

# Characteristics, dynamic changes, and prognostic significance of TCR repertoire profiling in patients with renal cell carcinoma

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## Abstract

The co-evolving tumour cells and the systemic immune environment are mutually dysregulated. Tumours affect the immune response in a complex manner. For example, although lymphocytes are mobilized in response to tumours, their function is impaired by tumour progression. This study aimed to explore how the baseline and dynamic renal cell carcinoma (RCC) tumour burdens affect the T-cell repertoire, and whether the baseline T-cell receptor  $\beta$ -chain (TCRB) diversity predicts prognosis. To characterise the TCRB repertoire, the baseline and follow-up peripheral TCRB repertoires of 45 patients with RCC and 2 patients with benign renal disease patients were examined using high-throughput TCRB sequencing. To explain the significance of TCRB diversity, 56 peripheral leukocyte samples from 28 patients before and after surgery were subjected to transcriptome sequencing. To validate the results, an advanced RCC patient's sample was subjected to single-cell RNA sequencing (scRNA, 10x Genomics). Higher TCRB diversity was found to be correlated with a higher lymphocyte-to-neutrophil ratio, especially indicating more naïve T cells. High-baseline TCRB diversity predicted a better prognosis for stage IV patients, and different tumour burdens exerted distinct effects on the immune status. The pre-operative TCRB diversity was significantly higher in benign and stage I (low tumour burden) RCC patients than in stage IV (high tumour burden) patients. After the tumour burden of advanced patients was mostly relieved, we observed that the TCRB diversity was restored, T-cell exhaustion was reduced, and naïve T-cells were mobilized. It was demonstrated that the circulating TCRB repertoire could reflect the immune status and predict prognosis, and to some extent that cytoreductive nephrectomy (CN) reduces the burden of the immune system in advanced patients, which might provide a good opportunity for immunotherapy.

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## Introduction

Accumulating evidence suggests that cancer is not only a genetic disorder but also a manifestation of immune dysfunction [1]. Although renal cell carcinoma (RCC) is considered a strongly immunogenic cancer [2], immunosuppression frequently occurs when immune cells are exhausted and inhibited by uncontrolled tumour progression. The dysfunction of T cells, which play an important role in adaptive immunity, is involved in carcinogenesis and tumour progression. A unique T-cell

receptor (TCR) composed of heterodimers ( $\alpha\beta$  or  $\gamma\delta$ ) is expressed on the surface of each T cell [3]. Along with the composition of T cell subsets, the diversity of the TCR repertoire reflects the adaptive immune status. The TCR repertoire is composed of more than 90% TCR $\alpha\beta$ , which recognizes self-antigen peptides and mutated or foreign antigen peptides [4], such as neoantigens on cancer cells. The most abundant TCR  $\beta$  chain (TCRB) comprises variable (V), diversity (D), joining (J), and constant (C) regions. The amount of TCRB diversity arises from the recombination of VDJ regions

and additional nucleotide deletions and/or insertions, specifically in the complementarity-determining regions (CDRs). CDR3 is the primary CDR responsible for recognizing antigen peptides; it straddles the V(D)J junction, composed of the 3'-end of the V region, the whole D region, and a partial J region. Profiling of the TCR repertoire is crucial for understanding the processes of carcinogenesis and cancer progression, especially in tumours with unknown antigenic triggers [5]. Several studies have suggested that TCR diversity might be a good biomarker for therapeutic monitoring and prognostic assessment [6–8].

However, the relationship between TCR diversity and prognosis is unclear. Several studies have shown that high infiltrating or circulating TCRB diversity predicts better prognosis in cervical and lung cancer [6,8], whereas low infiltrating TCRB diversity predicts better prognosis in bladder cancer [9]. These contradictory results highlight the need for further studies to confirm if the connection between TCR diversity and prognosis is cancer specific. Therefore, a precise method for TCR repertoire construction and sequencing is required. Currently, the construction of a TCR library for sequencing utilizes multiplex polymerase chain reaction (PCR) with multiple V and J gene primers based on genomic DNA [10] or complementary DNA (cDNA) [11]. Although these systems are considered bias-controlled during the matching of multiple primers for V and J genes, mismatch induced by polymerase chain reaction (PCR) amplification is inevitable. In 2014, Chudakov *et al* [12,13] reported a Switch Mechanism At the 5'-end of RNA Templates (SMART)-based error-free immune repertoires detection method, which was adopted by many commercial VDJ kits (e.g., SMARTer Human scTCR a/b Profiling Kit of TaKaRa) afterwards. This method uses the unique molecular index (UMI)-inserted template-switching oligo (TSO) to correct the amplification-induced mismatch and quantitative bias. We modified this method by introducing two universal TCR C gene primers to guarantee the specificity during the reverse transcription and the PCR amplification, and by simplifying the sequencing library construction steps.

Tumours exert pressure on the immune response, mobilizing lymphocytes while impairing their function. However, little is known about alterations in the repertoire of responding T cells and at what threshold the tumour load begins to impact the TCR repertoire. Furthermore, to explore the effects of tumours on TCRB diversity, we assessed the tumour burden by the tumour-node-metastasis (TNM) stage according to the American Joint Committee on Cancer staging system and tumour burden changes following nephron-sparing surgery, radical nephrectomy, or cytoreductive nephrectomy (CN). In the era of targeted therapy, the treatment of synchronous metastatic RCC patients with CN is controversial. We first evaluated the influence of CN by examining alterations in TCR diversity and immune cell subtype activity. Compared with tumour-infiltrating lymphocytes, peripheral T cells are not only easily obtained but also fundamental to sustaining adult human naïve T cells [14]. Although the intratumour heterogeneity of

the infiltrating TCRB repertoire in RCC has been reported [10], the characteristics of the circulating TCRB repertoire, especially the dynamic changes that occur after surgery, are unclear.

