BMC Cancer

RESEARCH

Open Access

Dynamic changes in immune repertoire profiles in patients with stage III unresectable non-small cell lung cancer during consolidation treatment with immunotherapy

Nareenart Iemwimangsa[1], Dulyathat Anantaya[2,3], Songporn Oranratnachai[4], Thanaporn Thamrongjirapat[2,3], Putthapoom Lumjiaktase[5], View-Hune Teoh[6], Khantong Khiewngam[7], Nanamon Monnamo[8], Pimtip Sanvarinda[9], Pimpin Incharoen[5], Angkana Charoenyingwattan[1], Insee Sensorn[1], Thitiya Dejthevaporn[2,3], Ekaphop Sirachainan[2,3], Wasun Chantratita[1], Thanyanan Reungwetwattana[2,3] and Narumol Trachu[8*]

Abstract

Background One-year of immune checkpoint inhibitor (ICI) treatment after concurrent chemoradiation (CCRT) in unresectable stage III non-small cell lung cancer (NSCLC) is a standard of care. The precise predictive biomarkers are under investigations either immunological markers or clinical characteristics. Here, we explored immune repertoire of T cell receptor β-chain (TCRβ) during ICI treatment.

Methods During August 2019 and September 2021, stage III NSCLC, post CCRT patients from Ramathibodi Hospital was enrolled. All patients were treated by durvalumab after CCRT. Blood samples were collected together with clinical data and tumor assessment every 3–4 months until disease progression or discontinuation of treatment due to adverse events. CDR3 region and *TCRB* polymorphisms was explored by RNA sequencing using Next-Generation Sequencing (NGS) TCR beta short-read assay. Bioinformatic analysis was performed to analyze clonal diversity, TCR convergence frequency and the Shannon diversity from each timepoint. Immune repertoire and clinical correlation were explored using Spearman's correlation and Pearson's correlation. RStudio software version 2021 build 372 was used for analyses. A significance level was at $P < 0.05$.

Results Forty-four blood samples from 12 patients were analyzed. Mean duration of durvalumab treatment was 284 days. After durvalumab treatment, increasing of TCR convergence frequency was found compared to baseline ($R = 0.36$). Interestingly, it was also significantly higher in non-progressive disease (non-PD) patients compared with progressive disease (PD) patients ($P = 0.011$). Furthermore, Shannon diversity was higher increasing in PD patients compared with non-PD patients. Taken together, our study found that increasing of TCR convergence with less T-cell diversity in non-PD patients probably demonstrated a T cell-specific clonal expansion response to durvalumab treatment in this population.

*Correspondence:
Narumol Trachu
narumol.tra@mahidol.ac.th

Full list of author information is available at the end of the article

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Conclusions TCRβ repertoire is the potential biomarker for predicting durvalumab treatment response in post CCRT stage III NSCLC patients. However, a larger cohort with long-read assay should be explored.

Keywords Stage III non-small cell lung cancer, Stage III NSCLC, TCR polymorphism, Immune repertoire, Immunotherapy

Background

More than half of patients with non-small cell lung cancer (NSCLC) in Thailand present with locally advanced or metastasis disease [1, 2]. Before the immunotherapy era, the standard treatment for unresectable stage III NSCLC was definitive concurrent chemoradiation (CCRT) with platinum-based doublet chemotherapy [3]. However, median progression-free survival (PFS) among patients who had received CCRT was approximately 8 months, with a very high 5-year recurrence rate of 76%, and only 15% of patients were alive at 5 years [4, 5].

Durvalumab is a selective, high-affinity, human IgG1 monoclonal antibody that blocks programmed death ligand 1 (PD-L1) binding to programmed death 1 (PD-1) and CD80, allowing T cells to recognize and kill tumor cells [6–8]. The PACIFIC study demonstrated significantly longer PFS and overall survival (OS) with consolidation of durvalumab after definitive CCRT regarding PD-L1 expression in stage III unresectable disease. Currently, this approach is the standard of care [9]. However, because of the high cost of immunotherapy, definitive CCRT remains the standard of care in many developing countries including Thailand. Thus, exploring effective predictive biomarkers and understanding the mechanism that drives the interaction between immunotherapy and CCRT warrants further investigation.

The diversity of T and B cells in terms of their receptor repertoire provides broad protection against a vast diversity of pathogens including cancer cells. Diversity is a measure of the number of unique T cell receptors or TCRs (richness) and their similarity in frequency [10, 11]. Targeted sequencing of the highly variable complementarity determining region 3 (CDR3) of the beta chain of the T cell receptor (TCRβ) can be used to identify T cell clones, their frequency, and the existence of antigenic responses within a repertoire [12]. Several studies suggested that patients with greater T cell clonal expansion (clonality), a characteristic of antigenic responses, exhibited improved clinical responses to immune checkpoint inhibitors (ICIs) in melanoma [13, 14]. Recent studies investigated protective immune responses in the lung by defining the role of neutrophils, antigen-presenting cells, and T cells [15, 16]. However, we still have limited knowledge of T cell repertoire attribution and the correlation with patient outcomes. Understanding specific T cell responses to immunotherapy treatment in NSCLC may help us to predict therapeutic efficacy and select patients that are most likely to benefit from ICI therapy [17, 18].

In this study, we investigated immune repertoire profiles and the association of TCRβ clonal expansion with treatment response in stage III unresectable NSCLC.

