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# Characteristics and prognostic significance of profiling the peripheral blood T-cell receptor repertoire in patients with advanced lung cancer

**Running title: Peripheral blood TCR in lung cancer patients**

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**Novelty and Impact:** Characterization of the T-cell receptor (TCR) repertoire by sequencing is a promising method for assessing tumor activity, directing therapy, and predicting prognosis; however, the importance of the TCR repertoire in lung cancer is unclear. In this systematic profiling of the peripheral blood TCR repertoire of advanced lung cancer patients, we found that dynamic change of the TCR repertoire was a good indicator of clinical outcome. This suggests that TCR repertoire analysis may be a useful biomarker to direct immunotherapy.

**Keywords:** T-cell receptor repertoire, lung cancer, immunotherapy, prognosis, high-throughput sequencing

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**Abbreviations:** T-cell receptor (TCR), complementarity determining region 3 (CDR3), immune checkpoint inhibitor (ICB), cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death-1 (PD-1)/programmed cell death-1 ligand (PD-L1), tumor mutational burden (TMB), Durable clinical benefit (DCB), T-cell receptor beta chain (TRB), progression-free survival (PFS), absolute lymphocyte count (ALC), neutrophil/lymphocyte ratio (NLR), lactate dehydrogenase (LDH)

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**Abstract**

Lung cancer is one of the greatest threats to human health, and is initially detected and attacked by the immune system through tumor-reactive T cells. The aim of this study was to determine the basic characteristics and clinical significance of the peripheral blood T-cell receptor (TCR) repertoire in patients with advanced lung cancer. To comprehensively profile the TCR repertoire, high-throughput sequencing was used to identify hypervariable rearrangements of complementarity determining region 3 (CDR3) of the TCR β chain in peripheral blood samples from 64 advanced lung cancer patients and 31 healthy controls. We found that the TCR repertoire differed substantially between lung cancer patients and healthy controls in terms of CDR3 clonotype, diversity, V/J segment usage, and sequence. Specifically, baseline diversity correlated with several clinical characteristics, and high diversity reflected a better immune status. Dynamic detection of the TCR repertoire during anti-cancer treatment was useful for prognosis. Both increased diversity and high overlap rate between the pre- and post-treatment TCR repertoires indicated clinical benefit. Combination of the diversity and overlap rate was used to categorize patients into immune improved or immune worsened groups and demonstrated enhanced prognostic significance. In conclusion, TCR repertoire analysis served as a useful indicator of disease

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development and prognosis in advanced lung cancer and may be utilized to direct future immunotherapy.

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**Introduction**

Lung cancer is the leading cause of cancer-related death worldwide, with an 18% 5-year overall survival rate in the United States.[1] Traditional anti-cancer treatment mainly focus on killing tumor cells directly. With the great success of immunotherapy especially immune checkpoint inhibitor (ICB) targeting cytotoxic T-lymphocyte associated protein 4 (CTLA-4) or programmed cell death-1 (PD-1)/programmed cell death-1 ligand (PD-L1) in improving cancer patients survival, recent cancer related researches pay a large of attention on the anti-cancer role of immune system.[2-4] As essential factors in the anti-cancer immune response, T cells participate in the cancer-cell killing activities of immunotherapy as well as traditional treatments, such as chemotherapy and radiotherapy.[5, 6] Therefore, in-depth analysis of T cells has the potential to provide essential insight for understanding an individual's tumor and immunity.[7]

T cells recognize antigen peptides via specific T-cell receptors (TCRs) expressed on the cell surface. The types of TCRs on each T cell are unique and vary within individuals and populations, permitting immune responses to a diverse range of foreign antigens.[8, 9] The specificity and diversity of TCRs are predominantly derived from the highly variable complementarity determining region 3 (CDR3) and are generated by random rearrangement and junction region mutation of V(D)J regions,

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which are three fragments located in TCR-coding genes.[10] The diversity of the TCR repertoire reflects the diversity of cellular immunity, and several studies have shown that CDR3 diversity is important in cancer diagnosis, therapy, and prognosis.[11-15]