This study is the first to profile the baseline and dynamic characteristics of the peripheral TCRB repertoire in patients with RCC treated with surgery or targeted therapy and to estimate the prognostic value using a SMART-based UMI-corrected TCR library.

## Materials and methods

Details of the methods used are provided in supplementary material, Supplementary materials and methods.

Eighty-seven samples of peripheral blood leukocytes were obtained from 45 patients with RCC and 2 patients with benign kidney disease, after which the RNA was extracted, and the SMART-based UMI-corrected TCRB libraries for sequencing were constructed. Among these patients, 40 of the 42 treatment-naïve patients with different tumour burdens had paired pre- and post-operative (seventh day after surgery) peripheral TCRB sequencing data and were grouped to analyse the effect of surgery (alteration of the tumour burden) on the immune status. This group is hereafter referred to as the surgery-associated cohort. Baseline TCRB sequencing data were obtained from 18 advanced RCC patients enrolled in this study and were used to predict prognosis. The detailed demographic information of the enrolled patients is shown in supplementary material, Table S1. The use of human samples and the experiments in the study were approved by the ethics committee of the Cancer Hospital of the Chinese Academy of Medical Sciences (CAMS, approval number NCC2016XQ-22). In addition, the patients were informed of the study and thoroughly understood the research.

The proportion of immune cells was calculated by deconvoluting the bulk RNA-seq data and validated by flow cytometry and single-cell RNA sequencing (scRNA) data. Data were analysed by the MIGEC, MiXCR, and CellRanger software programmes and the R packages 'tcr', 'GSVA', 'CIBERSORT', and 'Seurat'.

The raw TCRB repertoire and transcriptome sequencing data generated in this study are deposited in the Genome Sequence Archive [15] at the BIG Data Center [16], Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under the accession number HRA000049 [<http://bigd.big.ac.cn/gsa-human>]. The bulk data and the raw scRNA sequencing data (not uploaded) are also available from the corresponding authors upon reasonable request.

## Results

### TCRB repertoire information of enrolled patients

To minimize PCR amplification-induced bias and error, universal primers containing UMIs were used rather than

multiplexed primers. All reads are grouped by their UMI, after which read groups with at least four to six reads (depends on the sequencing depth of each sample) are assembled. For the 80 samples in the surgery-associated cohort, a total of 10 072 661 TCRB clonotypes were detected, which included  $115\,428 \pm 62\,953$  (mean  $\pm$  standard deviation, SD) TCRB clonotypes per sample in the pre-operative TCRB repertoire and  $136\,388 \pm 48\,815$  (mean  $\pm$  SD) TCRB clonotypes in the post-operative TCRB repertoire. All 64 V genes and 13 J genes were detected. The detailed quality control result of the SMART-based UMI-corrected TCRB sequencing platform developed in house is shown in supplementary material, Figure S1. It is feasible to quantify the TCRB clonotype abundance by this method.

### The immune status is reflected by the baseline TCRB diversity

It is unclear whether high or low TCR diversity corresponds to a better immune status. We herein defined the immune status as the physiological homeostasis maintained by the immune system. To investigate how the TCRB diversity reflects the immune status in the baseline samples, we evaluated the relationships among the Malta's stemness index [17], immunophenotypic scores obtained from bulk RNA-seq data analysis, and the TCRB diversity index (represented by the Efron–Thisted estimator) of 28 pre-operative treatment-naïve patients.

We observed that a higher peripheral blood stemness index was correlated with higher TCRB diversity (Pearson correlation analysis,  $R = 0.54$ ,  $P = 0.0027$ , Figure 1A), which indicates that a high TCRB diversity might be related to the diverse naïve T cells, which are characterised with multipotent differentiation potential and proliferative capacity. The gene set enrichment analysis (GSEA) scores of the *c7* gene sets from Molecular Signatures Database also revealed that wealthy TCRB diversity is associated with high numbers of T cells, especially CD4- and CD8-positive naïve T cells, whereas more neutrophils or pro-tumour Treg cells appeared when TCRB decreased in the peripheral blood (Figure 1B). Consistently, genes that were positively correlated with TCRB diversity were clustered into 162 gene ontology (GO) terms, and the top 20 significant terms were found to be related to biosynthesis and metabolic process. Especially, the genes in the top five terms are mostly ribosomal genes, which were reported to be highly expressed in naïve T cells [18] (Figure 1C). Furthermore, some terms associated with T-cell selection and development were also included in the enriched GO terms (supplementary material, Table S2). In addition, the scRNA sequencing data suggested that TCR clonotype of naïve T cells is unique, but TCR clonotype of effector T cells was expanded (detail results are shown below). These results suggest that a higher TCRB diversity may accord with a relatively more powerful anti-tumour potential given that diverse naïve T cells

are the reserve force for identifying neoantigens on tumour cells.

Conversely, genes that were negatively correlated with TCRB diversity were enriched in the inflammatory response and coagulation process, especially in the activation of neutrophil and platelet (Figure 1C), which have been reported as risk factors for worse prognosis by the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) [19] and supporters serve to circulating tumour cells survive [20]. In addition, the TCRB diversity and neutrophil/lymphocyte ratio (NLR) were negatively correlated (Pearson's correlation analysis,  $R = -0.57$ ,  $P = 0.0019$ , Figure 1D); the NLR predicts a poor prognosis [21].