Methods

Patients and study design

This was a cross-sectional study. The inclusion criteria included patients with histologically confirmed stage III NSCLC according to the 8th edition of the American Joint Committee on Cancer (8th AJCC) staging, who were aged 18 years or older and diagnosed at Ramathibodi Hospital between August 2019 and September 2021. Cut-off survival follow-up was in September 2022. Eligible patients had no disease progression after receiving definitive CCRT and received consolidation treatment with durvalumab every 2 weeks for 1 year. Patients with allergies or contraindications to durvalumab were excluded. Blood samples were collected at baseline or within 2 months from starting durvalumab and then every 3–4 months during the treatment, and clinical status was gathered corresponding to changes in *TCRβ* polymorphisms at each timepoint (Fig. 1) until disease progression. Computed tomography scans were performed every 3–4 months to monitor disease status until disease progression or discontinuation of treatment due to toxicity. Treatment response was evaluated by the investigator according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1. The experimental protocol was approved by the Human Research Ethics Committee of Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (Institutional Review Board number COA. MURA2019/730). All methods were performed in accordance with relevant guidelines and local regulations.

Next generation sequencing

Buffy coat (from peripheral blood), DNA, and RNA were extracted and amplified from blood samples by polymerase chain reaction technique with specific primers to generate the TCR library. High throughput sequencing generated TCR sequencing data that could be analyzed with bioinformatics tools based on different research objectives.

RNA extraction was performed from peripheral blood mononuclear cells using the MagMAX mirVana total RNA isolation kit (Thermo Fisher Scientific, Waltham, MA, USA). The RNA concentration was determined using the Qubit™ Fluorometer technique by Qubit™ RNA

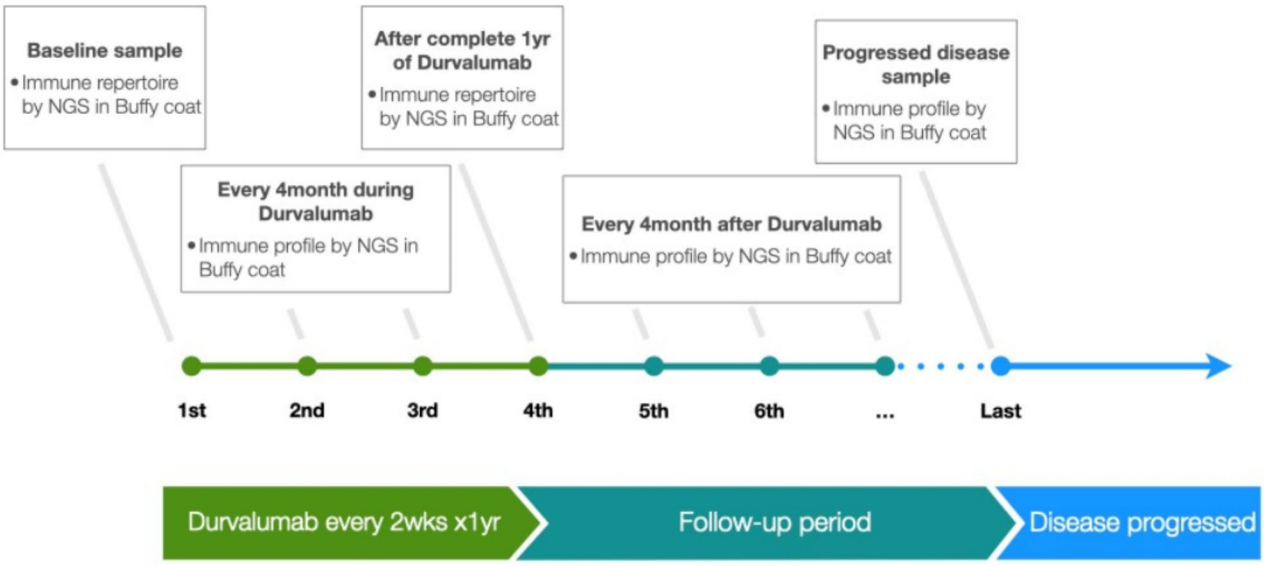

Fig. 1 Blood sample collection schedule

HS Assay Kit, then NGS was run using the Oncomine™ TCR Beta-SR Panel on the Ion AmpliSeq™ according to the manufacturer's instructions. Clonal diversity assessment and bioinformatic analyses were completed using Ion Reporter software as per the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis

Baseline characteristics, tumor characteristics, clinical outcomes, and adverse events were reported as descriptive statistics. Normality of data was assessed using the Shapiro-Wilk test. Based on distribution results, parametric tests (Pearson's correlation) were used for normally distributed data, while non-parametric tests (Spearman's correlation) were applied for non-normally distributed data.

Disease progression was defined as unequivocal progression of existing non-target lesions, progression in target lesions of 20%, or more new lesions according to the RECIST criteria version 1.1.

Shannon diversity was explored in each patient and in all pooled samples correlated to disease status using Pearson correlation. The Shannon diversity index measured the diversity among TCR clones: greater Shannon diversity indicated greater diversity of TCR clones. TCR convergence is defined as the aggregate frequency of TCR clones sharing a CDR3 and variable genes. Meanwhile, evenness indicates the commonness of TCR clones. R Studio software version 2021 build 372 was used for downstream analyses. Changes in TCR convergence ratio, and evenness were calculated and compared between the non-PD and PD groups using the Spearman's correlation. *P*-values of <0.05 were considered statistically significant.

Results

Baseline characteristics

A total of 15 patients diagnosed with unresectable stage III NSCLC and who received treatment with definitive CCRT followed by durvalumab as consolidation therapy were included in this study. Two out of 15 patients had inadequate buffy coat for NGS analysis. One patient was excluded because of necrotizing fasciitis during durvalumab treatment, which interfered with the immune repertoire interpretation. Ultimately, 12 patients were included in the study, with a total of 44 samples collected and analyzed through pooled analysis. The baseline characteristics are listed in Table 1. Most patients were male (83.3%), with a median age of 68 years, and were mainly current smokers (41.7%) with ECOG 0–1 (91.7%), clinical T3–4 (66.6%), and clinical N2 disease (66.7%). The prevalence of stage IIIA, IIIB, and IIIC disease was 25%, 66.7%, and 8.3%, respectively. Adenocarcinoma was the most frequently detected cell type (58.4%), while squamous cell carcinoma was identified in only 8.3% of patients. Most patients received concurrent CCRT with weekly carboplatin and paclitaxel (91.7%). During durvalumab treatment, one patient developed immune-related pneumonitis grade 3 and one patient developed immune-related hepatitis grade 3, which were improved with drug interruption and steroid treatment. At the time of data cut-off on September 30, 2022, the median follow-up time was 25.5 months (range, 12–44 months), 5 out of 12 patients (42%) had disease progression, and 7 out of 12 patients were still alive without disease progression.

