using the Chi-squared test or Fisher's exact test. Statistical tests were two-sided, and P values less than 0.05 ($P<0.05$) were considered significant. All data were analyzed using R software version 4.0.2.

Results

Characteristics of the patient population

Characteristics of the 47 NSCLC patients with *KRAS* mutation are displayed in *Table 1* and *Figure 1A*. Their median age was 60 (range, 35–77) years and 21 (44.7%) patients were younger than 60 years. Of these, 24 (51.1%) were male, and 20 (42.6%) were former or current smokers. Across the entire cohort, the vast majority had lung adenocarcinoma (n=46, 97.9%), and only one patient had lung squamous cell carcinoma. According to the 8th AJCC TNM staging system, 66.0% (n=31) of patients were at stage I, 4.3% (n=2) at stage II, 10.6% (n=5) at stage III, and 19.1% (n=9) at stage IV.

TMB data was available for 44 patients (93.6%). Using a cut-off value of 10 mut/Mb, 34 (72.3%) patients were TMB-low (<10 mut/Mb) and 10 (21.3%) were TMB-high (≥10 mut/Mb). Patients with TMB-high were more likely to be male (90.0% *vs.* 35.3%; P=0.0033) and have a smoking history (90.0% *vs.* 26.5%; P=0.0005). Other characteristics including age, tumor stage, histology, and PD-L1 expression, were not significantly different between the TMB-high group and TMB-low group.

Given that the majority of the included patients in this study have operable, early-stage disease at presentation, PD-L1 expression was determined in 14 of the 47 patients. Among them, eight patients had positive PD-L1 expression, and six had negative PD-L1 expression, considering 1% as the cut-off. Moreover, no significant correlations

Table 1 Patient characteristics

Characteristic	Patients (N=47)
Age (years), n (%)	
<60	21 (44.7)
≥60	26 (55.3)
Sex, n (%)	
Male	24 (51.1)
Female	23 (48.9)
Smoking history, n (%)	
Ever	20 (42.6)
Never	27 (57.4)
Tumor stage, n (%)	
I	31 (66.0)
II	2 (4.3)
III	5 (10.6)
IV	9 (19.1)
Histology, n (%)	
Adenocarcinoma	46 (97.9)
Squamous cell carcinoma	1 (2.1)
TMB, n (%)	
<10	34 (72.3)
≥10	10 (21.3)
Unknown	3 (6.4)
PD-L1, n (%)	
<1%	8 (17.0)
≥1%	6 (12.8)
Unknown	33 (70.2)

TMB, tumor mutation burden; PD-L1, programmed cell death-ligand 1.

were found between PD-L1 expression and other clinical characteristics.

Correlation between clinical and molecular features and KRAS mutation status

Typical frequencies of distinct *KRAS* mutation subtypes are shown in *Figure 1B*. Most mutations (n=41, 87.2%) caused a change in the amino acid residue at codon 12 (G12X), and the most common *KRAS* mutation subtype was G12C (n=15), followed by G12D (n=10), G12V (n=10), G12A (n=5), and G12S (n=1). Considering the specific subtype G12C is of particular clinical significance for *KRAS* targeted therapy, we divided patients into two groups according to their mutation subtypes. Patients with *KRAS* G12C mutation were included in the G12C cohort, and others with *KRAS* non-G12C mutations were included in the non-G12C cohort. A higher percentage of patients with a smoking history was observed in the G12C cohort than in the non-G12C cohort (66.7% *vs.* 31.3%; P=0.0299), while there were no significant differences in age, sex, tumor stage, or histology between these two cohorts.

The distribution of *KRAS* concomitant mutations is presented in *Figure 1C*, and the four most common concomitant mutations were *TP53* (n=16, 36.7%), *STK11* (n=8, 16.3%), fat atypical cadherin 2 (*FAT2*) (n=6, 12.2%), and RNA binding motif protein 10 (*RBM10*) (n=6, 12.2%). Among these genes, *TP53*, *STK11*, and *KEAP1* have been reported to be the most common and clinically significant concomitant mutations (16). Given there were only two patients with *KEAP1* mutation in our cohort, we focused our analysis on *TP53* and *STK11* mutations. We classified *TP53* and *STK11* variants according to a five-category somatic variant classification system and found four and twelve *TP53* variants were classified as class 1 and class 2 mutations respectively, and 6 and 2 *STK11* variants were classified as class 1 and class 5 mutations respectively (37). To determine whether these variants are oncogenic/loss of function mutations, we undertook a comprehensive analysis by manually navigating COSMIC, ClinVar, and OncoKB databases. Oncogenic/likely oncogenic variants were determined as oncogenic/likely oncogenic reported by at least one database, and not reported variants were classified as variants not reported by any of the three databases. The results indicated that 15 and 1 *TP53* variants were classified as oncogenic and likely oncogenic mutations respectively, and 6 and 2 *STK11* variants were classified as oncogenic and not reported mutations respectively. Patients harboring *TP53* or *STK11* mutations were more likely to be male (P=0.0305; P=0.004) and former or current smokers (P=0.0134; P=0.0071). In comparison with patients with early-stage (stage I and II) NSCLC, *STK11* mutation was more frequently observed in those with advanced (stage III and IV) disease (P=0.0054).

