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ORIGINAL RESEARCH

Circulating pre-treatment T-cell receptor repertoire as a predictive biomarker in advanced or metastatic non-small-cell lung cancer patients treated with pembrolizumab alone or in combination with chemotherapy

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Background: The circulating T-cell receptor (TCR) repertoire is a dynamic representation of overall immune responses in an individual.

Materials and methods: We prospectively collected baseline blood from patients treated with first-line pembrolizumab monotherapy or in combination with chemotherapy. TCR repertoire metrics were correlated with clinical benefit rate (CBR), progression-free survival (PFS), overall survival (OS) and immune-related adverse events (irAEs). We built a logistic regression classifier by fitting all four TCR-β repertoire metrics to the immune checkpoint inhibitor (ICI) CBR data. In the subsequent receiver operating characteristic (ROC) analysis of the resulting logistic regression model probabilities, the best cut-off value was selected to maximise sensitivity to predict CBR to ICI.

Results: We observed an association between reduced number of unique clones and CBR among patients treated with pembrolizumab monotherapy (cohort 1) [risk ratio = 2.86, 95% confidence interval (CI) 1.04-8.73, $P = 0.039$]. For patients treated with pembrolizumab plus chemotherapy (cohort 2), increased number of unique clones [hazard ratio (HR) = 2.96, 95% CI 1.28-6.88, $P = 0.012$] and Shannon diversity (HR = 2.73, 95% CI 1.08-6.87, $P = 0.033$), and reduced evenness (HR = 0.43, 95% CI 0.21-0.90, $P = 0.025$) and convergence (HR = 0.41, 95% CI 0.19-0.90, $P = 0.027$) were associated with improved PFS, while only an increased number of unique clones (HR = 4.62, 95% CI 1.52-14.02, $P = 0.007$) were associated with improved OS. Logistic regression models combining the TCR repertoire metrics improved the prediction of CBR (cohorts 1 and 2) and were strongly associated with PFS (cohort 1, HR = 0.38, 95% CI 0.19-0.78, $P = 0.009$) and OS (cohort 2, HR = 0.20, 95% CI 0.05-0.76, $P < 0.0001$). Reduced TCR conversion was associated with increased frequency of irAEs needing systemic steroid treatment.

Conclusion: Combined pre-treatment circulating TCR metrics might serve as a predictive biomarker for clinical outcomes among patients with advanced non-small-cell lung cancer treated with pembrolizumab alone or in combination with chemotherapy.

Key words: T-cell receptor repertoire, non-small-cell lung cancer, immunotherapy, chemotherapy, biomarkers

INTRODUCTION

T-cell recognition of tumour antigens underpins the efficacy of immune checkpoint inhibitors (ICIs) for the treatment of cancer. ICIs such as pembrolizumab have transformed the treatment of many cancers, including non-small-cell lung cancer (NSCLC).[1,2] Although many patients experience clinical responses to ICI, unfortunately, many show primary resistance. Predictive biomarkers for ICI efficacy are largely related to tumour immunogenicity, such as programmed death-ligand 1 (PD-L1) positivity,[1,3] tumour-infiltrating lymphocytes,[4] human leucocyte antigen (HLA) heterozygosity,[5] interferon-γ gene signature,[6] tumour mutational burden[7] and others. One promising area of interest has been examining the quality and quantity of the pre-existing T-cell response since it may represent the presence and immune recognition of tumour neoantigens.[8]

T-cell receptors (TCRs) interact with HLA molecules on cancer cells to initiate an immune response. TCRs are

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highly diverse heterodimers and comprise α and β chains ($\alpha\beta$ TCR). The variable region of TCR-α is encoded by multiple variable (V) and joining (J) segments, while TCR-β is additionally encoded by a diversity (D) segment.[9-11] Each TCR chain contains three hypervariable loops in its structure referred to as the complementarity determining regions (CDR1-3). CDR1 and 2 are required for interaction of the TCR with the HLA complex and encoded by V genes. The CDR3 of the TCR-β is encoded by the junctional region between the V, J or D genes, thus containing most of the diversity.[12] It is estimated that up to 25×10^6 different TCR-$\alpha\beta$ combinations exist due to the process of gene recombination and junctional nucleotide insertion or deletion.[13] TCR repertoire is commonly measured using multiple metrics, such as number of unique clones, evenness, Shannon diversity and convergence.[14-16]

The pre-treatment TCR repertoire has been found to be associated with clinical outcomes in NSCLC and other cancers. High intratumoural TCR diversity before therapy was reported to be associated with worse survival among 15 NSCLC patients.[16] Although tumour infiltrate TCR clonality is potentially more informative of the tumour-specific response,[15,17] studies have shown that sequencing peripheral blood TCR repertoire can be used alternative to intratumoural TCR to predict clinical outcome among patients with NSCLC or other cancer types.[17-23] Increased diversity (as Shannon index) in peripheral circulating programmed cell death protein 1-positive (PD-1+) CD8+ T cells among 25 NSCLC patients treated with anti-PD-1/PD-L1 antibodies was associated with better response to therapy and improved progression-free survival (PFS).[19] Looney et al. (2020) showed that increased total circulating TCR convergence and clonality predicted response to anti-cytotoxic T-lymphocyte associated protein 4 (CTLA-4), among a mixed cohort of 22 cancer patients.[22] Low peripheral diversity (diversity index—DE50) was shown to be associated with treatment response and prolonged PFS among 38 melanoma patients treated with anti-PD-1, but not among 42 patients treated with anti-CTLA-4.[20] However, no study so far has investigated the TCR repertoire in patients treated with combination immunochemotherapy.