Table 1 Demographic and clinical characteristics

Characteristic	Proportion *n* = 12 (%)
Median age at diagnosis, years (range)	68 (47–77)
Male sex, no. (%)	10 (83.3)
Smoking status, no. (%)	
–Current smoking	5 (41.7)
–Former smoking	4 (33.3)
–Never smoked	3 (25)
Histological type, no. (%)	
–Adenocarcinoma	7 (58.4)
–Squamous cell carcinoma	1 (8.3)
–Non-small cell carcinoma, NOS	4 (33.3)
Tumor stage, no. (%)	
–T1	1 (8.3)
–T2a	2 (16.8)
–T2b	1 (8.3)
–T3	4 (33.3)
–T4	4 (33.3)
Nodal stage, no. (%)	
–N0	1 (8.3)
–N1	1 (8.3)
–N2	8 (66.7)
–N3	2 (16.7)
Clinical staging, no. (%)	
–IIIA	3 (25)
–IIIB	8 (66.7)
–IIIC	1 (8.3)
ECOG performance status, no. (%)	
–0	3 (25)
–I	8 (66.7)
–II	1 (8.3)
CCRT regimen, no. (%)	
–Carboplatin and paclitaxel	11 (91.7)
–Cisplatin and etoposide	1 (8.3)
Disease progression at 3 years	
–No	7 (58.3)
–Yes	5 (41.7)
Immune-related adverse events, no. (%)	
–Pneumonitis	1 (8.3)
–Hepatitis	1 (8.3)

ECOG: Eastern Cooperative Oncology Group, CCRT: concurrent chemoradiotherapy

Dynamic changes in immune repertoire and clinical correlations pre- and post-durvalumab treatment

We conducted analyses of the immune repertoire and its clinical correlations both before and after durvalumab treatment. To assess the dynamic changes in the immune repertoire and its clinical correlations, it was necessary to compare baseline immune repertoire profiles with those obtained after the completion of durvalumab treatment. Consequently, two out of seven patients in the non-progressive disease (non-PD) group were excluded due to the absence of baseline immune repertoire profiling. In the progressive disease (PD) group, three out of five patients were excluded from the subset analysis: one lacked baseline immune repertoire data due to enrollment during the COVID-19 pandemic, one developed brain metastases and subsequently died, and one experienced pneumonitis and death without immune repertoire data following durvalumab treatment. Therefore, a total of seven patients were included in the subset analysis of dynamic changes in the immune repertoire and its clinical correlations before and after durvalumab treatment.

Table 2 Median duration of ICI therapy and changing immune repertoire according to disease status the non-PD group exhibited a smaller increase in Shannon diversity, with p-value of 0.381, higher evenness observed in the PD group indicates a less specific T cell clonal response to immune checkpoint inhibitors

	Non-PD group *N* = 5	PD group *N* = 2	*P*-value
Median duration of ICI therapy, months (range)	15 (3.1–18)	12.35 (2.3–18)	0.040
Median change in immune repertoire (range)			
Shannon diversity	+ 0.738 (-1.744–4.272)	+ 3.132 (1.414–4.850)	0.381
Evenness	-0.155 (– 0.305–0.066)	+ 0.149 (0.061–0.238)	0.191
TCR convergence ratio	–0.197 (– 0.206–0.602)	+ 0.254 (0–0.508)	0.952

Changes in Shannon diversity in non-PD patients and PD patients were + 0.738 and + 3.132, respectively. The non-PD group exhibited a smaller increase in Shannon diversity, with p-value of 0.381, suggesting a less diverse T cell population compared to the PD group. In contrast, the evenness of T cell clones decreased by – 0.155 in the non-PD group, while it increased by approximately + 0.149 in the PD group (Table 2). The higher evenness observed in the PD group indicates a less specific T cell clonal response to immune checkpoint inhibitors (ICIs).

TCR convergence was calculated as the aggregate frequency of sharing a variable gene and CDR3 amino acid sequence with at least one other identified clone in the same sample. Although TCR convergence has limitations in its interpretation due to the varying number of TCR clones across individual samples, we used the TCR convergence ratio, defined as the TCR convergence number divided by the total TCR clones in the same sample. The TCR convergence ratio in the non-PD group decreased (– 0.197) while it increased by approximately 0.254 in the PD group. This showed a less diverse TCR clonal response to durvalumab treatment in the PD group.