In summary, patients with low TCRB diversity may have severe inflammatory response and relatively hypercoagulable state; conversely, high TCRB diversity indicates a homeostatically balanced immune system armed with abundant naïve T cells. Accordingly, it is possible that the difference in TCR diversity is correlated with their tumour burden.

### Differences in the baseline immune statuses of patients with different tumour burdens

Although tumours with antigenic characteristics could recruit T cells, the accumulated T cells have defective killing functions, termed tumour-induced T-cell dysfunction [22]. The tumour burden at different clinical stages may have a distinct immunogenic effect on the host. The circulating T-cell repertoire is a fundamental source for recruitment [23]. Thus to explore the pressures exerted by different tumour burdens on the immune system, we profiled and compared the baseline circulating TCRB diversities and the functional statuses of various immune cell subtypes in treatment-naïve RCC patients with different tumour burdens in the surgery-associated cohort.

Four types of indicators for T-cell diversity unanimously represented pre-operative TCRB diversity in benign and stage IRCC patients, which was significantly more abundant than that in stage IV patients. For instance, the counts of all observed clonotypes in stage I patients were higher than those in stage IV patients (Wilcoxon test,  $P = 0.0057$ , Figure 2A,B). Furthermore, the total proportion of the top 25 000 clonotypes in stage I patients was lower than that in stage IV patients (Wilcoxon test,  $P = 0.025$ , Figure 2C,D), and the TCRB diversity estimated by Efron–Thisted and Chao1 in stage I patients was consistently higher than that in stage IV patients (Wilcoxon test,  $P = 0.0011$  and  $P = 0.0014$ , respectively, Figure 2E and supplementary material, Figure S2A). In addition, the pre-operative TCRB diversity estimated by the Shannon–Wiener index showed a gradually decreasing trend from stages I to IV, but the differences were not significant (supplementary material, Figure S2A). Overall, the pre-operative TCRB diversity of stage IRCC patients was significantly higher than that of stage IV patients. However, for the post-operative TCRB diversity, there were no statistically