Analyzing TCR diversity not only enhances our understanding of the mechanisms of effective or ineffective anti-cancer immunity but also provides improved direction for anti-cancer treatment.[16-20] Nevertheless, conclusions regarding beneficial aspects of the TCR repertoire are inconsistent and sometimes contradictory.[7, 16-22] Moreover, the existence of differences in TCR patterns between cancers and tissue or blood samples highlight the necessity of additional detailed studies for specific malignancies, at both the baseline and treatment-response levels.[13-15, 20, 22-27]

Our knowledge of the TCR patterns in lung cancer is very limited. The TCR repertoire is heterogeneous, differing between regions within the same tumor in lung cancer and many other malignancies.[13, 15, 24-28] Furthermore, tumor tissue is difficult to obtain and dynamically monitor, especially in patients with advanced disease. The use of blood samples is an attractive alternative to overcome these problems.[19] However, before TCR diversity in blood samples can be used to direct disease monitoring and therapy, we must attain a better understanding of the impact of lung cancer on TCR diversity. Therefore, in the current study, we conducted the first systematic analysis of CDR3 diversity of TCR β chains in blood from lung cancer patients and healthy

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controls to characterize the TCR diversity associated with lung cancer and to determine which aspects of the TCR repertoire may predict clinical prognosis.

## Materials and Methods

### Subject cohorts

Peripheral blood samples were obtained from 64 patients with lung cancer (48 treatment-naïve and 16 relapsed) who were hospitalized for anti-cancer treatment in the Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). Peripheral blood samples were also obtained from 31 healthy controls. Subjects with past or concurrent autoimmune disease or AIDS were excluded from the study. Peripheral blood was collected at the beginning of the first or a new cycle of treatment. One to two additional blood samples were obtained after treatment to assess dynamic changes in 26 patients. Circulating tumor DNA (ctDNA) was used to detect tumor mutational burden (TMB) using plasma from the same blood samples obtained from patients. Clinical information was collected from the hospital's case management system, and the clinical response to treatment was evaluated by physicians according to the Response Evaluation Criteria in Solid Tumors (RECIST). Follow-up data were obtained from hospital records or telephone calls. Durable clinical benefit (DCB) was defined as

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progression-free survival (PFS) beyond 6 months. [20]

The study was conducted in compliance with the Helsinki Declaration of 1975 and was approved by the Institutional Review Board of Tongji Medical College of Huazhong University of Science and Technology. All subjects provided written informed consent.

**High-throughput sequencing of T-cell receptor β genes**

Multiplex PCR amplification of CDR3 of the TCR β chain (TRB) was conducted including PCR1 and PCR2, inclusively and semi-quantitatively. To amplify all possible V(D)J combinations, a set of 32 V forward and 13 J reverse primers was used to perform multiplex PCR1 assays as follows: 1 cycle at 95°C for 15 min, 10 cycles of denaturation at 94°C for 30 s, and 10 cycles of annealing at 60°C for 90 s and extension at 72°C for 30 s. In the second round, PCR2 was performed using universal primers. Sequencing libraries were loaded onto the Illumina XTen System, and reads of 151-bp fragments were obtained.

The CDR3 sequence was defined as the amino acids between the second cysteine of the V region and the conserved phenylalanine of the J region, according to the ImMunoGeneTics (IMGT) V, D, and J gene references. The CDR3 sequences were identified and assigned using the MiXCR software package.[29]

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Shannon's entropy was calculated on the clonal abundance of all productive TCR sequences. The normalized Shannon's entropy (Shannon index) was determined by dividing Shannon's entropy by the natural logarithm of the number of unique productive TCR sequences.[30] Morisita's overlap index was used to determine similarities between samples; the index ranged from 0 to 1, with 0 and 1 representing minimal and maximal similarity, respectively.[16]

**Statistical analysis**

Differences between groups were compared using the Mann-Whitney or Kruskal-Wallis tests. Correlations between variables were analyzed using Spearman's rank test. Relationships between clinical benefit and the TCR repertoire were determined using Fisher's exact test. The log-rank test and Cox proportional hazards regression model were used to compare differences in PFS between groups. All statistical analyses were calculated using R (V3.4.4), GraphPad Prism 7.0, or SPSS 24.0. A two-sided p value $< 0.05$ was considered statistically significant.