Dynamic changes in immune repertoire and clinical correlation from first-dose durvalumab until data cut-off

Longitudinal dynamic changes in the immune repertoire from first-dose durvalumab until data cut-off were

observed in our study in every patient while receiving immunotherapy. These change in the immune repertoire profiles can be categorized into four groups according to the completeness of treatment (1 year of durvalumab treatment) and disease status at the data cut-off date (Table 3, Supplement Table 1 and supplement Fig. 1):

1. Complete treatment without disease progression. This group of patients (5/12) had a long median follow-up period of 21 months, and the results showed that the trends of Shannon diversity were increased while the trends of evenness were decreased in each patient. This probably indicated that the immune response was adapting tumor neoantigen-specific TCR clones from prior durvalumab treatment, corresponding with an increased TCR convergence ratio.
2. Complete treatment with disease progression. Only one patient was classified in this group with a median follow-up time of 12 months. Shannon diversity and evenness were increased, which probably indicated that there was no specific immune response to tumor neoantigens, as well as no change in TCR convergence ratio over time.
3. Incomplete treatment without disease progression. There were two patients in this group with a median follow-up time of 7.5 months. The treatments for these two patients were stopped early because of acute kidney injury and severe hepatitis. We also found increased Shannon diversity and TCR convergence and decreased evenness, as seen in the complete treatment without disease progression group. This finding probably indicated that even a short exposure time to ICIs can lead to durable immunological responses in some patients.
4. Incomplete treatment with disease progression. There were four patients in this group with a median follow-up time of 5 months. Durvalumab treatment was stopped because of disease progression. The data might be difficult to interpret because some patients had just one sample before disease progression, but we can still observe the trend in changing immune repertoire profiles. Shannon diversity and TCR convergence ratio were increased while evenness decreased, indicating no specific immune response to durvalumab treatment.

We also performed a pooled samples analysis in all patients to explore the trend of TCR convergence frequency. Overall, over a longer follow-up period, TCR convergence frequency was higher after receiving durvalumab ($R = 0.39$) and was significantly higher in non-PD patients compared with PD patients ($P = 0.0052$) (Fig. 2). Increased TCR convergence represented a T-cell specific clonal expansion response to durvalumab treatment in non-PD patients. We identified a pattern difference

Table 3 The immune repertoire profiles based on treatment and patterns of clinical response

	Demographic data		Durvalumab data			Changing of immune repertoire		
No.	**Sex/ age**	**Stage**	**Total cycle**	**Dose (mg)**	**Stoppage reason**	**Shannon diversity**	**Evenness**	**TCR convergence ratio**
Complete treatment with non-PD								
2	M 61	3 A	25	500	Complete	+2.096	−0.201	+0.394%
3	M 51	3B	28	800	Complete	+8.746	+0.132	+1.540%
6	M 59	3B	25	640	Complete	+3.160	−0.162	+0.152%
7	F 59	3 A	26	800	Complete	+6.670	−0.073	+1.224%
11	M 69	3B	26	500	Complete	+4.272	−0.180	−0.583%
Complete treatment with PD								
1	M 70	3B	25	700	PD (liver)	+1.414	+0.242	0.000
Incomplete treatment with non-PD								
5	M 66	3B	10	690	Non-PD (AKI)	+0.620	-0.416	+0.650%
13	F 76	3B	1	530	irAE (hepatitis gr 3)	NA	NA	NA
Incomplete treatment with PD								
8	M 75	3B	5	600	PD (LN, pleura, brain)	NA	NA	NA
9	M 47	3 C	1	1000	irAE (pneumonitis gr 3)	+0.850	+0.061	+0.500%
14	M 77	3 A	12	600	PD (local recurrence LUL)	+0.918	-0.020	+0.184%
15	M 72	3B	1	450	Dead from infection (pneumonitis)	NA	NA	NA

M: Male, F: Female, LN: Lymph nodes, AKI: Acute kidney injury, irAE: immune related adverse event, gr: grade, LUL: Left upper lobe of lung, TCR: T cell receptor, PD: Progressive disease, NA: Not applicable

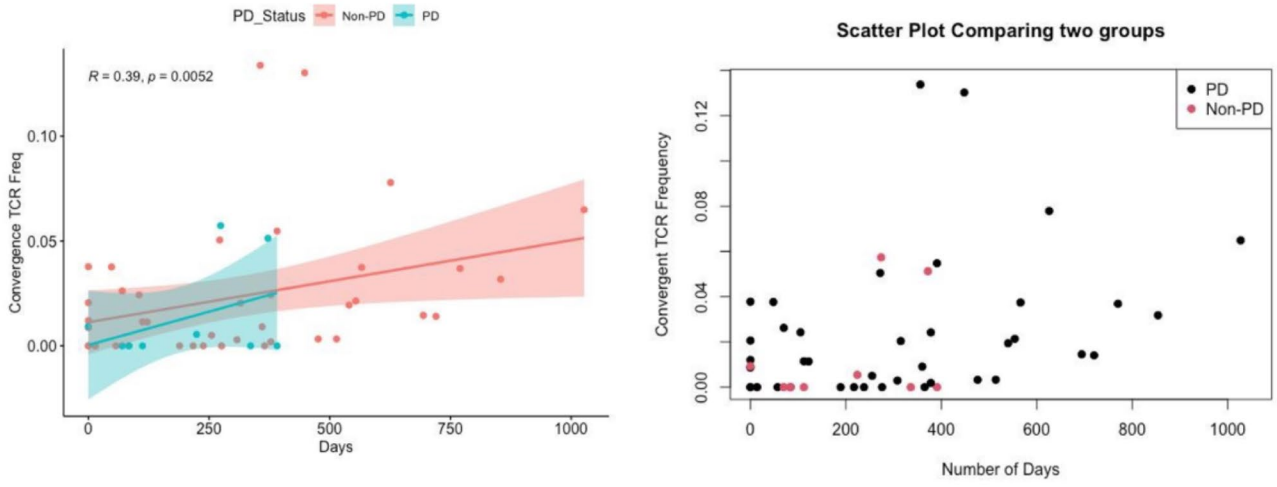

Fig. 2 Treatment scatterplot indicating the relationship between the PD and non-PD groups and dynamic changes in TCR convergence frequency according to treatment response by using the analysis Spearman correlation. PD: Progressive disease, TCR: T cell receptor