We subsequently analyzed the relationship between *KRAS* mutation subtypes and the two most heavily studied biomarkers for immunotherapy, TMB and PD-L1 expression. The Kruskal-Wallis test indicated no significant

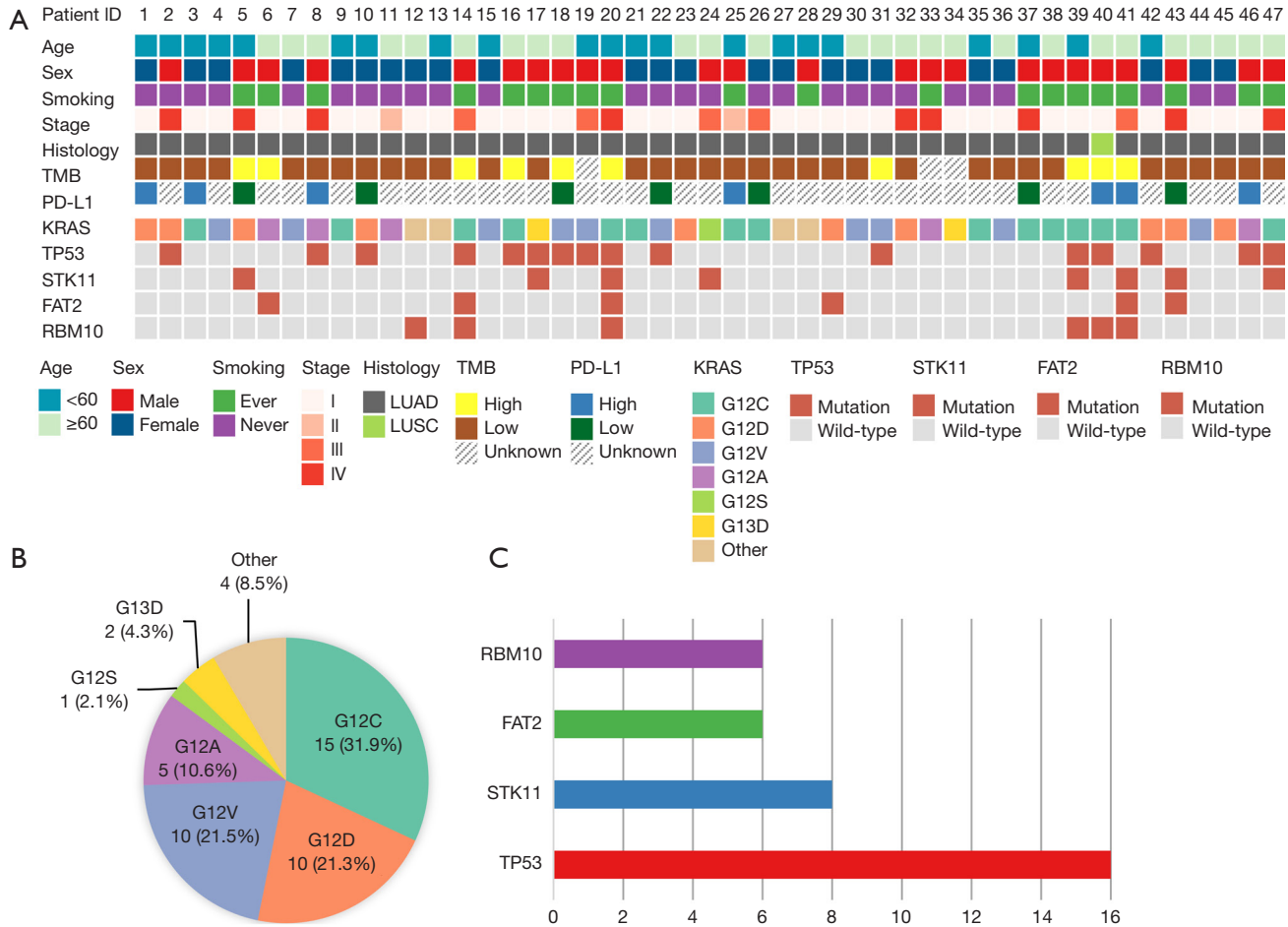

Figure 1 Overall clinical and molecular features of patients with *KRAS* mutant NSCLC. The mutation spectrum and corresponding clinical and molecular features for each patient. (A) Each row represents an individual characteristic, and each column represents an individual patient; (B) pie chart depicts the proportion of each *KRAS* mutation subtype; (C) bar diagram shows the four most common *KRAS* concomitant mutations. TMB, tumor mutation burden; PD-L1, programmed cell death-ligand 1; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; *TP53*, tumor protein p53; *STK11*, serine/threonine kinase 11; *FAT2*, fat atypical cadherin 2; *RBM10*, RNA binding motif protein 10; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer.

difference in TMB value between the four different subtypes of *KRAS* mutation (*Figure 2A*). Similarly, no significant differences in TMB value and PD-L1 expression were observed between the G12C and non-G12C cohort (*Figure 2B,2C*).

To evaluate potential relationships between *KRAS* concomitant mutations and the efficacy of immunotherapy, we also analyzed their association with TMB and PD-L1 expression. It was shown that the presence of *TP53* mutation was associated with higher TMB compared to the *TP53* wild-type counterparts (P=0.0064, *Figure 2D*). Likewise, patients with *STK11* mutation displayed higher TMB compared to those with wild-type *STK11* (P=0.0053, *Figure 2E*). Significant correlations between PD-L1 expression with *TP53* or *STK11* were not seen (P=1, *Figure 2F,2G*), which may be explained by the overall limited number of patients tested for PD-L1 expression.

TCR repertoire metrics and their associations with KRAS mutation status

TCR sequencing was performed on 47 tumors and 37 PBC specimens from 47 patients included in our cohort. The Shannon index in tumor and PBC ranged from 2.6909