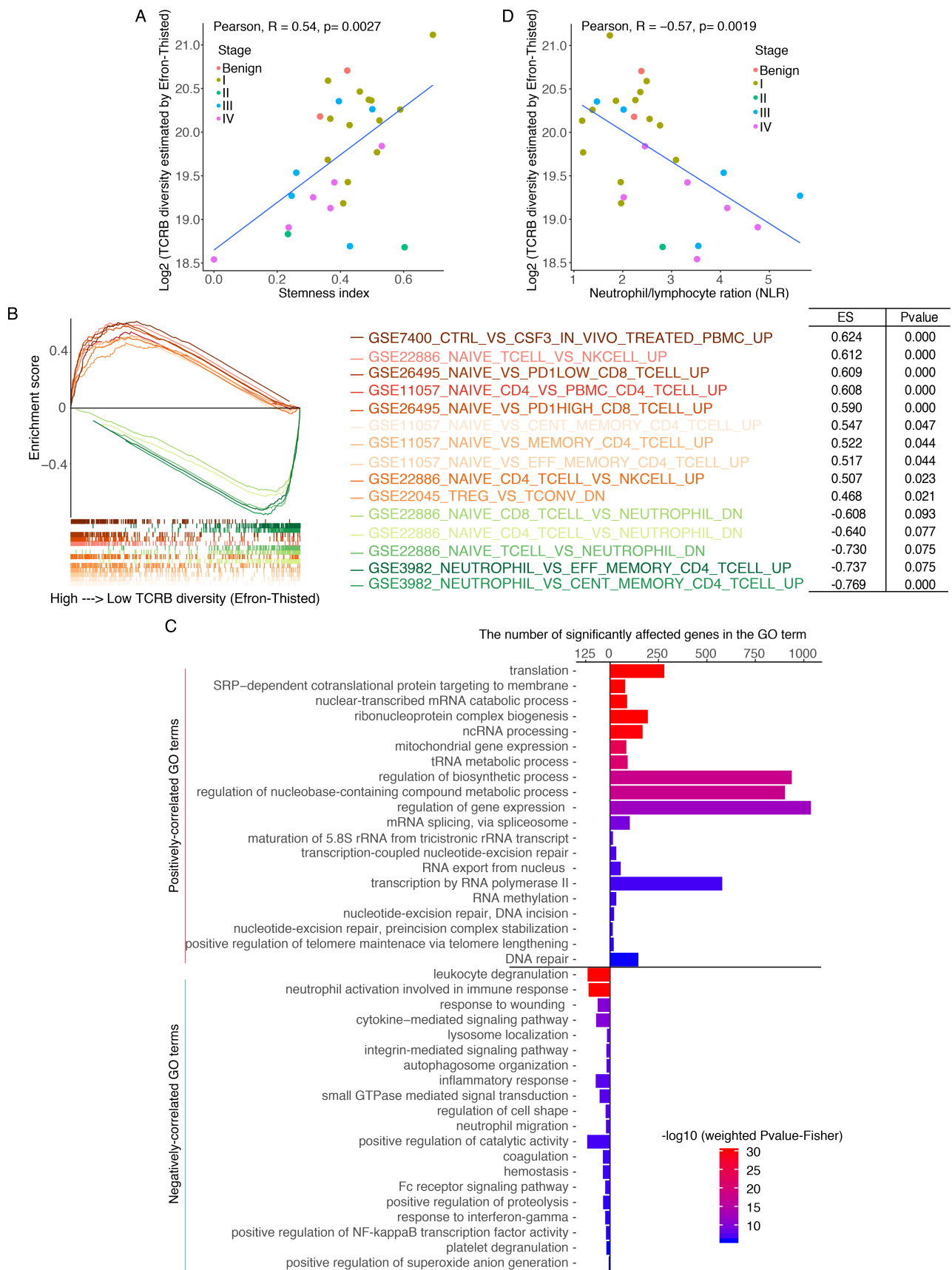


Figure 1. Correlation between basic TCRB diversity and other indexes. (A) The relationship between TCRB diversity and stemness indexes. (B) Correlation between the GSEA scores of c7 immune terms and TCRB diversity. Multiple significant GSEA mountain plots were merged into a single plot, with the orange lines indicating positively correlated terms and the green lines representing negatively correlated terms. Each term was attached to an enrichment score (ES) and a Pvalue. (C) Enriched GO terms for genes correlated with TCRB diversity. The plot includes the top 20 positive and top 20 negative GO terms according to the weighted Fisher's P value. (D) Relationship between TCRB diversity and the NLR.





could predict the prognosis of local RCC (stage I–III). Furthermore, to confirm this speculation, implementation of a large-scale, rigorous trial for peripheral TCRB repertoire sequencing in RCC patients is necessary.

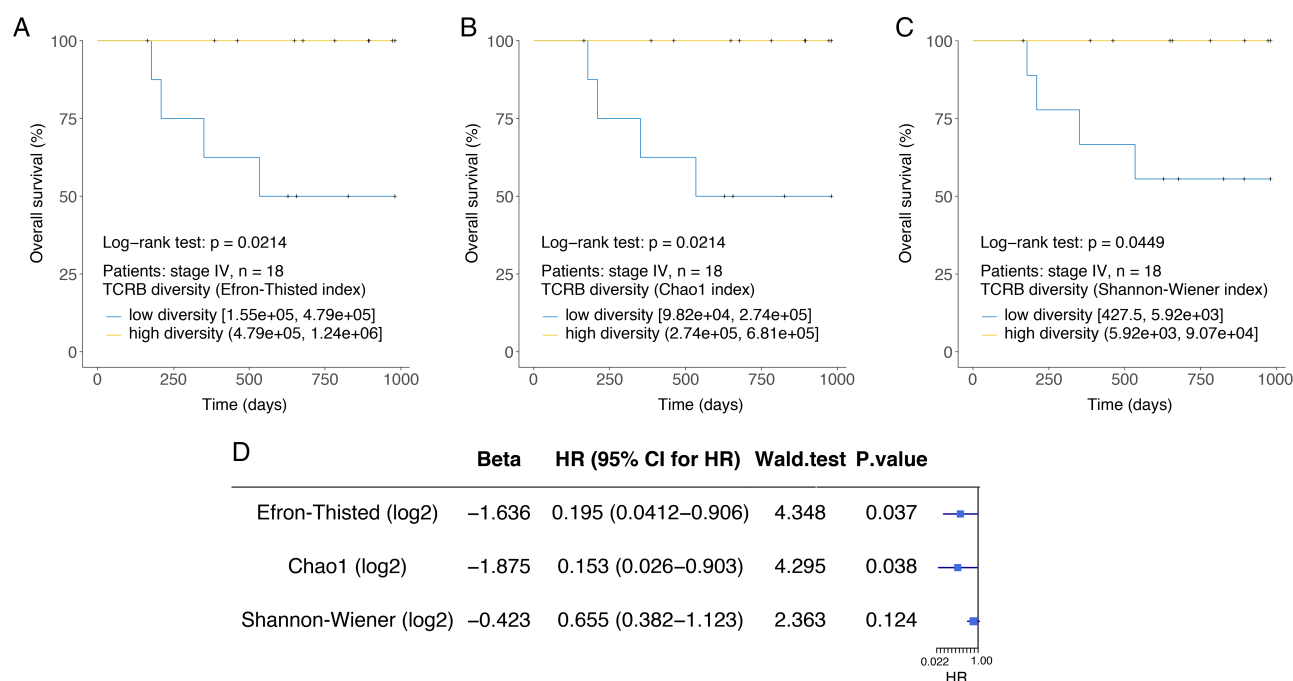
### The reduced tumour burden restores the immune statuses of patients with advanced RCC

Next, to investigate the impact of tumour burden alterations on the TCR repertoire and global immune status, we compared alterations in paired circulating TCRB diversity levels to the activities of immune cell subsets before and after surgery. Different diversity indexes consistently indicated that the TCRB diversity in stage IV patients significantly increased after most of the tumour burden (immunogenicity) was relieved by surgery. For example, the number of observed TCRB clonotypes increased after surgery (paired Wilcoxon test,  $P = 0.0039$ , Figure 4A), and the TCRB diversity indexes (Efron–Thisted, Chao1, and Shannon–Wiener) also consistently increased after surgery (paired Wilcoxon test,  $P = 0.0039$ ,  $P = 0.0039$ , and  $P = 0.0067$ , Figure 4B and supplementary material, Figure S3) in stage IV patients. Similarly, the stemness indexes indicated more stem naïve T cells after surgery in stage IV patients (Figure 4C). The immunophenotypic scores of immune cell subsets estimated by bulk RNA-seq showed similar results, as exhausted T-cell numbers were reduced (Figure 4E). These findings indicated that the TCR repertoire was restored when the overwhelming number of immunogens was removed by CN in stage IV patients.