## Results

**Subject characteristics**

A total of 124 samples from 48 treatment-naïve patients, 16 individuals who relapsed

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after several lines of therapy, and 31 healthy controls were analyzed in this study. In addition to the baseline samples, follow-up samples were obtained from 26 untreated patients after treatment: one additional sample from 23 patients and two additional samples from three patients. Most patients (95.3%) had advanced-stage disease, and disease stage was uncertain in three patients. Adenocarcinoma was the leading subtype of lung cancer. Anti-cancer treatment included chemotherapy, radiotherapy, tyrosine kinase inhibitor (TKI) therapy (also known as targeted therapy), surgery, and anti-angiogenic therapy. These therapies were often used in combination in individual patients. Detailed patient characteristics and outcomes are summarized in Supporting Information Tables S1 and S2.

**Differences in TCR repertoires between patients and healthy controls**

Diversity of the TCR repertoire can be measured using the Shannon index and the CDR3 clonotype. The stability of these methods for detection of TCR repertoire diversity from blood samples has been previously reported.[12] As diversity is variable by nature, we collected paired blood samples from healthy volunteers at a one-year interval to evaluate alteration of TCR diversity over time. We found that the paired samples showed high coherence in TCR diversity according to the Shannon index (Supporting Information Fig. S1). We then compared TCR diversity between all

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patients with lung cancer (treatment-naïve and relapsed) and healthy controls. Interestingly, lung cancer patients showed significantly lower clonotype number and diversity compared to healthy controls (Supporting Information Figs. S2a and S2b). Further, among lung cancer patients, diversity in the relapsed group was slightly lower than that in the untreated group (Supporting Information Figs. S2c and S2d). Thus, to exclude the impact of previous therapy, we compared the TCR repertoire of only treatment-naïve patients and healthy controls and found that treatment-naïve lung cancer patients displayed reduced TCR diversity compared to healthy controls (Figs. 1a and 1b, Supporting Information Figs. S2e and S2f). Subsequent analyses focused on treatment-naïve patients.

Vβ and Jβ fragments can exhibit preferential usage in both health and disease. Analysis of Vβ and Jβ fragments revealed that 18 Vβ and 2 Jβ fragments exhibited differential usage frequencies between lung cancer patients and healthy controls (Figs. 1c and 1d, Supporting Information Fig. S3). Of these fragments, TRBV5-1 was more frequent in patients with lung cancer, whereas other fragments were more frequent in healthy controls.

The rate of overlap was further used to compare treatment-naïve patients and healthy controls. Although not completely separate, most individuals from the same group clustered together with their respective cohort (Supporting Information Fig. S4).

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These data demonstrate that the TCR repertoire in peripheral blood samples from patients with lung cancer can be clearly differentiated from the repertoire of healthy individuals in several aspects. Moreover, these changes may reflect an altered immune status associated with lung cancer.

**Correlation between the TCR repertoire and clinical characteristics**

We assessed the relationship between CDR3 diversity and several clinical characteristics in treatment-naïve patients. Both clonotype and diversity were similar in males and females; however, diversity was significantly lower in patients 60 years of age or older compared to younger patients (Supporting Information Fig. S5). Accurate information regarding tumor size and metastases was available for 32 and 45 patients, respectively. In these patients, clonotype and diversity were lower when the tumor diameter was larger than 3 cm or the number of metastatic organs was greater than three (Fig. 2). These results suggest that patients with lung cancer exhibit limited TCR repertoire diversity and that this diversity is further limited in patients with advanced age, larger tumors, or more metastatic sites. Interestingly, these factors are often associated with a poor immune status.