Here we investigate the pre-treatment circulating TCR-β repertoire in NSCLC patients to predict clinical outcomes. We analysed two cohorts one treated with single-agent pembrolizumab and another treated with pembrolizumab in combination with chemotherapy. We constructed logistic regression models to identify the most informative characteristics to predict response to therapy. Furthermore, we also evaluated the association of the circulating TCR-β repertoire and the development of immune-related toxicities.

MATERIALS AND METHODS

Patients

Patients with unresectable locally advanced or metastatic NSCLC were recruited prospectively from Sir Charles Gairdner and Fiona Stanley Hospitals in Western Australia from June 2018 to July 2021. All procedures were approved by the Human Research Ethics Committees at Edith Cowan University (ECU) (No. 18957) and Sir Charles Gairdner Hospital (No. 2013-246 and RGS0000003289) in compliance with the Declaration of Helsinki. All patients provided informed consent to the study. Patients were 18 years or older and treated with pembrolizumab monotherapy or pembrolizumab in combination with chemotherapy in the first-line setting. Patients' demographics were collected from the medical record. These included the following: age, sex and smoking history. Clinical information included: tumour stage, histological subtype, PD-L1 expression, molecular characteristics, Eastern Cooperative Oncology Group (ECOG) performance status and type and start date of treatment. Baseline pre-treatment haematology results included absolute numbers of neutrophils and lymphocytes. These were used to calculate neutrophil/lymphocyte ratio (NLR).

Information about clinical response, date of disease progression, date of death and immune-related adverse events (irAEs) was collected to assess clinical outcomes.

DNA extraction and TCR analysis

Pre-treatment blood was collected in K_2 EDTA tubes. It was stored either as whole blood or white cell pellets at -80°C. High-quality DNA was extracted using the QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany). DNA was quantified using a Nanophotometer NP80 (Implen, Westlake Village, CA) and 200 ng of DNA was used to construct libraries with the Oncomine TCR Beta-SR Assay (Thermo Fisher Scientific, Waltham, MA) kit, including the optional library amplification step. TCR libraries were quantified using the Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific), diluted to 25 pM and loaded into an IonChef for template preparation and into an Ion 540 chip before sequencing on an Ion S5 (Thermo Fisher Scientific). We aimed for approximately two million raw reads per sample, which were down-sampled to one million within the TCR analysis workflow on IonReporter (Thermo Fisher Scientific). TCR repertoire metrics (number of unique clones, evenness, Shannon diversity and convergence) were calculated using the Oncomine TCR Beta-SR—w1.4—DNA—Single Sample workflow on IonReporter.

Statistical analysis

Patients were stratified into two groups: cohort 1 received pembrolizumab monotherapy and cohort 2 received pembrolizumab in combination with chemotherapy. Because the efficacy of pembrolizumab alone was confirmed mainly in patients with PD-L1 $\geq$50%,[1] cohort 1 only included NSCLC patients with PD-L1 $\geq$50%. Responders represent patients who experienced clinical benefit including complete response (CR), partial response (PR) or those who had stable disease (SD) for 6 months or more, and non-responders as patients with progressive disease (PD)

within 6 months from commencing treatment. Clinical benefit rate (CBR) is the ratio of responders comparing to the study participants. PFS was defined as the time between the start of treatment and disease progression or death. Overall survival (OS) represents the time between the start of immunotherapy and death.

Demographics and disease characteristics at baseline were compared using chi-square test.

A Kruskal–Wallis test was used to compare downsampling values for the number of clones (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.102066). To ensure saturation of clones, one million downsampling values were used for all the analyses. We compared TCR-β repertoire metrics (number of unique clones, evenness, Shannon diversity and convergence) between responders versus non-responders, and between irAE classifiers using a Mann–Whitney *U* test.

We used the online cut-off finder developed by Budczies et al. (2012)[24] to identify the best cut-off for each TCR metric based on PFS, rather than response, as it is a better surrogate of survival.[25] We used the identified cut-offs to determine the association of TCR-β repertoire metrics with clinical benefit or irAEs using Fisher's exact test. The association between pre-treatment TCR-β repertoire metrics and PFS or OS was assessed using Mantel–Cox log-rank and Gehan–Breslow–Wilcoxon tests, with the data dichotomised based on the above cut-offs.

To compare the TCR gene usages among responders versus non-responders in both cohorts and among those who experienced irAEs versus no irAEs, we carried out Mann–Whitney *U* tests.