To validate these results, another stage III RCC patient with inferior vena cava tumour thrombosis was

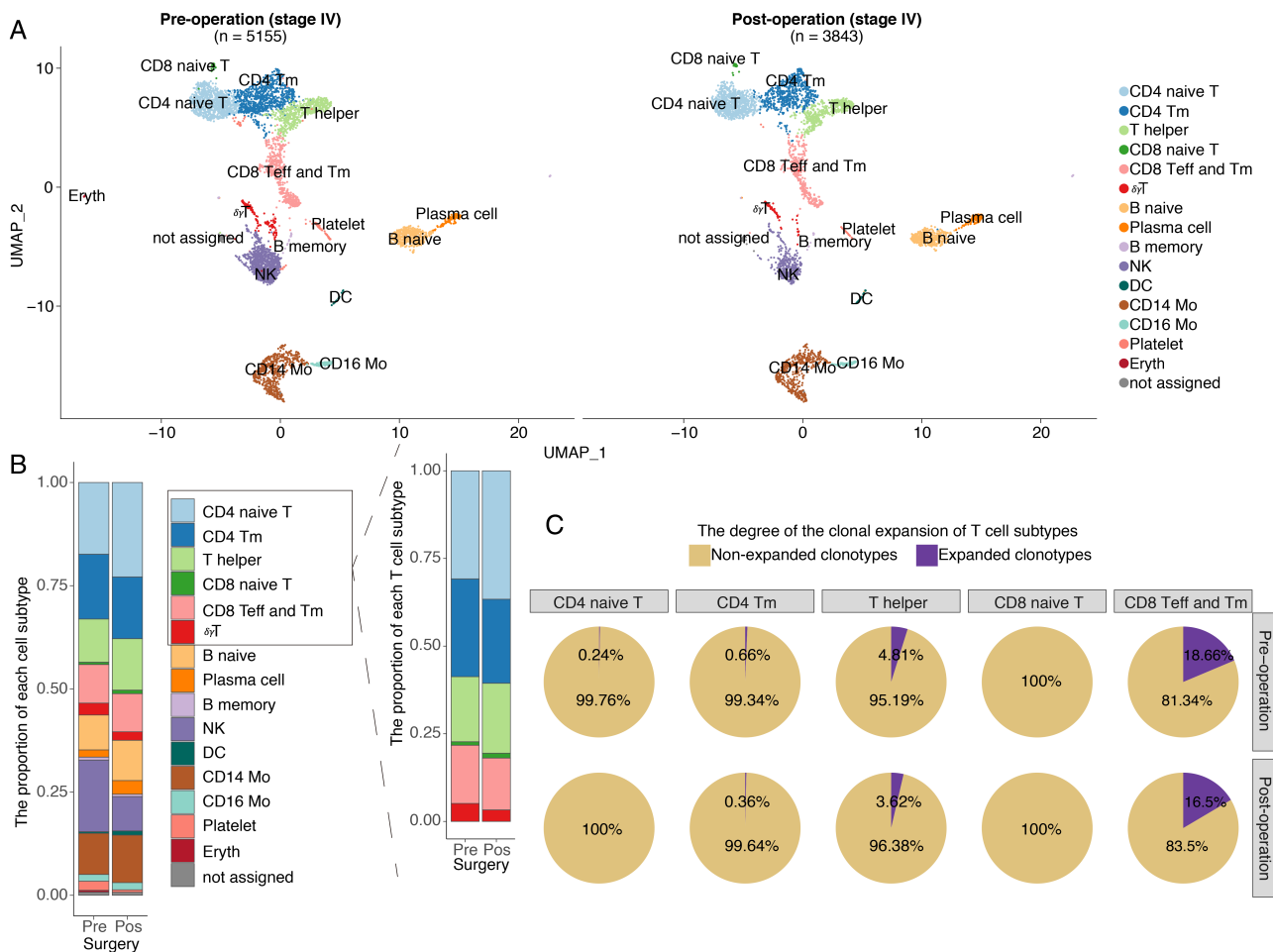
enrolled to analyse the changes in exhausted T cells before and after surgery. Our results suggested that the proportions of PD-1- and TIM3-positive cells among CD8-positive T cells were decreased after surgery (supplementary material, Figure S4E, F). In addition, the immune repertoire and gene-expression profiling detected by scRNA-seq ( $n = 8998$ , details available in the supplementary materials and methods) of a treatment-naïve stage IV patient indicated that the proportions of CD4- and CD8-positive naïve T cells were increased after CN (Figure 5A, B). The key feature markers used to confirm the identified clusters are shown in supplementary material (Figure S5). Integrated with the corresponding single-cell immune repertoire data, we confirmed the high proportion of clonal expansion (defined as more than one T cells shared an identical  $\alpha$ - $\beta$  TCR pair) in the helper T cells and effector T cells and the high clonal diversity of naïve T cells (Figure 5C).

TCRB diversity was, in addition, positively correlated with CD4 naïve T cells (estimated by deconvolution of the RNA-seq data, the model for estimating CD8 naïve T cells was not provided) (supplementary material, Figure S6), which indicated that higher TCRB diversity was associated with more naïve T cells. The increased TCRB diversity in stage IV patients after removal of the primary tumour with CN was potentially attributed to tumour-associated antigens being released into the blood upon the destruction of tumour cells during surgery, and to the fact that more naïve T cells were mobilized to enhance the probability of recognizing the newly released antigens, which further initiate an immune response.