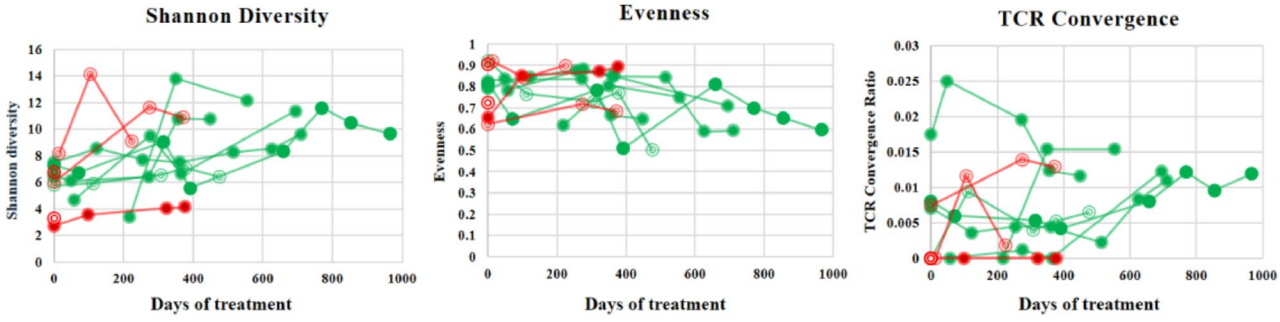

Fig. 3 Changes in immune repertoire profiles during immunotherapy treatment between patients with progressive disease (red color) and non-progressive disease (green color). (**A**) Shannon diversity, (**B**) evenness, and (**C**) TCR convergence ratio. TCR: T cell receptor

in changing immune repertoire in patients between the PD and non-PD groups, and Shannon diversity in each patient was increased and persistent in the non-PD group, indicating greater diversity in the TCR response to treatment. The evenness of the non-PD group tended to decrease more compared with the PD group, showing more diversity in TCR changes during durvalumab treatment. We also found that the TCR convergence ratio increased in the non-PD group, which can be explained by the specific clonal expansion response to tumor neoantigens (Fig. 3).

Case demonstration

Patient with disease progression

Case #1 (patient number 9) A 47-year-old male heavy smoker was diagnosed with cT4N3M0 squamous cell lung cancer. He stopped durvalumab after one cycle of treatment because of grade 3 pneumonitis and had a local recurrence of lung cancer within 1 year. We monitored the frequency of CDR3, which determined different T cell clone specificities and distinguished them from differences in amino acid sequences at the time of starting durvalumab and 6 and 12 months after stopping durvalumab. Before the patient received durvalumab, some amino acid sequences were higher compared with others. However, after durvalumab was started and stopped after the first cycle, the variations in CDR3 frequency were lost during the follow-up period (Fig. 4). The less clonal-specific T cells in this patient referred to an increase in both Shannon diversity and evenness, which correlated with disease progression at 10 months after stopping durvalumab.

Patient without disease progression

Case #2 (patient number 2) A 61-year-old male heavy smoker was diagnosed with cT2aN2M0 lung adenocarcinoma. He had completed 1 year of durvalumab treatment, and he had no evidence of disease progression at the cut-off date. Changes in *TCRβ* polymorphisms at each time-point showed a specific clone of T cells with an amino acid in the CDR3 region of "ASSQDRRDNQPQH" (brown line) that increased 8 months after starting durvalumab and continued to increase even after the completion of durvalumab for 8 months (Fig. 5). Moreover, the CDR3

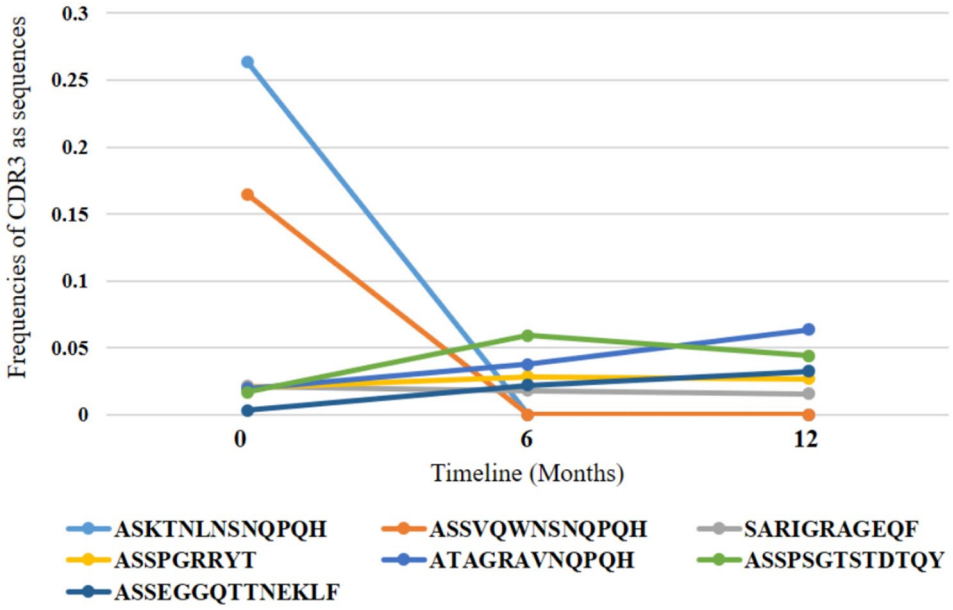

Fig. 4 Case study 1 with progressive disease showing the trend in CDR3 amino acid sequence frequencies changing over time with durvalumab treatment. CDR3: complementarity determining region 3