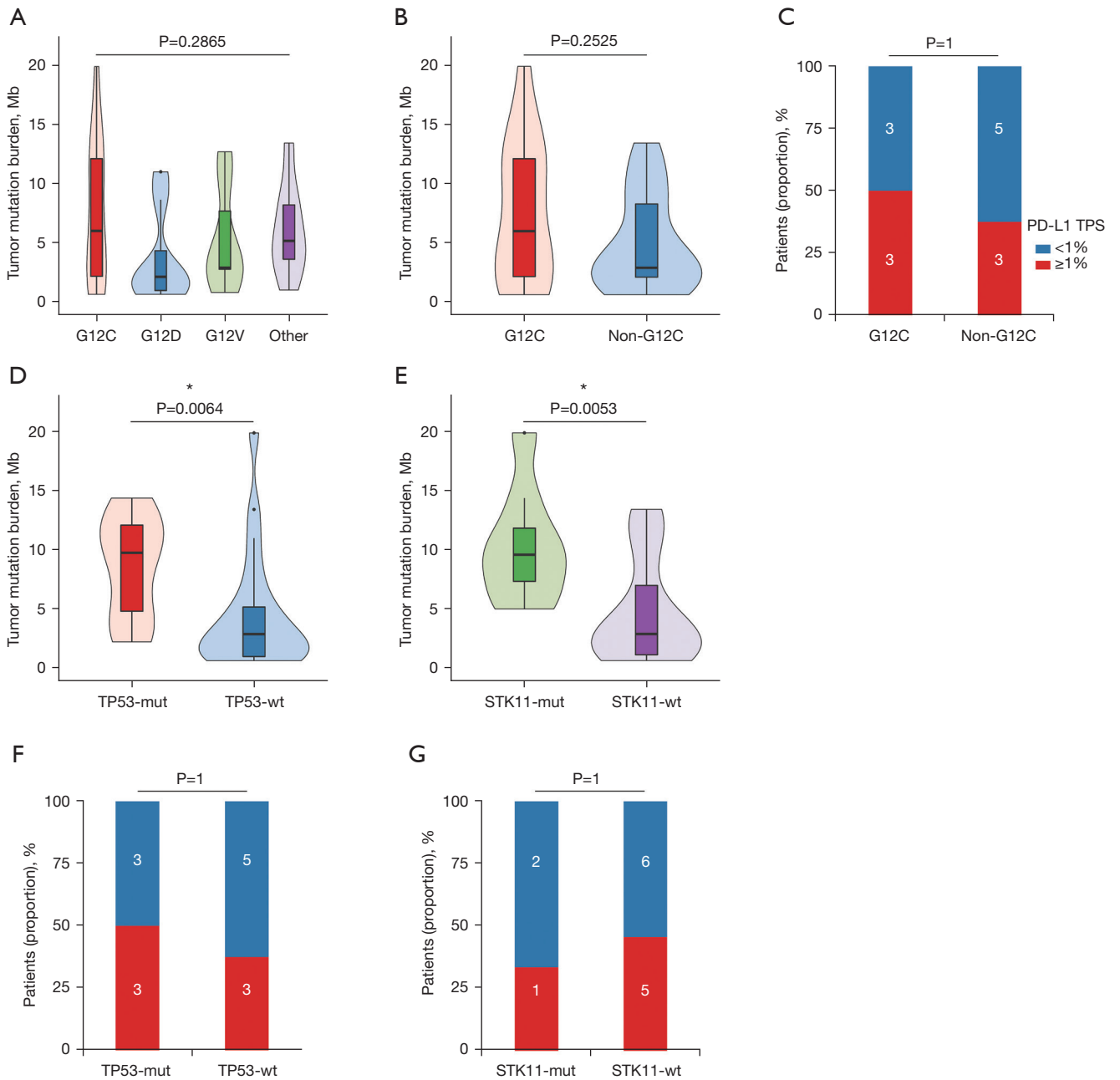

Figure 2 Comparison of tumor mutation burden and PD-L1 expression in patients harboring different *KRAS* mutation status. (A) Tumor mutation burden distribution based on four major mutation subtypes; (B) comparison of tumor mutation burden; (C) PD-L1 expression in *KRAS* G12C mutant versus non-G12C mutant patients; tumor mutation burden distribution based on *TP53* (D) and *STK11* (E) mutation status; PD-L1 expression distribution based on *TP53* (F) and *STK11* (G) mutation status. *, P<0.05. PD-L1, programmed cell death-ligand 1; TPS, tumor proportion score; *TP53*, tumor protein p53; *STK11*, serine/threonine kinase 11; *TP53*-mut, *TP53* mutant; *TP53*-wt, *TP53* wild-type; *STK11*-mut, *STK11* mutant; *STK11*-wt, *STK11* wild-type; *KRAS*, Kirsten rat sarcoma viral oncogene homolog.

to 7.8073 (median, 6.3522) and 2.823 to 9.4832 (median, 7.3313), respectively. Median values of Clonality in tumor and PBC were 0.157 (range, 0.0642–0.526) and 0.1959 (range, 0.0613–0.6382), respectively. The range of MOI values was from 0.002 to 0.7591, and the median MOI was 0.0936. We subsequently evaluated the potential association between TCR repertoire metrics and clinical features, and the results indicated TCR repertoire metrics were independent of age, sex, smoking history, and tumor stage (Figures S1-S4).

T cells use highly diverse TCRs to recognize tumor antigens arising from genetic mutations and promote the renewal and activation of adaptive antitumor immune responses. Therefore, we were particularly interested in exploring the relationship