To further investigate how peripheral blood diversity reflects immune status in lung cancer, we first evaluated the relationship between absolute lymphocyte count (ALC)

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and CDR3 diversity and found a positive correlation between the two factors (Supporting Information Fig. S6). We then evaluated two systemic inflammation biomarkers, neutrophil/lymphocyte ratio (NLR) and lactate dehydrogenase (LDH), which represent a worsened prognosis and immune status at high levels.[31] We observed significant negative correlations between both clonotype and diversity of CDR3 with NLR as well as LDH (Fig. 3). After categorizing patients using previously described cut-off values[32, 33] for NLR and LDH, we found that both high NLR and LDH groups exhibited lower clonotype and diversity (Supporting Information Fig. S7).

A recent study demonstrated that high NLR and LDH correlated with poor outcomes after ICB treatment in lung cancer.[32] It has also been reported that the efficacy of ICB treatment depends on suitable immune phenotypes.[34, 35] Based on the correlation between CDR3 diversity and NLR or LDH and the usefulness of diversity in immune status assessment, TCR diversity may be a potential biomarker for immunotherapy. Therefore, we further analyzed the relationship between TCR diversity and other immune checkpoint biomarkers. For example, gene mutations are associated with the efficacy of ICB. Although blood TMB was not significantly correlated with diversity in our study (data not shown), clonotype and diversity weakly correlated with the number of gene mutations detected in the blood, as previously suggested (Supporting

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Information Fig. S8).[15] In addition, lower clonotype and diversity were also present in patients with mutated TP53 (Supporting Information Fig. S9), which was reported recently to be a negative predictor for response to ICB treatment.[36]

Taken together, these findings indicate that the TCR repertoire is closely associated with several clinical characteristics, and high repertoire diversity reflects favorable immune status in patients with lung cancer, which may be exploited to direct anti-cancer treatment.

**Prognostic significance of peripheral blood TCR repertoire**

As immune status can be reflected by CDR3 diversity, we continued to explore the prognostic value of the TCR repertoire. TCR clonotype and diversity are often similar, and clonotype is included in diversity. Therefore, we focused on diversity in the next analysis. Unfortunately, baseline diversity of the treatment-naïve group did not demonstrate prognostic significance in terms of DCB or PFS (Supporting Information Fig. S10). To determine whether patients with poor prognosis demonstrated differences in diversity, we identified six patients whose blood samples were collected within 3 months of their death. Although five of these patients did show low diversity, the diversity of one patient (P015) was extremely high (Supporting Information Fig. S11). Interestingly, the first blood sample of one patient (P022) was collected before

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treatment and showed higher diversity than the median, but diversity of the post-treatment sample decreased dramatically, and the patient died quickly thereafter (Supporting Information Fig. S11). This diversity change could be due to the continuous development of the immune system during tumor evolution, which is further modulated by anti-cancer treatment.[7, 37] Indeed, the relapsed group exhibited lower TCR diversity than the untreated group, indicating a weak immune system (Supporting Information Fig. S2d).

Serial blood samples were obtained from 26 patients and included one sample prior to treatment and either one or two samples after treatment. However, simply dividing these patients into increased- or decreased-diversity groups did not account for patients with a poor prognosis (data not shown). For example, in five patients whose second blood sample was collected around the time of disease progression, diversity changes were inconsistent or small in magnitude (Fig. 4, Supporting Information Fig. S12). One of the five patients exhibited a definite increase in TCR diversity, but upon review of clinical information, we found that this patient received a blood transfusion one month before the second blood sample was obtained. Data from the second sample for this patient were thus excluded during subsequent analyses. Recent transfusions did not occur in any of the other patients. In patients with a total of three blood samples, slight TCR diversity change sustained for a prolonged time was

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accompanied by disease progression, whereas an obvious increase in diversity, even after a short period of slight change, suggested disease control (Supporting Information Fig. S13a). A previous study also indicated that peripheral blood CDR3 diversity changes within 10% represent a stable-diversity status and reflect a poor prognosis.[19] Some low frequency T-cell clones may only be detected once even in the same sample, which may introduce bias to the analysis. To determine the effect of low frequency T-cell clones, we collected three blood samples from different individuals and divided each sample into three to extract DNA. Each DNA sample was then analyzed twice, generating six replicates from each of the three individuals. The results demonstrated that diversity did not fluctuate seriously (Supporting Information Fig. S14). A maximum diversity error was calculated for each sample, and the average diversity change was approximately 10% for the three individuals (Supporting Information Fig. S14). These results confirm that diversity change within 10% reflects stable TCR repertoire diversity.[19]