**Figure 3.** Higher baseline TCRB diversity is associated with better prognosis of in stage IV patients. Kaplan–Meier plots of the overall survival of patients with stage IV RCC (A–C,  $n = 18$ ) stratified by the baseline TCRB diversity index. Cox regression analysis for overall survival of patients with stage IV RCC (D).





**Figure 5.** The proportion of naïve T cells increased after CN as determined by scRNA. scRNA data from a validated stage IV treatment-naïve patient are shown. (A) Pre-operative peripheral blood mononuclear cell (PBMC, n = 5155 cells) and post-operative PBMC (n = 3843 cells) samples from a validated stage IV treatment-naïve RCC patient are shown. Each dot represents a cell. Clusters were identified by principal component analysis and visualized with uniform manifold approximation and projection (UMAP). CD4 naive T: CD4 naive T cells; CD4 Tm: CD4-positive memory T cells; T helper: T helper cells; CD8 naive T: CD8 naive T cells; CD8 Teff and Tm: a cluster including CD8-positive effector T cells and memory T cells;  $\gamma\delta$ T: T cells with TCR $\gamma$  and  $\delta$  chains; B naive: naïve B cells; Plasma cell: plasma cells; B memory: memory B cells; NK: nature killer cells; DC: dendritic cell; CD14 Mo: classic monocyte; CD16 Mo: monocyte expressing CD16; Platelet: platelet; Eryth: erythrocyte; not assigned: cells were not assigned. (B) The stack bar plot shows the proportions of all subtypes in all peripheral blood and T cell populations. (C) Pre-operative (n = 2592) and post-operative (n = 2208)  $\alpha\beta$ T cells are shown. The pie chart represents the distribution of TCRB clonotypes with different abundances in different T-cell subtypes. A clonotype that is detected more than once is defined as a clonotype that has undergone clonal expansion. Clonal expansion occurs in the memory and effector T cells and the naïve T cells exhibited high clonal diversity.

IV patients after surgery. To further confirm whether this pattern existed universally in all persistent clonotypes, we assessed alterations in all persistent clonotypes among the 40 patients. We defined persistent clonotypes with a fold change of no more than 1.2 (post- to pre-operative concentrations) as stable clonotypes; conversely, clonotypes with a fold increase of more than 1.2 were defined as increased, and the rest clonotypes were defined as decreased. Examples are shown in Figure 6F. We found that the proportion of decreased clonotypes was greater than that of increased clonotypes in stage IV patients, which was distinct from the observed trend in stage I patients (Wilcoxon test,  $P = 0.013$ , Figure 6G). However, no differences in the proportions of stable clonotypes were observed among the different stages (Figure 6G). Moreover, the mean concentrations of both the persistent and emerging

clonotypes were significantly decreased in stage IV patients after surgery (paired Wilcoxon test,  $P = 0.0051$  and  $P = 0.018$ , respectively, Figure 6H).

Overall, after the immunogenic burden was reduced by surgery, the concentration of persistent clonotypes (high abundance) in stage IV patients decreased, and the proportion and number of emerging clonotypes (moderate and low abundance) in stage IV patients increased, which was accompanied by decreases in the concentration of each clonotype.

## Discussion

The treatment of synchronous metastatic RCC patients with CN is controversial. During the era of cytokine