between TCR repertoire metrics and the different *KRAS* mutation subtypes. Our results suggested all TCR repertoire metrics including the Shannon index, Clonality, and MOI showed no significant differences among the major *KRAS* mutation subtypes (*Figure 3A-3E*). Similarly, no significant differences between the G12C cohort and the non-G12C cohort were observed (*Figure 3F-3J*). These findings indicated NSCLC harboring an identical major driver mutation might induce a comparable degree of the breadth and strength of the T cell immune response.

The impact of *KRAS* concomitant mutations on the efficacy of ICIs has been widely reported in NSCLC. To further explore the effect of these concomitant mutations on the antitumor immune response, comparisons of differences in the TCR repertoire metrics between patients with and without specific concomitant mutations were performed. The results showed patients with *KRAS/TP53* comutation had similar Shannon index, Clonality, and MOI with those of wild-type *TP53* (*Figure 4A-4E*). We subsequently performed the same analysis for *STK11* mutant patients, and again, both the Shannon index and Clonality did not differ between patients with *KRAS/STK11* comutation and those with wild-type *STK11* (*Figure 4F-4I*). Intriguingly, patients with *KRAS/STK11* comutation showed significantly higher MOI compared to their *STK11* wild-type counterparts (P=0.012, *Figure 4J*). The higher MOI represented more similarity between tumor tissue and paired peripheral blood, indicating *KRAS/STK11* comutation in NSCLC may induce distinctive immunological features.