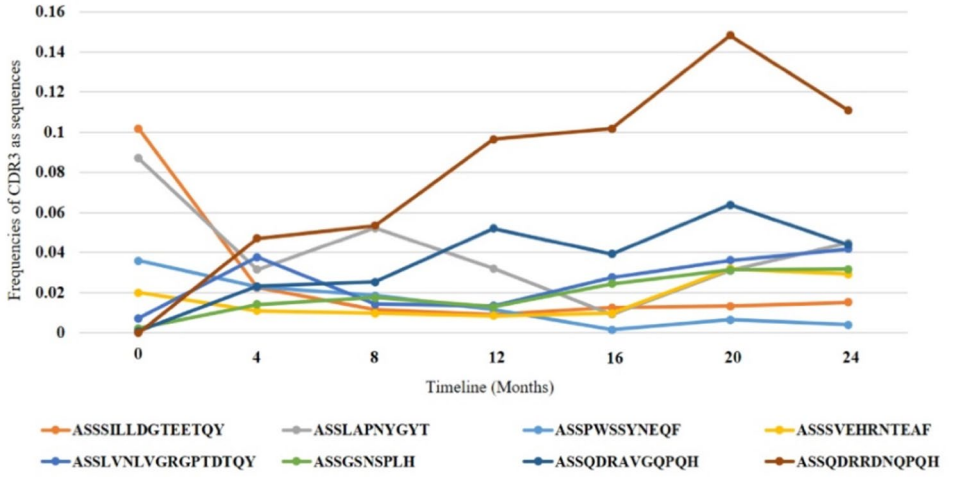

Fig. 5 Case study 2 with non-progressive disease showing the trend in CDR3 amino acid sequence frequencies changing over time with durvalumab treatment. CDR3: complementarity determining region 3

region of *TCRβ* also showed an amino acid sequence of "ASSQDRAVGQPQH" (deep blue line) that increased in the same trend. However, both slightly decreased after completing 12 months of durvalumab. While there was only a small difference in the amino acid between them (ASSQDRRDNQPQH vs. ASSQDRAVGQPQH), these T cell clones showed an immune repertoire response against cancer neoantigens.

Case #3 (patient number 6) A 59-year-old female former light smoker was diagnosed with cT4N2M0 NSCLC. She completed 1 year of durvalumab treatment without treatment interruption. The CDR3 region of *TCRβ* showed an amino acid sequence of "ASSYGDTQY" (blue line) (Fig. 6) that was sustainably high before durvalumab initiation and that maintained a higher frequency compared with others during and even after completing durvalumab treatment for more than 1 year. This finding demonstrated the long immune response to tumor neoantigens prior to durvalumab and the long specific T-cell clone response to immunotherapy in responding patients.

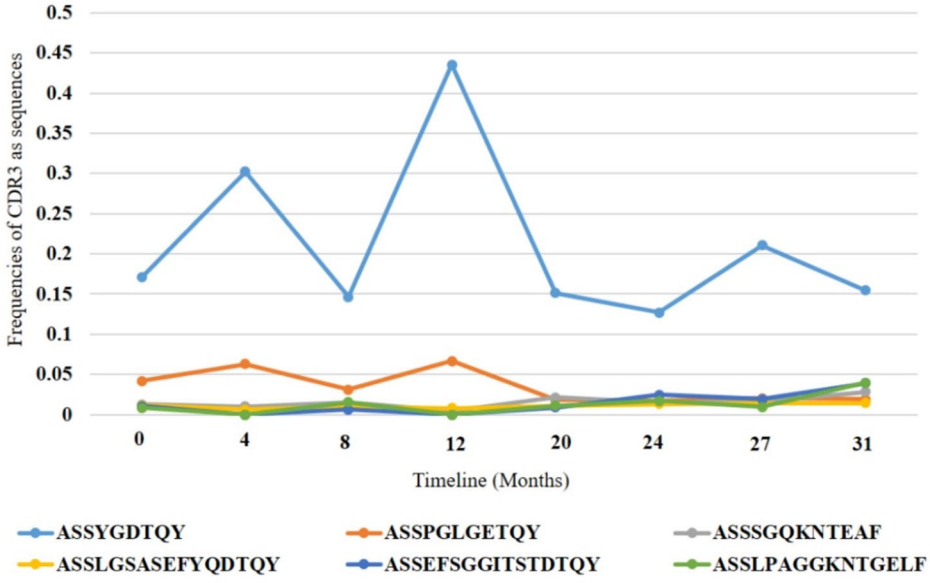

Fig. 6 Case study 3 with non-progressive disease showing the trend in CDR3 amino acid sequence frequencies changing over time with durvalumab treatment. CDR3: complementarity determining region 3

Discussion

This prospective study of the *TCRβ* short-read assay, which identified CDR3 region *TCRβ* polymorphisms in patients with stage III unresectable NSCLC treated with definitive CCRT followed by 1 year of durvalumab treatment, demonstrated dynamic changes in immune repertoire profiles between non-PD patients and PD patients. These included the trend in increasing levels of Shannon diversity value in the non-PD group compared with the PD group, together with the trend in decreased evenness of T cell clones in non-PD patients. These results suggested the development of specific T cell clones after the use of ICIs in responding patients. Moreover, TCRβ convergence frequency was higher after receiving durvalumab ($R = 0.36$) and was significantly higher in non-PD patients compared with PD patients in a pooled samples analysis. Increased TCRβ convergence represented a T cell-specific clonal expansion response to durvalumab treatment in non-PD patients.

Previous studies have reported varying results for TCR analyses in different tissue or blood samples and cancer types. Prospective studies in patients with advanced NSCLC have suggested that an increase in the percentage of TCR frequency increases the likelihood of more tumor-specific T cells that can control tumor proliferation [19] and a decrease in diversity is observed when a subset of TCR clones expand and dominate, which has been correlated with treatment response in some studies using ICIs as a monotherapy [20]. The blockade of PD-L1 enables more antigen-specific T cell clones to survive apoptosis, resulting in a decrease in T cell diversity. The interpretation of the 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. Studies on the dynamic changes of the TCR repertoire have also reached conflicting conclusions [21, 22]. These inconsistencies highlight the need for further precise studies on the peripheral TCR repertoire.