The response classification accuracy of each of the TCR-β repertoire metrics to the ICI CBR data was evaluated by calculating the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. We then built a logistic regression classifier by fitting all four TCR-β repertoire metrics to the ICI CBR data. In the subsequent ROC analysis of the resulting logistic regression model probabilities, henceforth referred to as the model score, the best cut-off value was selected to maximise sensitivity to predict CBR to ICI.

For internal cross-validation, the dataset was split into three different partition settings, i.e. three training/testing splits in 60/40, 70/30 and 80/20 across 100 randomised runs. For each run, the logistic regression model is constructed with the training set and validated by the testing set.

The best cut-off value for the model score was selected to maximise sensitivity to predict response to ICI and used to dichotomise the cohorts and correlate with PFS and OS using Mantel–Cox log-rank and Gehan–Breslow–Wilcoxon tests. Multivariate Cox regression analyses were carried out for OS incorporating age, sex, ECOG, smoking, histology, NLR and the model score as categorical metrics. PD-L1 expression was only included for the analysis of cohort 2.

Statistical analyses were carried out using GraphPad Prism version 9.3.1 (San Diego, CA), IBM SPSS Statistics, Version 28.0 (Armonk, NY), R Statistical Software v4.1.2 (R Core Team 2021) and RStudio 2022.07.1 (RStudio Team 2022).

RESULTS

Study cohorts and baseline characteristics

A total of 63 patients initially considered for treatment with single-agent pembrolizumab were recruited for cohort 1. Of those, 15 patients were excluded (Figure 1). Median follow-up of the final cohort of 48 patients was 524 days, with 3 patients achieving CR, 16 PR and 9 SD for 6 or more months.

Recruitment into cohort 2, pembrolizumab with chemotherapy, included 65 patients. Of those, 12 were excluded (Figure 1). All patients received pembrolizumab with carboplatin in combination with pemetrexed (34 patients), paclitaxel (18 patients) or vinorelbine (1 patient). Median follow-up of the final cohort of 53 patients was 335 days, with 1 patient obtaining a CR, 19 PR and 22 SD for 6 or more months.

Patients' characteristics at baseline were comparable between cohort 1 and 2, with no statistically significant difference between the groups (Table 1). As per inclusion criteria, all patients in cohort 1 express PD-L1 $\geq$50%. Most patients in cohort 2 expressed PD-L1 $<$50%.

Prognostic value of TCR repertoire and clinical outcomes

Among cohort 1, 48 patients treated with pembrolizumab alone, there was a statistically significant difference in the median of unique clones among responders and non-responders (1607 versus 2485, respectively; $P = 0.037$) (Supplementary Figure S2A, available at https://doi.org/10.1016/j.esmoop.2023.102066). None of the other TCR diversity metrics (evenness, Shannon diversity or convergence) were significantly different between the responders and non-responders in either of the cohorts (Supplementary Figure S2B-D, available at https://doi.org/10.1016/j.esmoop.2023.102066).

For survival analysis, we used Cutoff Finder to identify a cut-off for each of the metrics able to predict PFS (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2023.102066). Statistically significant cut-offs were obtained for cohort 2, but not for any of the variables in cohort 1. As a result, we used this cut-off to correlate TCR metric with clinical outcomes in both cohorts 1 and 2. Using these cut-offs, patients with a low number of unique clones were more likely to respond to pembrolizumab than those with a high number of unique clones [risk ratio = 2.86, 95% confidence interval (CI) 1.04-8.73, $P = 0.039$]. Eighty percent (12/15) compared to 48% (16/33) of patients with low versus high TCR responded to pembrolizumab (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.102066). No association between treatment response and any of the TCR metrics was found among patients in cohort 2 treated with pembrolizumab in combination