Relationship between TCR repertoire metrics and existing biomarkers for immunotherapy

As PD-L1 expression and TMB are commonly used in clinical practice to further screen the dominant population of immunotherapy, we further analyzed the relationship between TMB, PD-L1 expression, and TCR repertoire metrics. These findings demonstrated neither TMB, nor PD-L1 expression were associated with TCR repertoire metrics, including the Shannon index, Clonality, and MOI (Figures S5,S6). Therefore, we speculated TCR repertoire metrics might provide a better understanding of the immunological characteristics of patients with *KRAS* mutation and should be considered complementary to TMB and PD-L1 expression.

Discussion

ICIs have significantly improved the therapeutic landscape of advanced NSCLC. Although both *EGFR* and *KRAS* are frequently mutated driver genes, patients with *KRAS* mutant NSCLC are more likely to benefit from ICIs (38-40). Therefore, it is very important to reveal the underlying immunological features to guide precision medicine in *KRAS* mutant NSCLC. In the current study, we applied high-throughput TCR sequencing of TCR β genes on tumor tissue and paired PBC from patients with *KRAS* mutant NSCLC. Our results revealed TCR repertoire metrics including the Shannon index, Clonality, and MOI showed no significant differences among major *KRAS* mutation subtypes, and the presence of *KRAS/TP53* comutation did not affect TCR repertoire metrics. However, patients with *KRAS/STK11* comutation showed significantly higher MOI compared to their *STK11* wild-type counterparts. Moreover, neither TMB nor PD-L1 expression was associated with TCR repertoire metrics. As far as we know, this is the first study evaluating the characteristics of TCR repertoires in NSCLC patients with different *KRAS* mutation subtypes and concomitant mutations.

KRAS mutations in NSCLC are dominated by single-base missense mutations and localized frequently at codon 12, codon 13, or codon 61 (2,41). It has been suggested that they are heterogeneous in many respects, including intrinsic guanosine triphosphatases activity, the affinity of effectors, and different sensitivities to targeted therapy (42,43). However, it remains unclear whether *KRAS* mutation subtypes affect the efficacy of immunotherapy. In our analysis, no significant differences in TCR repertoire metrics including Shannon index, Clonality, and MOI were found among the major *KRAS* mutation subtypes. Likewise, TMB and PD-L1 expression were independent