We then compared the prognosis of patients with CDR3 diversity increases greater than 10% to patients with stable or decreased diversity. The sampling intervals were longer than two cycles of anti-cancer treatment, and longer interval pairs of data were selected for patients with three blood samples. In the group where diversity increased more than 10%, all patients obtained DCB, whereas only 63.6% (7/11) in the

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stable-diversity group and 50% (3/6) of patients in the decreased-diversity group exhibited DCB (Fig. 5a). Furthermore, PFS was significantly longer in the increased-diversity group than in the stable- or decreased-diversity group (Fig. 5d, p = 0.032). However, most patients in the stable-diversity group had a favorable prognosis, suggesting that stable diversity may have less clinical utility (Supporting Information Fig. S15).

Although changes in diversity do not consider CDR3 sequence information, this information is not ignored when calculating overlap of paired samples (Supporting Information Fig. S13c). In the three patients with three blood samples, a high overlap rate was consistent with disease control (Supporting Information Fig. S13b). Overlap of paired samples was generally lower in patients with quick progression (0.503 ± 0.168, whereas the median paired-sample overlap of all available data was 0.705) (Fig. 4). When patients were categorized by the median overlap rate, 91.7% (11/12) of patients with a high CDR3 overlap before and after treatment attained DCB, whereas 53.8% (7/13) of patients with a low CDR3 overlap exhibited DCB (Fig. 5b). The difference in PFS between the two groups was also significant (Fig. 5e, p = 0.0002). Next, TCR diversity and paired-sample overlap were combined to divide patients into either improved- or worsened-immune status groups. Patients with increased TCR diversity (>10%) were assigned to the improved-immune status group, and those with

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decreased TCR diversity (>10%) were assigned to the worsened-immune status group. Finally, the median paired-sample overlap rate was used to divide the stable-diversity group into the following two groups: high-overlap patients were assigned to the improved-immune status group, and low-overlap patients were assigned to the worsened-immune status group. DCB was achieved in 100% (11/11) of patients in the improved-immune status group and only in 50% (7/14) of those in the worsened-immune status group (Fig. 5c). Importantly, the difference in PFS between the two groups was highly significant (Fig. 5f, $p = 0.0001$).

To validate the stability of the TCR prognosis method, additional subgroups, uni-variable, and multi-variable analyses related to potential prognostic factors were further conducted (Supporting Information Tables S3 and S4). The results of these analyses were consistent with our previous findings. These data suggest that the combination of paired peripheral blood CDR3 diversity changes and paired-sample overlap provides valuable prognostic information.

## Discussion

It is widely accepted that the immune system is one of the most powerful and complex anti-cancer weapons. T cells are the main component of the anti-cancer immune system, and the T-cell repertoire of an individual can be accurately profiled

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using deep sequencing.

Previous studies showed that results of TCR analysis of tissue or blood samples and cancer types can vary. The interpretation of TCR analysis is further complicated by the fact that both high and low diversity in tumor tissue or blood TCR repertoires can be associated with better prognosis.[17, 20-22] Studies regarding dynamic changes in the TCR repertoire have also reached conflicting conclusions.[16, 19] Together, these inconsistencies highlight the necessity for more precise studies of specific tumor types. To our knowledge, no previous study has systematically analyzed the characteristics and clinical significance of the TCR repertoire in the peripheral blood of patients with advanced lung cancer. Furthermore, results from dynamic measurements may be more accurate due to the heterogeneity of anti-cancer immunity within tumors, which constantly changes over time.[7, 15, 28]

In this study, we analyzed the TCR repertoire of peripheral blood cells from patients with advanced lung cancer and healthy controls. These two groups showed differing TCR repertoires in terms of CDR3 clonotype, diversity, V/J segment usage, and sequence. Compared with healthy individuals, patients with lung cancer had lower CDR3 diversity. Further analysis revealed that CDR3 diversity in patients with a more severe disease state or poorer immune status was substantially lower. These results indicate that defective anti-tumor immunity in lung cancer can be reflected by

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decreased diversity in the peripheral TCR repertoire. Conversely, increased peripheral CDR3 diversity also predicted better clinical outcomes with traditional anti-cancer therapy.