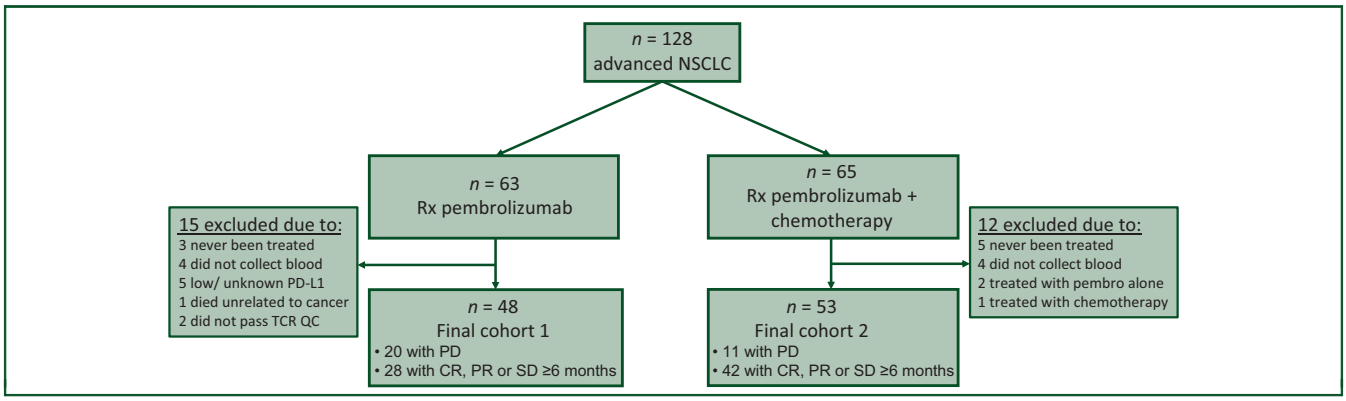

Figure 1. Flow diagram describing patient participation in the study.
Chemo, chemotherapy; CR, complete response; NSCLC, non-small-cell lung cancer; PD, progressive disease; pembro, pembrolizumab; PR, partial response; QC, quality control; Rx, treatment; SD, stable disease; TCR, T-cell receptor.

with chemotherapy (Supplementary Table S1 and Figure S2, available at https://doi.org/10.1016/j.esmoop.2023.102066).

Table 1. Demographics and disease characteristics of patients at baseline

	Pembrolizumab only (cohort 1), *n* (%)	Pembrolizumab + chemotherapy (cohort 2), *n* (%)	*P* value
Age, years			
≥65	34 (71)	39 (70)	0.826
<65	14 (29)	14 (30)	
Sex			
M	26 (54)	34 (64)	0.309
F	22 (46)	18 (34)	
ECOG			
≤1	40 (83)	47 (89)	0.567
>1	8 (17)	6 (11)	
Smoking			
Yes	39 (81)	47 (89)	0.517
No	7 (15)	4 (7)	
Unknown	2 (4)	2 (4)	
Stage			
II	1 (2)	0	0.453
III	5 (10)	8 (15)	
IV	43 (88)	45 (85)	
Histopathology			
Adenocarcinoma	38 (79)	38 (72)	0.665
SCC	9 (19)	13 (24)	
Others	1 (2)	2 (4)	
Molecular status[a]			
KRAS mutant	21 (54)	14 (35)	0.343
KRAS wild type	16 (41)	22 (55)	
KRAS unknown	1 (3)	3 (7)	
EGFR, ALK or ROS-1	1 (3)	1 (3)	
PD-L1 expression			
≥50%	48 (100)	10 (19)	<0.0001
1%-49%	0	18 (34)	
0%	0	22 (42)	
Unknown	0	3 (6)	
Total	48	53	

P value represents the statistical difference between the two cohorts.
ALK, echinoderm microtubule-associated protein like-4-anaplastic lymphoma kinase (EML4/ALK) fusion; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; F, female; KRAS, Kirsten Rat Sarcoma GTPase; M, male; NSCLC, non-small-cell lung cancer; PD-L1, programmed death-ligand 1; ROS1, echinoderm c-ros oncogene 1 fusion; SCC, squamous cell carcinoma.
[a]Molecular status was only examined in NSCLC with non-squamous cell carcinoma histology (39 patients in cohort 1 and 40 in cohort 2).

Despite the association between the low number of unique clones with response to pembrolizumab, no statistically significant association with PFS or OS (Figure 2) was observed for any of the metrics among those treated with pembrolizumab. On the contrary, patients treated with a combination of pembrolizumab and chemotherapy (cohort 2) were more likely to have longer PFS if they had a high pre-treatment number of unique clones [hazard ratio (HR) = 2.96, 95% CI 1.28-6.88, $P = 0.012$], low evenness (HR = 0.43, 95% CI 0.21-0.90, $P = 0.025$), high Shannon diversity (HR = 2.73, 95% CI 1.08-6.87, $P = 0.033$) or low convergence (HR = 0.41, 95% CI 0.19-0.90, $P = 0.027$) (Figure 2I-L). In terms of association of TCR metrics with OS, only a high pre-treatment number of unique clones were associated with longer OS among patients treated with pembrolizumab in combination with chemotherapy (HR = 4.62, 95% CI 1.52-14.02, $P = 0.007$) (Figure 2M). Low evenness was significantly associated with longer OS in cohort 2 when evaluated using the Gehan—Breslow—Wilcoxon test (HR = 0.38, 95% CI 0.14-1.03, $P = 0.032$), which gives more weight to events that occur at early time points, but not when using Mantel—Cox log rank.

An exploratory analysis to assess TCR gene usage among responders versus non-responders in both cohorts showed that responders among cohort 1 patients had lower usage of TRBV6-9 compared to non-responders ($P = 0.032$) (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2023.102066). Among patients in cohort 2, TRBV4-2 ($P = 0.011$), aTRBV7-3 ($P = 0.010$) and TRBV 11-2 ($P = 0.030$) were more frequent among non-responders compared to responders (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2023.102066).