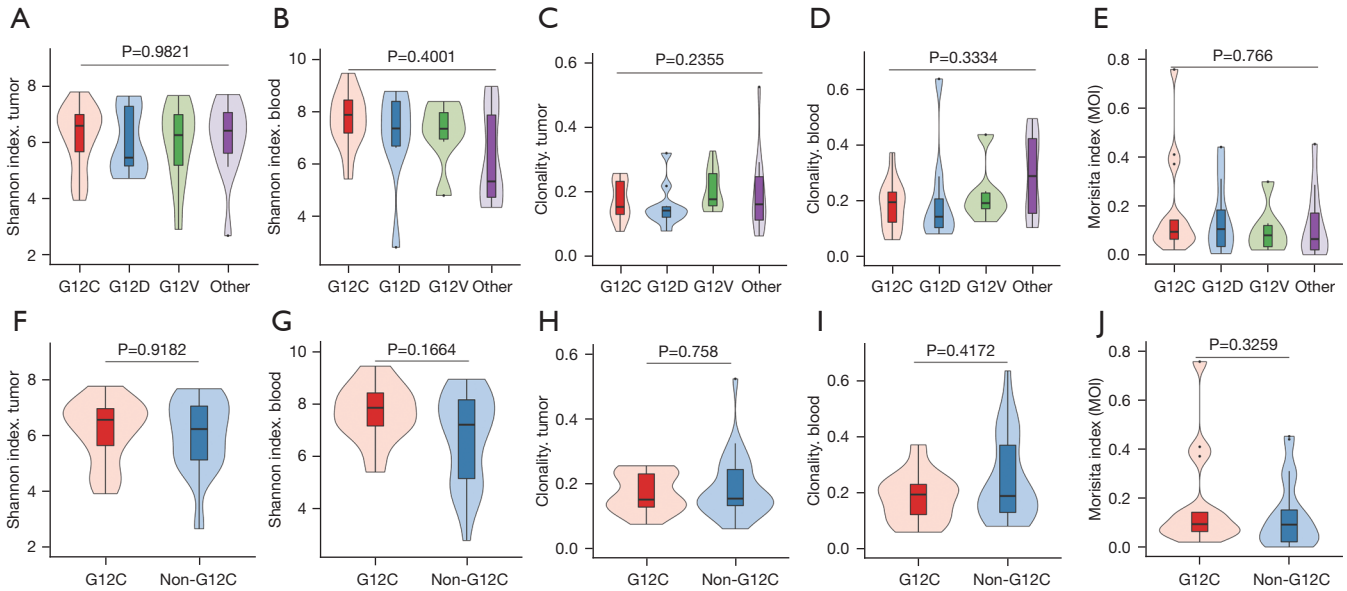

Figure 3 Correlation between TCR repertoire metrics and the status of *KRAS* mutation subtypes. Comparison of TCR repertoire metrics among four major mutation subtypes: (A) Shannon index in tumor; (B) Shannon index in paired PBC; (C) clonality in tumor; (D) clonality in paired PBC; (E) Morisita index. Comparison of TCR repertoire metrics in *KRAS* G12C mutant versus non-G12C mutant patients. (F) Shannon index in tumor; (G) Shannon index in paired PBC; (H) clonality in tumor; (I) clonality in paired PBC; (J) Morisita index. TCR, T cell receptor; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; PBC, peripheral blood cell.

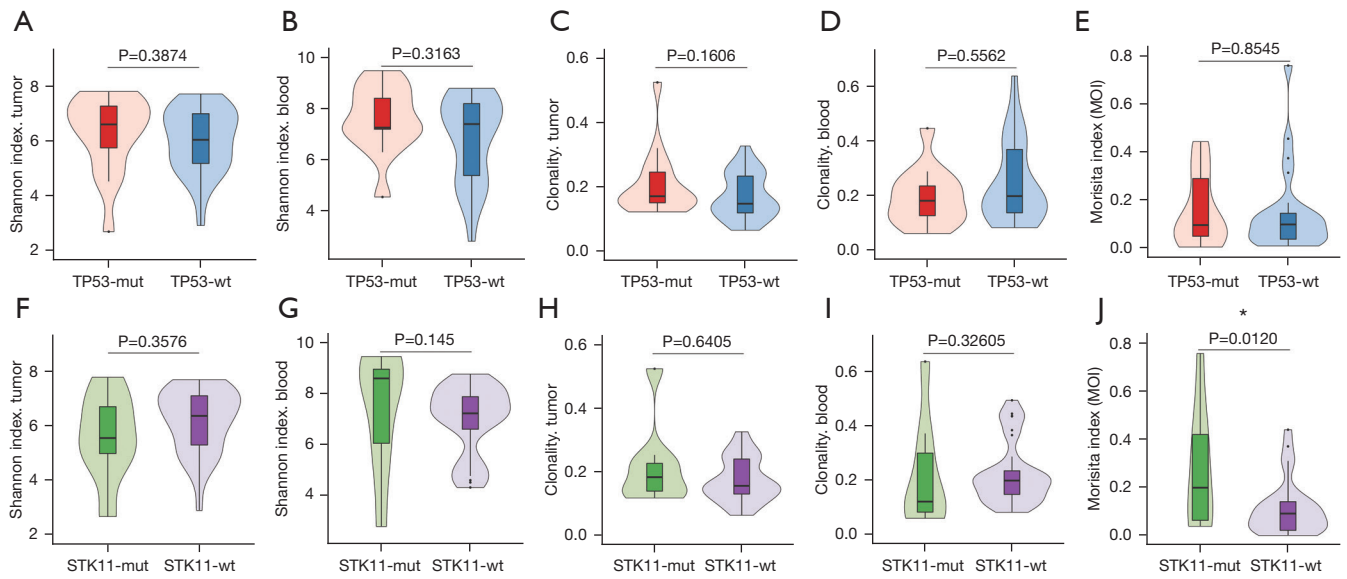

Figure 4 Correlation between TCR repertoire metrics and the status of *KRAS* concomitant mutations. Comparison of TCR metrics according to *TP53* mutation status: (A) Shannon index in tumor; (B) Shannon index in paired PBC; (C) clonality in tumor; (D) clonality in paired PBC; (E) Morisita index. Comparison of TCR metrics according to *STK11* mutation status: (F) Shannon index in tumor; (G) Shannon index in paired PBC; (H) clonality in tumor; (I) clonality in paired PBC; (J) Morisita index. *, P<0.05. *TP53*, tumor protein p53; *STK11*, serine/threonine kinase 11; *TP53*-mut, *TP53* mutant; *TP53*-wt, *TP53* wild-type; *STK11*-mut, *STK11* mutant; *STK11*-wt, *STK11* wild-type; TCR, T cell receptor; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; PBC, peripheral blood cell.

