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Thorough Lymphadenectomy Adversely Impacts the Outcome After Immunotherapy for Postoperative Intrathoracic Recurrence of Non-Small Cell Lung Cancer

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Posted Date: 20 May 2025

doi: 10.20944/preprints202505.1530.v1

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Thorough Lymphadenectomy Adversely Impacts the Outcome After Immunotherapy for Postoperative Intrathoracic Recurrence of Non-Small Cell Lung Cancer

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Simple Summary: Patients with non-small-cell lung cancer (NSCLC) often experience recurrence after surgery. Immune checkpoint inhibitors (ICIs) have become an effective treatment for patients with recurrence; however, the factors influencing the outcome are not fully understood. In this study, we analyzed patients who received ICI monotherapy for postoperative recurrence. We found that patients with high programmed death-ligand 1 (PD-L1) expression levels in surgically resected samples responded better to ICIs and had longer survival. Interestingly, patients who had a greater number of lymph nodes dissected during surgery showed poorer outcomes with ICIs, particularly those with recurrence within the thoracic cage. This effect was particularly clear in patients with recurrence limited to the chest. These results suggest that preservation of lymph nodes is beneficial to achieve better outcomes after ICI therapy, providing a rationale for recommending preoperative ICI treatment when the lymphatic immune system is intact. (142)

Abstract: Background: Immune checkpoint inhibitors (ICIs) have emerged as a standard treatment for recurrent non-small cell lung cancer (NSCLC). However, the predictive factors for their efficacy in postoperative recurrence remain unclear. Methods: We retrospectively analyzed 30 patients who underwent complete surgical resection of NSCLC and subsequently received ICI monotherapy for recurrence. Clinicopathological factors, including the programmed death ligand 1 (PD-L1) expression and number of dissected lymph nodes (DLNs), were evaluated for their association with the treatment response and prognosis. Results: The high expression of PD-L1 (≥50%) in surgical specimens was significantly associated with higher response rates, prolonged progression-free survival (PFS), and overall survival (OS) after the initiation of ICI therapy. In contrast, patients with ≥15 DLNs had a significantly shorter PFS than those with <15 DLNs, particularly in patients without extrathoracic recurrence. A multivariate analysis identified the expression of PD-L1, the number of DLNs, and the presence of extrathoracic metastasis as independent prognostic factors for ICI-PFS. Conclusion: Extensive lymphadenectomy may worsen the prognostic outcomes of ICI monotherapy, possibly by impairing tumor-draining lymph node-mediated immunity. These findings highlight the importance of lymphatic preservation and support the clinical rationale for neoadjuvant ICI therapy for resectable NSCLC. (193)

Keywords: non-small cell lung cancer; immune checkpoint inhibitor; lymph node dissection; tumor-draining lymph node; recurrence; neoadjuvant therapy; PD-L1 expression

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1. Introduction

Non-small cell lung cancer (NSCLC) is one of the most aggressive malignancies, accounting for the majority of cancer-related deaths worldwide [1]. Despite complete resection, approximately half of stage II and two-thirds of stage III patients experience recurrence, and the prognosis is poor [2]. There is an urgent need to develop more curative treatment strategies for both patients with metastatic NSCLC and those with recurrent NSCLC.

Immune checkpoint inhibitors (ICIs) have been used in the treatment of NSCLC, starting with a clinical trial of nivolumab for unresectable squamous cell carcinoma and non-squamous cell carcinoma in 2015 [3,4]. Since then, several clinical trials have been conducted on the combination of ICIs and chemotherapy for unresectable NSCLC [5–9], and this systemic therapy is currently used as the first-line treatment for patients with unresectable NSCLC with negative driver gene mutations.