Logistic regression to predict the best model that correlates with CBR, PFS and OS

We constructed logistic regression models combining all four TCR repertoire metrics to predict treatment response. The AUC for the model score was 0.693 for cohort 1 and 0.760 for cohort 2. These moderate predictive powers were confirmed by internal cross-validation (Supplementary

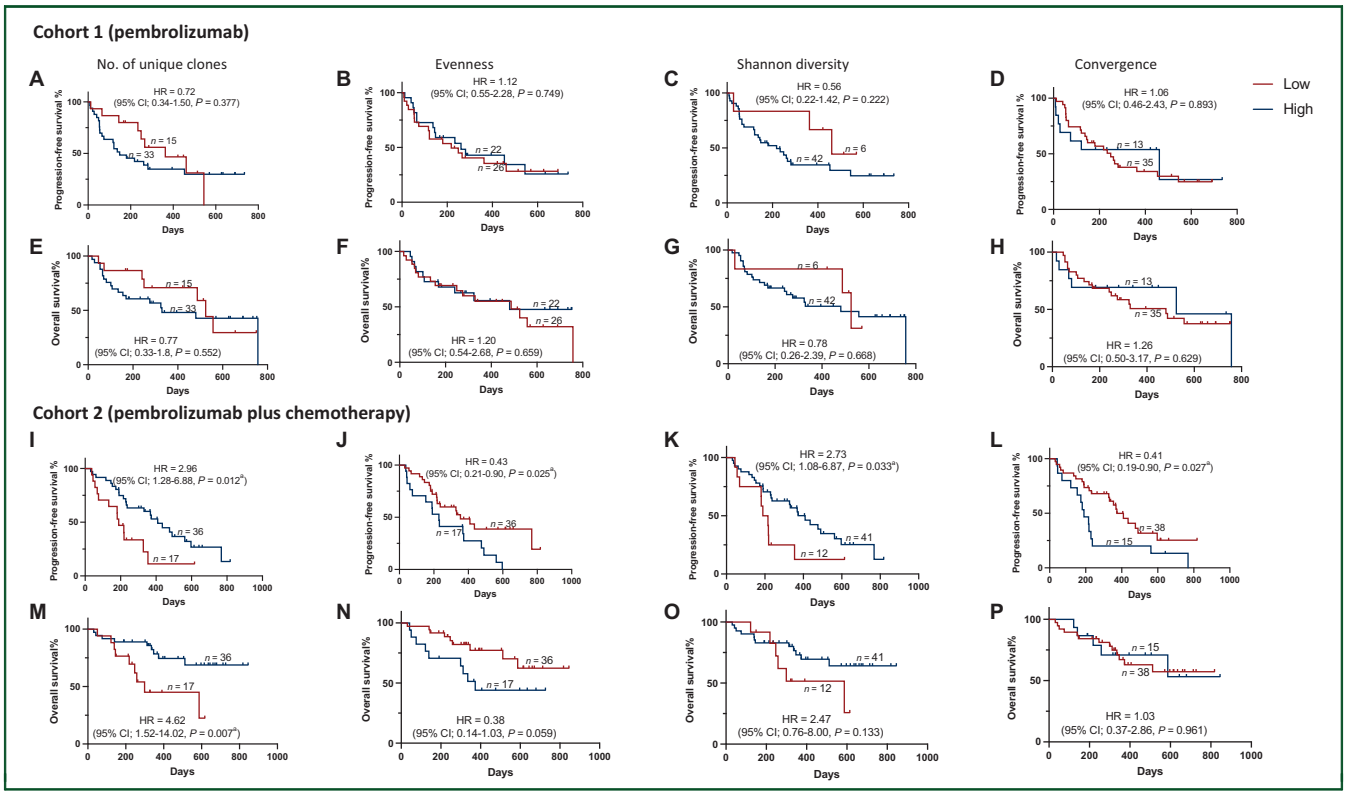

Figure 2. Kaplan–Meier estimates of progression-free survival and overall survival based on TCR-β CDR3 diversity metrics of cohort 1 ($n = 48$) (A-H) and cohort 2 ($n = 53$) (I-P). (A, E, I and M) No. of unique clones, (B, F, J and N) evenness, (C, G, K and O) Shannon diversity and (D, H, L and P) convergence.
CDR, complementarity determining region; CI, confidence interval; HR, hazard ratio; TCR, T-cell receptor.
[a]Statistically significant result using log-rank (Mantel–Cox) test.

Figure S4, available at https://doi.org/10.1016/j.esmoop.2023.102066). Results across the three partition settings are very similar, which shows stability in the model performance. The AUCs for cohort 2 were higher than for cohort 1, with ~10% higher AUCs for the training sets than for the testing sets.

The median model score was significantly higher in patients who responded to treatment in cohort 1 ($P = 0.0027$) and cohort 2 ($P = 0.0037$) (Figure 3B and F).

In cohort 1, a cut-off of 0.554 optimally distinguished responders from non-responders and provided a sensitivity of 61% and a specificity of 80% [positive predictive value (PPV) = 59%, negative predictive value (NPV) = 81%]. At this cut-off, we found a statistically significant association of high model score with improved PFS (HR = 0.38, 95% CI 0.19-0.78, $P = 0.0089$) and a trend towards improved OS (HR = 0.47, 95% CI 0.21-1.02, $P = 0.0591$) (Figure 3C and D). Multivariate analysis of 45 patients (3 patients were excluded due to the absence of demographic data) that included potential co-founder factors identified ECOG and NLR, but not the TCR-based model score, as the main predictors (Table 2).