Sheng J et al. reported 12 patients with advanced stage NSCLC treated with atezolizumab explored the CDR3 of *TCRβ* from patients' peripheral blood mononuclear cells. The dynamic change in the immune repertoire of the response group showed increased evenness of the TCR repertoire after receiving atezolizumab compared with the non-response group, and after disease progression, the evenness index had clearly decreased. This result demonstrated the variety in T cell clones that increased after immunotherapy treatment in responding patients [19]. Another prospective study from Norway [20] reported a decreased Shannon diversity index in responding patients with advanced-stage NSCLC who were treated with immunotherapy in combination with SBRT. This study observed greater TCR clone expansion in the responder group, which could be attributed more to the effects of radiotherapy than immunotherapy. The findings suggest that T cell responses may differ depending on the treatment modality. Furthermore, the radiation-induced expansion of T cells may enhance the effects of immunotherapy, supporting the idea that a less diverse but more targeted immune response could be associated with favorable treatment outcomes in these patients. The reduction in Shannon diversity observed in the Norwegian study might reflect a contraction of the immune repertoire as clonal expansion occurs in response to

treatment modalities, with some clonally expanded T cells more effectively targeting the tumor. Both results suggested two possible hypotheses. First, specific T cell clones respond to specific neoantigens. Second, tumor heterogeneity causes a variety of neoantigens to be produced after immunotherapy treatment, and also activates various T cell clones to attack each specific antigen. In addition, a Chinese study also reported a significantly higher level of TCR pool singleton frequency [19], which reflected the clonal proliferation of the TCR repertoire in the response group compared with the non-response group after immunotherapy treatment. A Spanish study of pembrolizumab monotherapy in advanced stage NSCLC also demonstrated increased TCR richness in the durable clinical benefit (DCB) group compared with the non-DCB group. These results were similar to our study and suggested that specific T cell clone frequency, evenness, and diversity of TCRβ during ICI treatment may be an important predictive biomarker for immunotherapy.

Limitation

Our study had several limitations. First, the small sample size was primarily due to the impact of the COVID-19 pandemic during patient enrollment, which resulted in patients being unable to provide samples at every appointment, thereby limiting the statistical power of the study. Although we attempted to extend the follow-up period, some of our patients experienced rapid clinical deterioration and early mortality, which resulted in a shorter follow-up duration than initially planned. Despite these limitations, this pilot study aimed to demonstrate the concept of dynamic changes in the immune repertoire profile and their potential impact on clinical outcomes. Second, all participants were recruited from a single institution, which may limit the generalizability of the findings to other populations. Lastly, the short-read sequencing technique had less specific region identification on TCR compare to the long-read technique which may affect the accuracy of TCR characterization.

Conclusions

In conclusion, our study confirmed the hypothesis that ICIs result in more tumor antigens being presented to T cells and more surviving tumor-specific T cells during the ICI treatment. The peripheral blood TCRβ repertoire in stage III unresectable NSCLC correlated with several clinical characteristics and patient immune statuses. Furthermore, dynamic changes in the TCR repertoire during immunotherapy may be useful predictive biomarkers for predicting response to ICI treatment. A larger cohort is needed to confirm this hypothesis.

Abbreviations

NSCLC	Non-Small Cell Lung Cancer
TCR	T Cell Receptor
TCRβ	Beta Chain of the T cell Receptor
CDR3	Complementarity Determining Region 3
PD	Disease Progression
non-PD	No disease progression
CCRT	Concurrent Chemo Radiation
PFS	Progression-Free Survival
PD-1	Programmed Death 1
PD-L1	Programmed Death Ligand 1
OS	Overall Survival
ICIs	Immune Checkpoint Inhibitors
RECIST	The Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic Acid
NGS	Next Generation Sequencing
ECOG	Eastern Cooperative Oncology Group
DCB	Durable Clinical Benefit

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13716-w.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

We thank the Division of Medical Oncology, the Department of Medicine, the Research Center, the Center for Medical Genomics, the Ramathibodi Lung Cancer Consortium (RLC), and the Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, for providing all samples and undertaking all tests for this research. We also thank all patients who participated in this study.

Author contributions

N.L.: Study design, methodology, formal analysis, Laboratory testing, software, validation, investigation, analysis, interpretation, resources, project administration, conclusion, writing-original draft and editing. D.A.: Methodology, formal analysis, investigation, data curation, interpretation, conclusion, writing-original draft and editing. S.O.: Methodology, formal analysis, investigation, software, data curation, interpretation, writing-review and editing. T.T: Investigation, data curation, analysis, interpretation, conclusion, writing-review and editing. P.L: Investigation, data curation, interpretation, writing-review and editing. V.T: Software, data curation, interpretation, writing-review and editing. K.K: Investigation, resources, data curation, writing-review and editing. N.M: Investigation, resources, sample collection. P.S.: Investigation, data curation, interpretation, writing-review and editing. P.l.: Investigation, sample preparation, writing-review and editing. A.C.: Investigation, sample preparation, software, writing-review and editing. I.S: Investigation, software, writing-review and editing. T.D: Investigation, resources, sample collection, data curation, writing-review and editing. E.S.: Investigation, resources, sample collection, data curation, writing-review and editing. W.C: Investigation, software, interpretation, conclusion, writing-review and editing. T.R: Study design, conceptualization, methodology, formal analysis, interpretation, investigation, resources, sample collection, data curation, conclusion, writing-review and editing, visualization, supervision, funding acquisition. N.T: Study design, conceptualization, methodology, formal analysis, laboratory testing, software, validation, investigation, interpretation, conclusion, resources, supervision, writing-review and editing.