Although studies regarding tumor-reactive T cells have mostly concentrated on tumor infiltrating lymphocytes, the anti-tumor function of peripheral T cells has also received recent attention.[38] Peripheral T cells shuttle back and forth between the tumor and systemic circulation. High diversity of the peripheral TCR repertoire limits the magnitude of immune escape by increasing the possibility of more tumor-specific T cells that are able to control the growth of cancer cells upon entry to the tumor site and recognize the corresponding antigens.[20, 39, 40] Healthy individuals with higher TCR diversity achieve adequate immune surveillance. Patients with low TCR diversity may have a severely impaired immune status, which is a potential negative predictor for ICB response.[20, 34, 35] The potential for patients with low TCR diversity to respond poorly to ICB is supported by the relationship of diversity with LDH, NLR, and TP53 mutation. Although the classic ICB biomarker, PD-L1, expression data was not available, a recent study found a reverse relationship between PD-L1 expression and aberrant p53 status.[41] The lower diversity in patients with a mutated TP53 gene in this study indirectly reflects a possible positive correlation between CDR3 diversity and PD-L1 expression. This correlation also supports the biomarker function of CDR3

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diversity. As both the immune system and tumors evolve constantly, dynamic monitoring over time is likely to improve the prognostic power of TCR diversity.[42, 43] Interestingly, patients with increased diversity after treatment in this study obtained better clinical outcomes due to improved anti-tumor immunity, which plays an important role in prolonged disease control, especially after therapy withdrawal. In contrast, decreased diversity was not conducive to therapy. It is also likely that the stable diversity observed in a subset of patients is a disadvantage, because the evolving tumor is likely not controlled by unchanged immunity.

A limitation of examining diversity is that it does not consider the specific CDR3 sequences. For this reason, the CDR3 overlap rate was used to compare the similarity of TCR clones before and after treatment. We found that patients with greater TCR repertoire similarities obtained better clinical outcomes; however, this does not necessarily contradict the immune evolution described above. A specific immune response is established during the course of tumorigenesis.[44] This part of anti-cancer immunity should be retained to continue tumor control. In addition to this pre-existing immune response, increasing diversity can then allow for the development or expansion of additional tumor-reactive clones to further enhance anti-cancer immunity.[16] An extremely low CDR3 overlap rate may be a result of an adverse effect of treatment, reflecting the possibility that most primary immune cells have been

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killed in anti-cancer treatment and replaced by newly generated cells that are unable to attack the tumor cells as efficiently. Indeed, the majority of patients with significantly increased or decreased TCR diversity also showed high or low CDR3 overlap rates, respectively, suggesting that increasing diversity improves anti-cancer immunity while concurrently maintaining pre-existing anti-tumor clones. Of note, the prognostic significance of changing diversity was slightly superior to that of the overlap rate in these two-part patients. Therefore, from a long-term perspective, increasing diversity is necessary to improve global anti-cancer immunity, but substantial fluctuations in the composition of the clonotype over a short time may not be beneficial. In patients with stable diversity, the prognostic significance of the TCR repertoire can be improved by categorizing the patients according to the overlap rate. Whether the diversity of these patients eventually changes requires further study. It is also possible that differences between individuals or therapies are responsible for the delayed change. Nevertheless, the combination of diversity and overlap provides a more comprehensive reflection of the immune status than either diversity or overlap alone.