In cohort 2, a cut-off of 0.695 was used to maximise sensitivity at 88%, with a specificity of 55% (PPV = 88%, NPV = 55%). At the specified cut-off, the low model score was associated with improved OS (HR = 0.20, 95% CI 0.05-0.76, $P < 0.0001$). The correlation between low model score and improved PFS was statistically significant only in the Gehan–Breslow–Wilcoxon test (HR = 0.47, 95% CI 0.18-1.23, $P = 0.008$) (Figure 3G and E). For the multivariate analysis we included 47 patients, as 6 patients lacked complete datasets for the variables included (Table 2). Backward stepwise regression multivariate analysis isolated the TCR-based model score as an independent predictive variable (HR = 0.32, 95% CI 0.11-0.95, $P = 0.039$) (Table 2).

Correlation between TCR repertoire and immune-related toxicity

Amongst all TCR-β metrics, only TCR convergence was statistically significantly different between patients who experienced irAEs needing steroids ($n = 23$) [≥grade (G) 3 or G2 irAE needing steroids, median = 0.00243] compared to those with G2 or less toxicity not requiring steroids ($n = 78$) (median = 0.00404) ($P = 0.040$) (Supplementary Figure S5, available at https://doi.org/10.1016/j.esmoop.2023.102066).

An exploratory analysis was conducted to assess TCR-β gene usage among patients who developed irAEs versus those who did not experience irAEs. Compared to those who did not develop irAEs, patients who developed irAEs had higher usage of TRBV9 ($P = 0.036$), TRBV5-7 ($P = 0.038$) and TRBJ1-3 ($P = 0.018$) and lower usage of TRBV20-1 ($P = 0.010$) (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2023.102066).

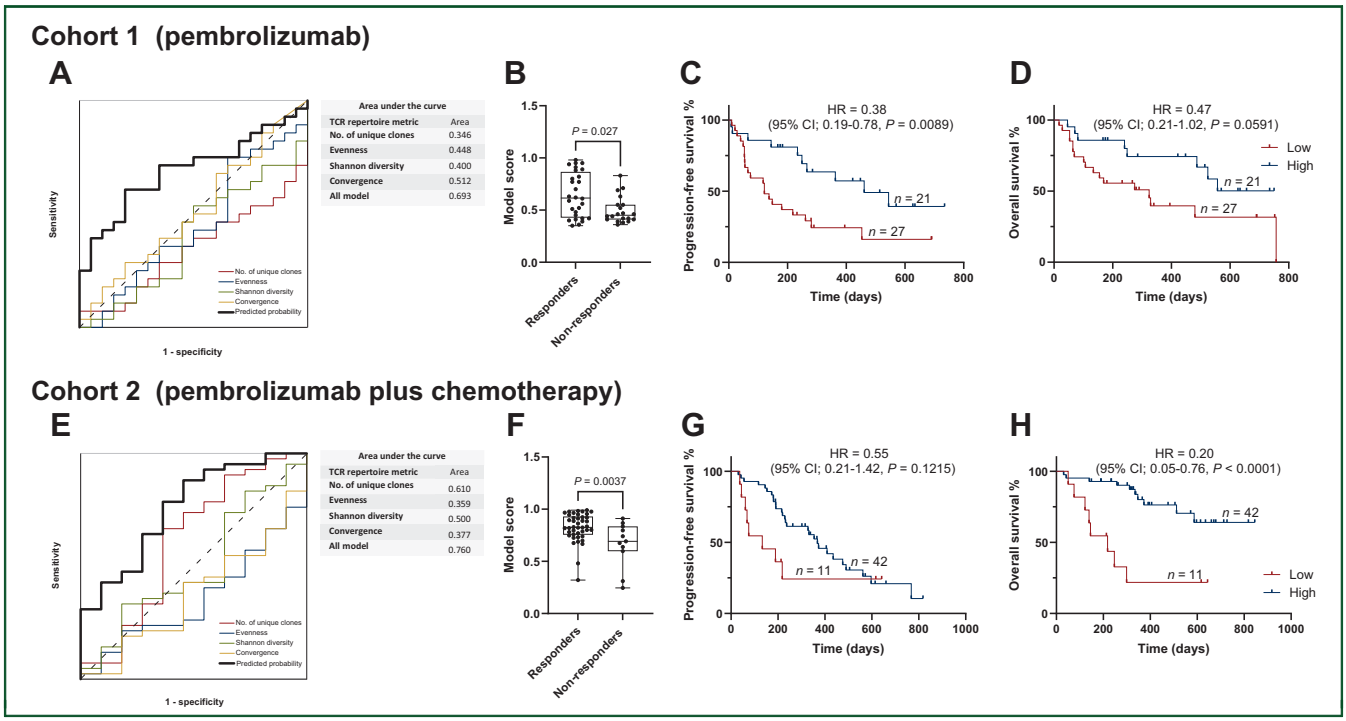

Figure 3. Model score combining TCR-β CDR3 repertoire metrics. (A-D) Cohort 1 ($n = 48$), cut-off = 0.554. (E-H) Cohort 2 ($n = 53$), cut-off = 0.695. (A and E) Receiver operating characteristic (ROC) curves of each TCR metric alone (number of unique clones, evenness, Shannon diversity and convergence) or the combination of all of them. Area under the curve for each ROC is indicated in the table. (B and F) The Mann—Whitney *U* test for the model score comparing responders with non-responders. (C and G) Kaplan—Meier curves of PFS of patients with high and low model score. (D and H) Kaplan—Meier curves of PFS of patients with high and low model score.