of *KRAS* mutation subtypes. Based on the above results, we speculated patients with different *KRAS* mutation subtypes may share similar overall immunological profiles and obtain comparable benefits from ICIs. A retrospective study suggested major *KRAS* mutation subtypes had no effect on the long-term prognosis of patients with advanced NSCLC who were treated with ICIs (44). Moreover, the IMMUNOTARGET study retrospectively evaluated the clinical efficacy of immunotherapy in 551 patients with NSCLC harboring specific driver mutations. When comparing *KRAS* G12C to other *KRAS* mutations or *KRAS* G12D versus other *KRAS* mutations, progression-free survival was not significantly different regarding *KRAS* mutation subtypes (38).

A growing number of clinical studies have revealed *KRAS* mutant NSCLC with different concomitant mutations could display heterogeneous biological features, immune profiles, and responses to ICIs (19). Of these concomitant mutations, *TP53* and *STK11* are the most investigated and clinically significant genes. Increasing evidence has suggested tumors with *KRAS/TP53* comutation were more likely to be PD-L1 positive and TMB-high (45). Moreover, *KRAS/TP53* comutation showed remarkable effects on facilitating $CD8^+$ T-cell infiltration and augmenting tumor immunogenicity, which may contribute to a probable sensitivity to ICIs (46). However, the associations of *KRAS/TP53* comutation with immunotherapy response in NSCLC remain controversial in clinical practice, and inconsistent results have emerged in different studies (17,45,47). We identified *KRAS/TP53* comutation was correlated with higher TMB in this study, consistent with a previous study (45). However, we did not find TCR repertoire metrics to be correlated with the *KRAS/TP53* comutation. The heterogeneity of the *TP53* mutation and other mechanisms that inactivate the *TP53* pathway may contribute to those results (48,49).

Contrary to *KRAS/TP53* comutation, co-occurring *KRAS/STK11* mutation is associated with primary resistance to ICIs and could even lead to hyperprogression on immunotherapy (20,50,51). Tumors with *KRAS/STK11* comutation are commonly negative for PD-L1 expression and contain abundant regulatory T cells instead of $CD8^+$ T cells (42). Beyond this, Skoulidis *et al.* identified that the *KRAS/STK11* comutation was characterized by a "cold" intratumoral immune microenvironment, despite harboring an intermediate or high TMB (20). Similarly, we also found that tumors with *KRAS/STK11* comutation displayed higher TMB than those with wild-type *STK11*. However, a higher TMB often correlates with a more favorable prognosis of immunotherapy, indicating patients with *KRAS/STK11* comutation may possess unique immunological features (52).

TCR-sequencing results suggested MOI was higher in patients with *KRAS/STK11* comutation, and we speculated this may partially account for the poor efficacy of ICIs in patients with that comutation. MOI measures the overlap of TCR repertoires between tumor tissue and paired peripheral blood. Chen *et al.* recently demonstrated MOI was higher in non-malignant nodules or lung adenocarcinoma tissues with ground-glass opacity components compared with lung adenocarcinoma tissues with solid nodules (53). Therefore, higher MOI might indicate a less active immune environment and lower T-cell expansion in this subgroup. Intriguingly, Zhang *et al.* performed TCR sequencing in patients receiving neoadjuvant PD-1 blockade in the CheckMate 159 trial and demonstrated that tumors with major pathological responses were enriched with migratory T cell clones that had peripherally expanded after treatment (54,55). Several studies using single-cell TCR sequencing have revealed the T cell response to immune checkpoint blockade relies on recruitment of novel and distinct T cell clones delivered from outside the tumor instead of reinvigoration of pre-existing tumor-infiltrating lymphocytes (TILs) that may have limited reinvigoration capacity (56,57). Our research results differed slightly from those reported by Han *et al.* (58). The TCR-based immunotherapy response index established in their study, which reflected the overlap of TCR repertoire between TILs and circulating PD-1^+CD8^+ T cells, showed significant positive correlations with the efficacy of ICIs. The discrepant findings might be attributed to two reasons. First, TCR-sequencing was performed in total PBC rather than in the isolated PD-1^+CD8^+ T cells in our study. Second, most patients enrolled in our study were diagnosed with operable early-stage NSCLC.