Funding

This research project is supported by Faculty of Medicine Ramathibodi Hospital Mahidol University, Bangkok, Thailand.

Data availability

The raw data (FASTQ files) relevant to our submission are now accessible as a reference in the Sequence Read Archive (SRA) under Project ID: PRJNA1026052 on NCBI. https://www.ncbi.nlm.nih.gov/sra/PRJNA1026052.

Declarations

Ethics approval and consent to participate

The Human Research Ethics Committee of Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, approved this study (IRB number COA. MURA2019/730). The study adhered to the Declaration of Helsinki to this effect in the 'Ethics approval and consent to participate' section or appropriate national guidelines. All methods were performed in accordance with the relevant guidelines and local regulations. Informed consent was obtained from all patients in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Center for Medical Genomics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[2]Division of Medical Oncology, Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[3]Faculty of Medicine Ramathibodi Hospital, Ramathibodi Lung Cancer Consortium (RLC), Mahidol University, Bangkok, Thailand
[4]Oncology Unit Sriphat Medical Center, Faculty of Medicine, Chiangmai University, Chiangmai, Thailand
[5]Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[6]Thermo Fisher Scientific, South San Francisco, CA, USA
[7]Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[8]Research Center, Faculty of Medicine Ramathibodi Hospital, Mahidol University, 270 Rama VI Road, Tungpayathai, Rajathewee, Bangkok 10400, Thailand
[9]Faculty of Science, Mahidol University, Bangkok, Thailand

Received: 5 September 2023 / Accepted: 11 February 2025
Published online: 24 February 2025

References

1. World Health Organization. 2021; 2021 [International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today/data/factsheets/populations/764-thailand-fact-sheets.pdf
2. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc. 2008;83(5):584–94.
3. Yoon SM, Shaikh T, Hallman M. Therapeutic management options for stage III non-small cell lung cancer. World J Clin Oncol. 2017;8(1):1–20.
4. Aupérin A, Le Péchoux C, Rolland E, Curran WJ, Furuse K, Fournel P, et al. Meta-analysis of concomitant versus sequential radiochemotherapy in locally advanced non-small-cell lung cancer. J Clin Oncol. 2010;28(13):2181–90.
5. Ahn JS, Ahn YC, Kim JH, Lee CG, Cho EK, Lee KC, et al. Multinational Randomized Phase III Trial with or without consolidation chemotherapy using Docetaxel and Cisplatin after Concurrent Chemoradiation in Inoperable Stage III Non-small-cell Lung Cancer: KCSG-LU05-04. J Clin Oncol. 2015;33(24):2660–6.
6. Stewart R, Morrow M, Hammond SA, Mulgrew K, Marcus D, Poon E, et al. Identification and characterization of MEDI4736, an antagonistic Anti-PD-L1 monoclonal antibody. Cancer Immunol Res. 2015;3(9):1052–62.
7. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
8. Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. J Clin Oncol. 2015;33(17):1974–82.
9. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after Chemoradiotherapy in Stage III non–small-cell Lung Cancer. N Engl J Med. 2017;377(20):1919–29.
10. Daly AJ, Baetens JM, De Baets B. Ecological diversity: measuring the unmeasurable. Mathematics. 2018;6(7):119.
11. Liu X, Wu J. History, applications, and challenges of immune repertoire research. Cell Biol Toxicol. 2018;34(6):441–57.
12. Robins HS, Campregher PV, Srivastava SK, Wacher A, Turtle CJ, Kahsai O, et al. Comprehensive assessment of T-cell receptor beta-chain diversity in alpha-beta T cells. Blood. 2009;114(19):4099–107.
13. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568–71.
14. Roh W, Chen PL, Reuben A, Spencer CN, Prieto PA, Miller JP et al. Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. Sci Transl Med. 2017;9(379).
15. Lavin Y, Kobayashi S, Leader A, Amir ED, Elefant N, Bigenwald C, et al. Innate Immune Landscape in Early Lung Adenocarcinoma by Paired single-cell analyses. Cell. 2017;169(4):750–e6517.
16. Kargl J, Busch SE, Yang GHY, Kim K-H, Hanke ML, Metz HE, et al. Neutrophils dominate the immune cell composition in non-small cell lung cancer. Nat Commun. 2017;8(1):14381.
17. Reuben A, Zhang J, Chiou S-H, Gittelman RM, Li J, Lee W-C, et al. Comprehensive T cell repertoire characterization of non-small cell lung cancer. Nat Commun. 2020;11(1):603.
18. Chapter 8 - The T Cell Receptor: Proteins and Genes. In: Mak TW, Saunders ME, Jett BD, editors. Primer to the Immune Response (Second Edition). Boston: Academic Cell. 2014. pp. 181–96.
19. Sheng J, Wang H, Liu X, Deng Y, Yu Y, Xu P et al. Deep sequencing of T-Cell receptors for Monitoring Peripheral CD8 + T cells in Chinese Advanced non–small-cell lung Cancer patients treated with the Anti–PD-L1 antibody. Front Mol Biosci. 2021;8.
20. Öjlert ÅK, Nebdal D, Snapkov I, Olsen V, Kidman J, Greiff V, et al. Dynamic changes in the T cell receptor repertoire during treatment with radiotherapy combined with an immune checkpoint inhibitor. Mol Oncol. 2021;15(11):2958–68.
21. Cha E, Klinger M, Hou Y, Cummings C, Ribas A, Faham M, et al. Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. Sci Transl Med. 2014;6(238):238ra70.
22. Akyüz N, Brandt A, Stein A, Schliffke S, Mährle T, Quidde J, et al. T-cell diversification reflects antigen selection in the blood of patients on immune checkpoint inhibition and may be exploited as liquid biopsy biomarker. Int J Cancer. 2017;140(11):2535–44.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.