CDR, complementarity determining region; CI, confidence interval; HR, hazard ratio; PFS, progression-free survival; TCR, T-cell receptor.

Statistically significant result ($P < 0.05$) using log-rank (Mantel—Cox) test.

DISCUSSION

This study highlights the potential of the peripheral pre-treatment TCR repertoire as a predictive marker for clinical outcome. To our knowledge, the herein study presents the largest number of locally advanced/metastatic NSCLC patients treated with pembrolizumab analysed for this purpose. Additionally, this is the first study to investigate the TCR repertoire among patients with advanced/

Table 2. Multivariate analysis for the correlation between the model score and OS among patients in cohort 1 ($n = 45$, all patients express PD-L1 of ≥50%) and in cohort 2 ($n = 47$)

Variable	Cohort 1 $n = 45$	Univariate				Multivariate (enter)				Multivariate (backward Wald)			
		P value	HR	95% CI		*P* value	HR	95% CI		*P* value	HR	95% CI	
Age (<65 versus ≥65 years)	12 versus 33	0.397	0.667	0.261	1.702	0.182	0.496	0.177	1.390				
ECOG (≤1 versus ≥2)	37 versus 8	**0.026**	0.348	0.137	0.884	**0.008**	0.195	0.058	0.652	**0.043**	0.339	0.119	0.969
Histology (SCC versus adeno)	9 versus 36	0.981	0.988	0.367	2.660	0.751	0.841	0.287	2.459				
Model score (<0.554 versus ≥0.554)	26 versus 19	0.114	0.502	0.213	1.180	0.059	0.378	0.137	1.038	0.069	0.401	0.150	1.073
NLR (<5 versus ≥5)	26 versus 19	**0.033**	0.407	0.179	0.930	**0.006**	0.263	0.101	0.681	**0.002**	0.233	0.091	0.597
Sex (F versus M)	20 versus 25	0.227	0.609	0.272	1.362	0.425	0.687	0.273	1.727				
Smoking (N versus Y)	7 versus 38	0.332	0.548	0.163	1.845	0.141	0.357	0.090	1.410				

Variable	Cohort 2 $n = 47$	Univariate			Multivariate (enter)			Multivariate (backward Wald)			
		P value	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	
Age (≥65 versus <65 years)	33 versus 14	**0.004**	0.244	0.093	0.645	0.076	0.349	0.109	1.118	0.065	0.367
ECOG (≤1 versus ≥2)	41 versus 6	0.283	0.499	0.140	1.776	0.306	0.468	0.110	2.001		
Histology (SCC versus adeno)	13 versus 34	0.946	1.040	0.336	3.222	0.774	1.197	0.350	4.093		
Model score (<0.695 versus ≥0.695)	36 versus 11	**0.002**	0.211	0.079	0.559	0.063	0.318	0.095	1.063	**0.039**	**0.324**
NLR (<5 versus ≥5)	24 versus 23	0.189	0.522	0.198	1.378	0.330	0.574	0.188	1.755		
PD-L1 (≥50% versus <50%)	9 versus 38	0.364	1.625	0.569	4.640	0.966	1.030	0.268	3.958		
Sex (F versus M)	14 versus 33	0.359	0.587	0.188	1.831	0.303	0.513	0.144	1.827		
Smoking (N versus Y)	3 versus 44	0.417	0.043	0.000	86.942	0.987	0.000	0.000			

Adeno, adenocarcinoma; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; F, female; HLA, human leukocyte antigen; HR, hazard ratio; M, male; N, no; NLR, neutrophil/lymphocyte ratio; NSCLC, non-small-cell lung cancer; OS, overall survival; PD-1/PD-L1, programmed cell death protein 1/programmed death-ligand 1; SCC, squamous cell carcinoma; Y, yes. Statistically significant results are bolded.

metastatic NSCLC treated with pembrolizumab in combination with chemotherapy as a separate group.

Here, we showed that a logistic regression model combining features of the circulating TCR-β repertoire can serve as a biomarker of clinical benefit, PFS and OS. In particular, our model score was strongly associated with OS in patients treated with pembrolizumab in combination with chemotherapy.