In addition to the MOI, several investigations have focused on TCR diversity and Clonality. Patients with higher diversity before the initiation of immunotherapy and more reduced TCR diversity after the first few treatment cycles may experience better prognosis and clinical outcomes (25,26). However, the trend in Clonality was completely the opposite (59-61). In our cohort, no significant differences in TCR diversity, and Clonality correlated to mutation subtypes or concomitant mutations were found, neither in the tumor nor in the blood. It was reported that TCR diversity correlated well with the efficacy of immunotherapy only in patients with *EGFR* and *ALK* wild-type NSCLC, suggesting that the influence of

the mutational landscape on tumor immune profiles cannot be neglected (62).

The efficiency of currently available biomarkers to select patients who are highly responsive to ICIs is still unsatisfactory (63). Our preliminary studies demonstrated TCR repertoire metrics were not associated with TMB or PD-L1 expression. Currently, it is generally accepted that TMB and PD-L1 are independent of each other, and the combination of TMB and PD-L1 expression could better predict the prognosis and response to ICIs in NSCLC (64). Therefore, the addition of the TCR repertoire might further improve the accuracy and effectiveness of the prediction results, particularly in neoadjuvant and adjuvant immunotherapy. Immunotherapy might be a promising approach to potentially reduce recurrence rates and improve survival based on the hypothesis that immune checkpoint inhibitors acts indirectly through modulating the immune system to promote immune recognition and eradicate micrometastases. Results from Checkmate 816, NADIM and IMpower 010 have indicated ICIs have clear utility in resectable NSCLC (65-67). However, more reliable and applicable biomarkers are required to determine the clinical benefit versus their potential risk. The NADIM study has shown that pretreatment tissue TCR repertoire evenness had better performance than TMB and PD-L1 expression in the prediction of complete pathologic responses after neoadjuvant chemoimmunotherapy (68). However, only 56 patients with resectable stage IIIA NSCLC were included, and just a subset of patients underwent T cell receptor sequencing. Thus, there is currently insufficient evidence to draw definitive conclusions on the predictive capability of TCR repertoire. More prospective clinical studies of neoadjuvant immunotherapy are warranted. Moreover, sampling at some specific time points to monitor dynamic changes of TCR repertoire is of great importance for fully evaluating its potential clinical applications.

There were some deficiencies in our study. First, the retrospective nature and limited number of patients potentially caused selection biases, and the results of this study need to be further confirmed by multi-center clinical trials. Second, the antigen-specific TCR repertoire was not assessed in our study. Third, most patients enrolled in this study were diagnosed with early-stage NSCLC and received radical surgery without neoadjuvant or adjuvant treatment. Therefore, prognostic information on immunotherapy was unavailable, which limited our ability to do further analyses. However, previous studies have focused on advanced cancer rather than early-stage disease, indicating our study has a certain value in understanding characteristics of the TCR repertoire and developing individualized therapeutic strategies in *KRAS* mutant operable early-stage NSCLC.

Conclusions

Taken together, this study presented a comprehensive analysis of the TCR repertoire in *KRAS* mutant NSCLC for the first time. Our results suggested *KRAS* mutation subtypes, along with *KRAS*/*TP53* comutation, TMB, and PD-L1 expression were not associated with TCR repertoire metrics. The *KRAS*/*STK11* comutation showed significantly higher MOI, indicating the distinctive immunological features in this subset of patients. The TCR repertoire could provide a new perspective on tumor immunity, and more relevant studies are warranted to validate and further explore its role in tumor immunotherapy.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-629/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-629/coif). YX, MS, and RC are current employees of Geneplus-Beijing Ltd. AP receives honoraria for lectures from OverGroup, AccMed, CongressLAB and Medica Editorial. AR declares consulting fees form AstraZeneca, participation on advisory board (Takeda), supporting for attending to meetings from Thermofisher and BMS. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted following the Declaration of Helsinki (as revised in 2013) and approved by the ethics committee of Peking Union Medical College Hospital (No. S-K1670). Written informed consent was obtained from all participants.

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