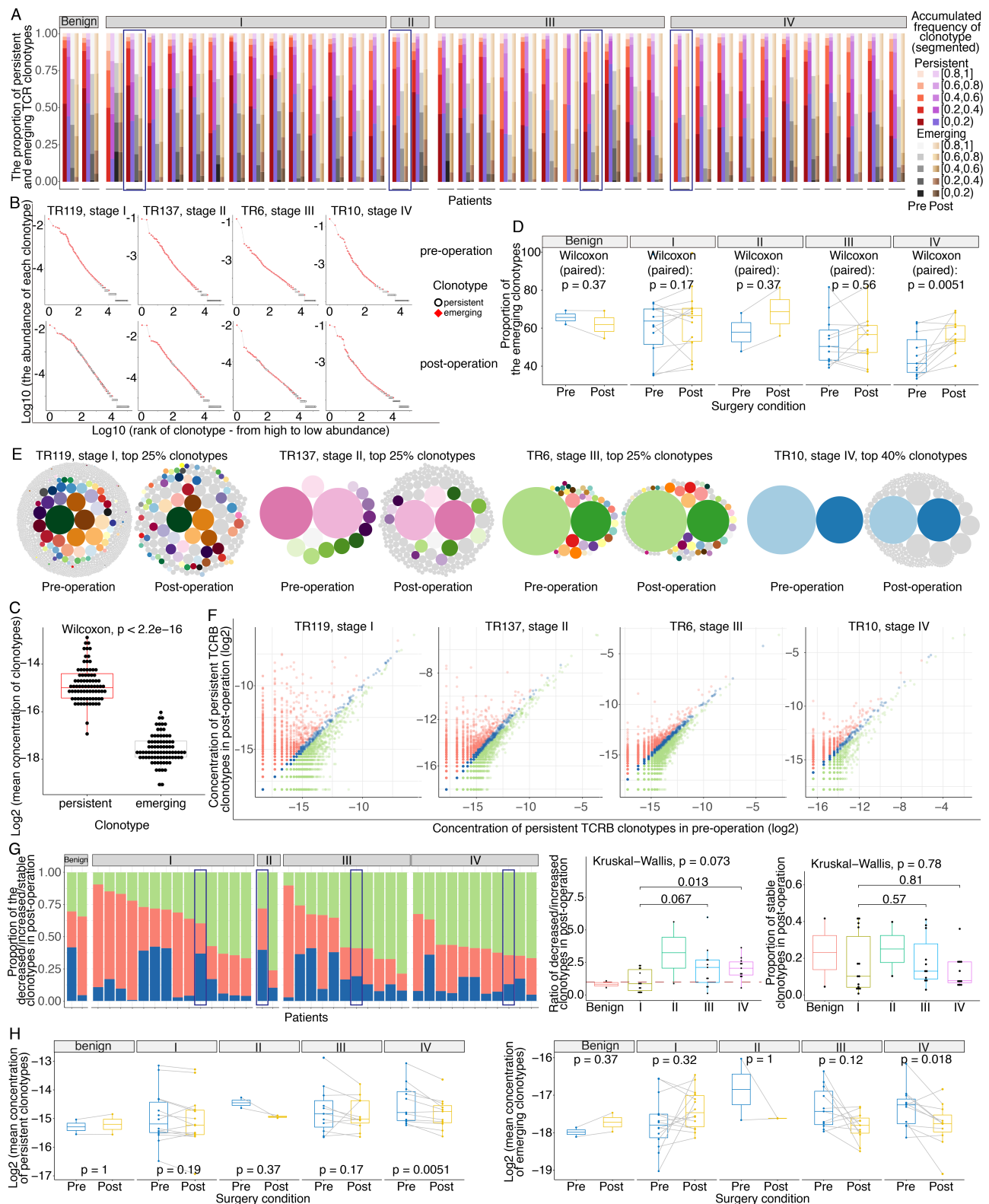


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therapy for advanced RCC, two convincing prospective randomized studies demonstrated that CN followed by cytokine therapy provided a better outcome than interferon alone [24,25]. Upon entering the era of targeted therapy, the value of CN has become controversial. One retrospective study of IMDC demonstrated that patients

treated with CN had improved IMDC prognostic profiles compared to those of patients who were not treated with CN [19]. However, another surprising newly published prospective study concluded that sunitinib alone was not inferior to CN followed by sunitinib for patients with advanced RCC [26].

To explore how tumour burden and its dynamic changes shape the global immune status of RCC patients, and whether CN is critical for improving the immune status in advanced patients, we profiled the characteristics and dynamic changes of the TCRB repertoire and the activity of RNA-seq-derived immune cell populations during tumour burden eradication or alteration by nephron-sparing or radical nephrectomy and CN. We found that the TCRB diversity was much lower in advanced RCC patients than that in stage I RCC patients, who had borne low tumour burdens. In addition, this reduced TCRB diversity was restored after removal of the primary tumour by CN. We inferred that when the tumour burden reached a certain threshold, it might exert significant pressure on the TCR repertoire.

The systemic environment co-evolves with tumour cells, and the two are mutually dysregulated [27]. Tumours elicit a series of autologous tumour antigen-specific T-cell clonotypes from their exposed neoantigens and immunogenic self-antigens while blocking the cytotoxicity of these T cells using tumour-derived PDL1 [28]. Because peripheral blood is a limited pool to store T cells, the expansion of one type of T-cell clonotype may lead to suppression of the others [29,30]. That is to say that there is not enough residence for the diversified naïve T cells. In addition, the immune checkpoint molecules on expanded T cells in patients with advanced RCC before surgery were higher than that on unexpanded T cells before surgery (supplementary material, Figure S7A). The adaptive immune system, as the keeper to protect host, produced plentiful inefficient hyperexpanded T-cell clonotypes, which occupied the space of naïve T cells. Consistently, bulk RNA-seq-derived immune cell activity data revealed that

exhausted T cell numbers were significantly reduced after CN. And the scRNA data also suggested the expression value of PD-1 on expanded T cells decreased after CN (supplementary material, Figure S7B). Those findings indicated that although the immune system dispatched large numbers of tumour-associated T cells with the same TCR, these immune cells not only failed to attack tumour cells but also disrupted the homeostasis of TCR diversity repertoire. The decreased TCRB diversity might be the tried-but-failed marker of the immune system to attack the tumour.

After the removal of the majority of the chronic and persistent immunogenic burden, which induced the immunosuppressive environment from the advanced RCC patients by CN, a part of invalid effector cells was eliminated and more naïve T cells appeared in their peripheral blood by peripheral proliferation [14] or homeostatic proliferation [31]. Furthermore, there is an acute and transient process for the immune system to fight, that is, the newly exposed tumour-associated antigens as the consequence of the destruction of tumour cells during surgery. Different from the chronic tumour-bearing stimuli before CN, the immune response caused by the transient antigens released by surgery is more similar to that of acute infection, and would also re-stimulate and mobilize the immune reaction. More clonotypes of naïve T cells appeared, and more opportunities to recognize the tumour-associated antigens would emerge. After antigen clearance, most of the effector cells with high-abundance clonotype were eliminated and a minor fraction of effector T cells survives to become memory T cells with low-abundance clonotype. As a result, TCR diversity is also increased.