The interaction between the initial T-cell repertoire and tumour-specific antigens in the lymphoid organs will result in the expansion of tumour-specific T cells.[26] Hence, reduced pre-treatment number of unique clones may represent an enrichment for tumour-specific T cells and therefore associated with response to treatment as demonstrated in our study. Notably, despite its association with response, the number of unique clones was not associated with survival. In fact, none of the TCR-β repertoire metrics tested were associated singularly with survival for patients treated with single-agent pembrolizumab.

Other studies have shown reduced evenness to be associated with favourable clinical outcomes as it represents the expansion of certain T cells over others.[20] Increased Shannon diversity[19] represents the relationship between clonality and evenness[27] and has been linked with good clinical outcomes.[19] Increased TCR convergence represents the merging of multiple amino acids to code the same CDR3 region and was found to be associated with improved outcomes.[22] All patients in the above-mentioned studies were treated with single-agent immunotherapy, anti-PD-1/PD-L1 or anti-CTLA-4 in the first- or the second-line setting. The discordance with previous studies might be explained by the differences in the methodologies and bioinformatics tools used to analyse the TCR repertoire.[28] Moreover, it might be due to the difference in the studied population in terms of cancer type, stage of disease, PD-L1 expression among patients and type of immunotherapy.[19,20,22,29,30] Other studies concentrate on the dynamic changes that occur in TCR repertoire before and after anti-PD-1/-L1 treatment.[18,19,23,31] The latter is a limitation to be addressed in future studies.

There have been no published studies regarding the correlation between pre-treatment TCR and clinical outcomes among patients with locally advanced or metastatic NSCLC treated with pembrolizumab in combination with chemotherapy (cohort 2), which is now a common standard of care in many countries. Low pre-treatment tissue TCR evenness was associated with pathological CR among patients with stage III NSCLC who received neoadjuvant chemoimmunotherapy.[32] Qian et al. (2021) investigated the role of TCR diversity among NSCLC patients treated with first-line pemetrexed-based chemotherapy.[33] Unlike immunotherapy studies, the authors showed that increased rather than reduced Shannon diversity was associated with favourable clinical outcome.[33] Interestingly, our results were consistent with those findings. We showed that high number of unique clones or Shannon diversity or low evenness or convergence was strongly correlated with improved clinical outcome among patients with locally advanced or metastatic NSCLC treated with pembrolizumab in combination with chemotherapy.

The difference in TCR-β metrics between cohort 1 and 2 might suggest the following: the use of chemotherapy will increase the release of a large number of tumour antigens that will need a higher number of pre-treatment TCR unique clones in order to improve recognition of cancer neoantigens and hence clinical outcome among patients treated with immunotherapy in combination with chemotherapy. The lower number of pre-treatment TCR unique clones will be favourable among those treated with pembrolizumab alone as it reflects the expansion of tumour-specific T cells and is associated with improved outcome.

Combining clonality and convergence was found to create a better predictive ability with an AUC of 0.89, compared to each alone[22] among a small cohort of patients treated with anti-CTLA-4. This was consistent with our all-model score that combined all four TCR metrics (number of unique clones, evenness, Shannon diversity and convergence) in each cohort separately. As we continue to analyse data from more patients and evaluate dynamic changes upon therapy commencement, we anticipate that our current model will serve as the foundation for more sophisticated models with improved prediction value.

Herein, we also evaluate the pre-treatment TCR and the development of irAE. Interestingly, reduced convergence was the only TCR metric associated with the development of irAE needing steroids. Further studies will be needed to validate this exploratory finding and query whether the association can be narrowed down to specific irAE.

Notably, there are some limitations to the present study. We have not assessed the concordance between the peripheral and intratumoural TCR profile in our samples. This was not possible due to the volume of obtained cancer tissue by fine needle aspiration which is only enough to examine the presence of targetable mutations. However, studies suggest that peripheral blood TCR repertoire can be reflective of the intratumoural TCR and can be used to predict clinical outcome.[17-23] Another limitation is the coherence of cohort 2 as PD-L1 is expressed in $\geq$50% of cancer cells among 19% of patients and it is unknown among 6% of patients. Although this might affect the results obtained, the cohort represents real-life patients seen in clinic and corresponds with real-life data.

CONCLUSION

The need to predict patients who will benefit from immunotherapy alone or in combination with chemotherapy is an ongoing challenge towards treatment personalisation. Circulating pre-treatment TCR logistic regression model might serve as an accessible predictive biomarker for clinical outcome among patients treated with pembrolizumab alone or in combination with chemotherapy. However, further studies are needed to

validate current findings and for the standardisation of the analysis techniques. Finally, comparisons between tumour and peripheral TCR repertoire across multiple metrics are important to establish how representative is the circulating TCR repertoire. Further investigations with large prospective cohorts will demonstrate whether the circulating pre-treatment TCR repertoire is a prognostic factor for immune checkpoint inhibition.

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DATA SHARING

All data are available upon reasonable request.

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DISCLOSURE

MM sits on advisory boards for Merck Sharp and Dohme (MSD), Bristol-Myers Squibb (BMS) and AstraZeneca (AZ). ESG has received travel support from MSD and Thermo Fisher Scientific. All other authors have declared no conflicts of interest.

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