In this study, the basic characteristics and clinical significance of the TCR repertoire in lung cancer were analyzed from various aspects. We propose that high baseline TCR diversity could serve as a preliminary predictor of a favorable response to ICB

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treatment, and further dynamic monitoring of the TCR repertoire is necessary to more accurately predict clinical benefits. We are currently conducting a study to verify these hypotheses. Furthermore, immune-related adverse events have recently attracted increasing attention, with serious events impacting patient survival.[45-48] For example, CDR3 diversity after CTLA-4 treatment is associated with toxicity.[21] Therefore, the relationship between the TCR repertoire and adverse events in patients with lung cancer requires exploration in future studies.

In conclusion, we show that the peripheral blood TCR repertoire of patients with advanced lung cancer is significantly different from that of healthy individuals. The peripheral blood TCR repertoire also correlates with several clinical characteristics and patient immune status. Furthermore, changes in the TCR repertoire during anti-cancer treatment may be a useful prognostic indicator and biomarker for future immunotherapy.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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**Figure legends**

**Figure 1. TCR repertoire in treatment-naïve patients with advanced untreated lung cancer and healthy controls.** Clonotype number (a) and diversity (b) of TCR CDR3s were compared between treatment-naïve patients (Pts, black points) and healthy controls (HCs, grey points). 18 V segments (c) and 2 J segments (d) were differentially used between treatment-naïve patients (black points) and HCs (grey points). Statistical analysis was performed using the Mann-Whitney test. Error bars indicate the mean and standard deviation (SD). Boxes depict the interquartile range with the line at the median, and whiskers depict the 5th–95th percentile. *p < 0.05, **p < 0.01, ***p < 0.001 ****p < 0.0001.

**Figure 2. TCR clonotype number and diversity stratified by tumor size and metastases in untreated lung cancer patients.** Comparison of clonotype number (a) and diversity (b) between patients with a primary tumor diameter greater than or less than 3 cm. Comparison of clonotype number (c) and diversity (d) between patients with metastases in more or less than three organs. Statistical analysis was performed using the Mann-Whitney test. Boxes depict the interquartile range with the line at the median, and whiskers depict the 5th–95th percentile. *p < 0.05, **p < 0.01, ***p < 0.001.

**Figure 3. Correlation between TCR repertoire and systemic inflammation biomarkers.** Correlation between neutrophil/lymphocyte ratio (NLR) and clonotype number (a) or diversity (b). Correlation between lactate dehydrogenase (LDH) and clonotype number (c) and diversity (d). Statistical analysis was performed using the Spearman's rank test.

**Figure 4. Dynamic analysis of diversity and overlap before and after treatment.** Blood samples were collected from five patients prior to treatment and around the time of disease progression. TCR diversity of each patient is shown on the left and overlap between pre- and post-treatment samples is depicted on the right. The frequency range (0–1) is divided into 1,000, and blue deepens as the frequency increases. Each point in the right graph represents a unique TCR clone.

**Figure 5. Prognostic significance of dynamic monitoring of the TCR repertoire in advanced lung cancer.** (a) Diversity change before and after anti-cancer treatment in 25 patients with durable clinical benefit (DCB; in blue) or no DCB (in red). A change of less than 10% represents stable diversity and is indicated by the dashed lines. (b) Distribution of patients according to time to progression and overlap of TCR clones

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before and after treatment. The vertical dotted line shows the median overall overlap. The horizontal dotted line divides patients with DCB or no DCB. Hollow dots represent patients with no progression. (c) Patients with increased diversity (>10%) after treatment or with both stable diversity and high overlap were defined as the immune improved group; the remaining patients were defined as the immune worsened group. Correlation of the combination of diversity change and overlap with clinical outcomes. (d) Progression-free survival (PFS) rate differences between patients with significantly increasing (↑) diversity and patients with significantly decreasing (↓) or stable (=) diversity. (e) PFS rate differences between patients with high and low overlap. (f) PFS rate differences between immune improved and immune worsened groups. Statistical analyses were performed using the Fisher's exact test and Log-rank test. HR, hazard ratio.