**Figure 6.** Characteristics of persistent and emerging clonotypes before and after surgery. (A) The composition ratios of the proportions of persistent and emerging clonotypes in segments with different cumulative frequencies. The red, purple, grey, and brown gradient-filled bar represents the segmented cumulative frequencies of clonotypes ranked from high to low abundance as follows: persistent clonotypes pre-operation, persistent clonotypes post-operation, emerging clonotypes pre-operation, and emerging clonotypes post-operation. Each dash represents a patient, and the detailed order of the patients is TR183, TR95, TR116, TR119, TR186, TR45, TR55, TR56, TR58, TR59, TR60, TR65, TR66, TR79, TR86, TR88, TR137, TR169, TR128, TR16, TR17, TR182, TR184, TR22, TR23, TR6, TR70, TRPT180, TRT141, TR10, TR102, TR123, TR15, TR156, TR179, TR24, TR27, TR63, TR83, and TRML21. The four representative patients are marked with rectangular frame. (B) Abundances of persistent clonotypes in four representative patients. The plot in the first row shows pre-operative TCRB clonotypes, and the plot in the second row shows post-operative clonotypes. The abscissa represents each individual clonotype, ranked from high abundance to low abundance, and the ordinate depicts the frequency (log<sub>10</sub>-transformed) of each clonotype. Red squares indicate persistent clonotypes, and white hollow circles indicate emerging clonotypes. The marked persistent clonotypes were concentrated among high-frequency clonotypes in both pre- and post-operative samples. (C) Difference in the mean concentrations of persistent and emerging clonotypes. (D) Paired changed proportions of emerging clonotypes before and after surgery in patients with different stages. (E) Changes in high-abundance clonotypes in the four representative patients before and after surgery. Each circle represents a clonotype. The diameter of the circle indicates the concentration of the clonotype. Persistent clonotypes are marked as colourful and bright circles, and a single colour represents one clonotype. Emerging clonotypes are uniformly marked as grey circles. For the representative patients in stages II–IV, the predominant high-abundance clonotypes that existed before surgery remained predominant high-abundance clonotypes after surgery, but their concentrations were reduced after surgery, especially in stage IV patients. (F) Concentration changes in persistent clonotypes in four representative patients before and after surgery. Each dot represents a persistent clonotype that is present in both pre- and post-operative samples. Post- versus pre-operative samples showing persistent clonotypes with decreased or increased abundance are marked as green and red dots, respectively, and stable persistent clonotypes are marked as blue dots. (G) The proportions of changes in persistent clonotypes post-versus pre-operation. The proportions of decreased, increased, and stable clonotypes are marked with green, red, and blue bars, respectively. The order of patients is TR183, TR95, TR66, TR58, TR116, TR86, TR56, TR79, TR60, TR55, TR119, TR88, TR186, TR59, TR45, TR137, TR169, TR128, TR182, TR17, TRT141, TR16, TRPT180, TR6, TR23, TR184, TR22, TR70, TR102, TRML21, TR156, TR27, TR83, TR15, TR123, TR179, TR10, TR24, and TR63. The middle boxplot shows the ratio of the proportions of decreased and increased clonotypes in post-operative samples in patients with different stages. The right boxplot shows the proportions of stable clonotypes in patients at different stages. The four representative patients are marked with rectangular frame. (H) Changes in the mean concentrations of persistent (left plot) and emerging (right plot) clonotypes before and after surgery in patients with different stages as determined by the paired Wilcoxon test.

Based on our TCR repertoire and RNA-seq-derived immune cell data, the global immune status improved after CN with respect to the increased TCR diversity by increased levels of naïve T cells. Similarly, another study illustrated that radiation therapy increases systemic responses to immune checkpoint inhibitors as a result of the radiation-induced exposure of immunogenic mutations to the immune system in lung cancer [32]. Thus, we cannot rule out the significance of CN for subsequent immune checkpoint inhibitors, especially because it decreases the tumour burden and improves the immune status.

As a whole, CN is effective in improving the diversity of the TCRB repertoire in patients with stage IV RCC. High-throughput circulating TCR repertoire sequencing is a valid method that reflects the dynamic changes in the immune status and predicts prognosis. However, it is necessary to further confirm whether CN is necessary for subsequent immune checkpoint inhibitor therapy in synchronous metastatic RCC patients by a prospective clinical trial.

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### Author contributions statement

LF, KTZ, and JZS designed the study. XGB, YJL, LW, and WXJ collected the samples and clinical information. LPG performed the experiments. LPG and LF performed the statistical analysis. LPG wrote the first draft of the manuscript, and LF, XGB, KTZ, JZS WZ, and JM edited sections of the manuscript. All authors reviewed the manuscript and approved the submitted version.

### Data availability statement

The raw TCRB repertoire and transcriptome sequencing data generated in this study are deposited in the Genome Sequence Archive at the BIG Data Center, Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under the accession number HRA000049 [http://bigd.big.ac.cn/gsa-human]. The bulk data and the raw single-cell RNA sequencing data (not uploaded) are also available from the corresponding authors upon reasonable request.

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## SUPPLEMENTARY MATERIAL ONLINE

### Supplementary materials and methods

**Figure S1.** Quality control validation of the in-house built SMART-based UMI-corrected TCRB sequencing platform

**Figure S2.** The TCRB diversity index across patients with different clinical stages and treatment statuses

**Figure S3.** Changes in the TCRB diversity estimated using (A) Chao1 and (B) Shannon–Wiener before and after surgery in benign samples and samples from patients with each clinical stage of cancer

**Figure S4.** Proportion of peripheral exhausted CD8 T cell was decreased after surgery

**Figure S5.** Identification of the subtype cell populations in PBMCs

**Figure S6.** Relationship between TCRB diversity and CD4 naïve T cells (estimated by the deconvolution of RNA-seq data)

**Figure S7.** The dynamic changes of the expression of immune checkpoint molecules on expanded and nonexpanded T cells before and after CN

**Table S1.** Characteristics of the enrolled patients

**Table S2.** All the significant GO terms enriched by the genes that were significant positively correlated with TCRB diversity

**Table S3.** Sequences of primers used in the TCRB library construction