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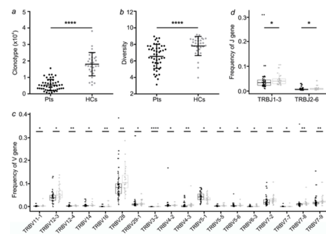


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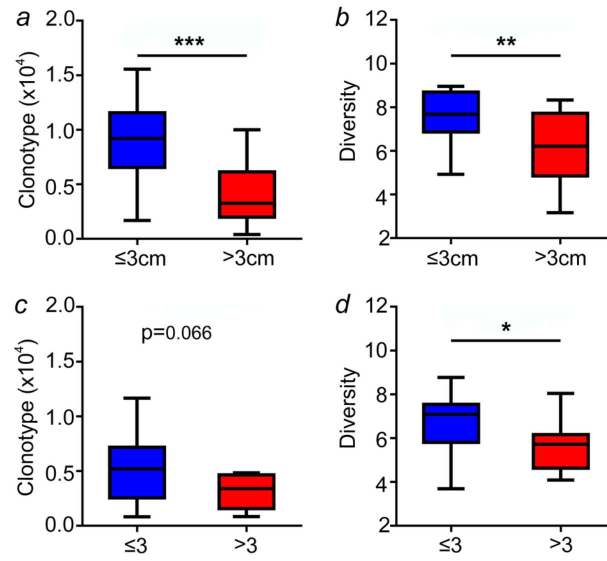


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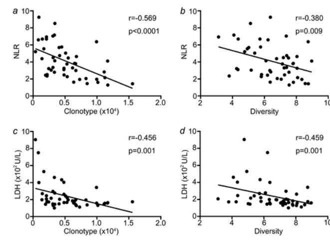


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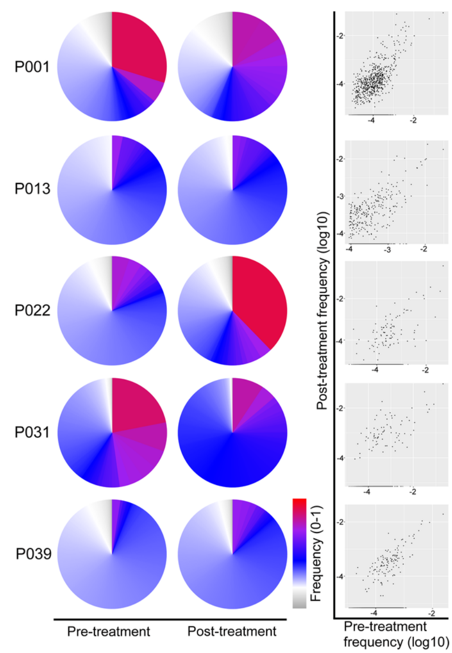


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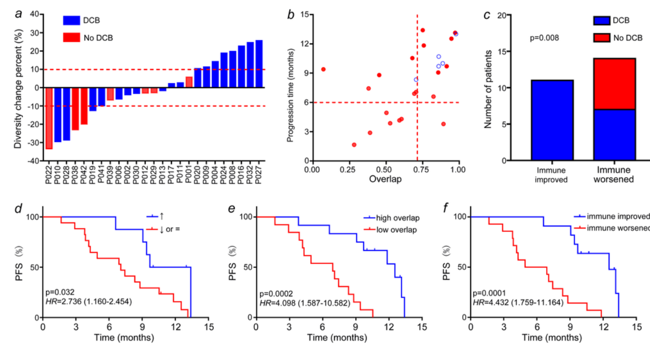


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T cells are essential players in the anti-cancer immune response. Characterization of the T-cell receptor (TCR) repertoire is a promising method for assessing tumor activity, directing therapy, and predicting prognosis; however, the importance of the TCR repertoire in lung cancer is unclear. This sequencing analysis found that the peripheral blood TCR repertoire of patients with advanced lung cancer was significantly different from that of healthy individuals. The peripheral blood TCR repertoire correlated with several clinical characteristics and patient immune status. Dynamic TCR repertoire analysis served as a useful indicator of disease development and may be utilized to direct future immunotherapy.

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