Recently, the perioperative treatment of resectable NSCLC has attracted attention. Currently, atezolizumab as adjuvant therapy after chemotherapy [10], neoadjuvant nivolumab and chemotherapy combination therapy [11], and neoadjuvant pembrolizumab and chemotherapy combination therapy followed by surgery and adjuvant pembrolizumab therapy are being used in clinical practice [12]. Several clinical trials are currently ongoing for the treatment of ICIs before and after surgery [13–15]. While neoadjuvant ICI therapy is expected to achieve the early control of micrometastases throughout the body [16], there is also the issue that a certain number of patients become inoperable due to progression during ICI treatment, and this remains a controversial issue.

The expression of programmed death-ligand 1 (PD-L1) is frequently used as a predictor of the efficacy of ICIs for unresectable NSCLC [17]. On the other hand, although there are some reports on predictors of the efficacy of ICIs for postoperative recurrence [18–20], these have not yet been fully elucidated. In this study, we analyzed various clinicopathological factors in patients treated with ICI monotherapy for recurrence after complete resection of NSCLC and clarified the factors that influenced the efficacy of ICI. Furthermore, we discuss the significance of neoadjuvant ICI treatment based on the results obtained.

2. Materials and Methods

2.1. Patient Selection

Thirty patients who underwent complete resection for NSCLC at the Department of Thoracic Surgery, Kagoshima University Hospital and received ICI monotherapy at any line of treatment for postoperative recurrence between January 2017 and December 2021 were included. We investigated whether clinicopathological factors, including those at the time of surgery, are related to the efficacy and prognosis of ICI monotherapy. Patients with known driver gene mutations and those who underwent radiotherapy before ICI therapy were excluded.

2.2. Data Collection and Ethical Approval

Patient clinicopathological information was retrospectively obtained through a review of medical records. This study was approved by the Ethics Committee of Kagoshima University Graduate School of Medicine and Dentistry (Approval No. 220178epi) and conformed to the principles outlined in the Declaration of Helsinki. The research participants and their relatives could opt out by viewing the research content hosted online.

2.3. Clinicopathological Factors and Grouping Criteria

Patients were stratified according to the following clinicopathological variables: sex, age at the start of ICI monotherapy (≥70 years or <70 years), Brinkmann Index (≥600 or <600), number of dissected lymph nodes (DLNs) (≥15 or <15), histological type (adenocarcinoma or other), PD-L1 expression rate in surgical specimens (classified into the high expression group [PD-L1 expression: ≥50%] and low expression group of [PD-L1 expression:<50%]), presence or absence of extrathoracic

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recurrent lesions at the initiation of ICI monotherapy, treatment line of ICI monotherapy after recurrence (≥3rd line or <3rd line), and period from surgery to the initiation of ICI monotherapy (≥2 years or <2 years). The efficacy of ICI therapy and prognosis after the initiation of ICI therapy were compared between the groups.

2.4. Radiological Evaluation Criteria

The radiological response to ICI monotherapy was evaluated using RECIST version 1.1, where a complete response (CR) was defined as the disappearance of all target lesions; a partial response (PR) was defined as a reduction in the sum of the diameters of the target lesions of ≥30% from the sum of the diameters at baseline; progressive disease (PD) was defined as an increase in the sum of the diameters of the target lesions of ≥20% or an absolute increase of ≥5 mm or the appearance of new lesions; and stable disease (SD) was defined as either a reduction sufficient to satisfy PR or an increase sufficient to satisfy PD [21].

2.5. Statistical Analysis

Owing to the small sample size, all analyses were considered exploratory. Associations between the objective response rate (ORR) and disease control rate (DCR) according to the radiological evaluation of ICI monotherapy and clinicopathological factors were analyzed using the chi-square test. ICI-progression-free survival (PFS) was defined as the interval from the initiation of ICI monotherapy to the date of disease progression or death from any cause, censored for patients without events at the last clinic visit. In addition, patients who discontinued ICI monotherapy owing to adverse events were censored at the date of discontinuation. ICI-overall survival (OS) was defined as the interval from the initiation of ICI monotherapy until the date of death from any cause and was censored for patients who were alive at the last clinic visit. Kaplan-Meier curves were plotted for ICI-PFS and ICI-OS, and differences between groups were analyzed using the log-rank test. Due to the limited number of patients in this study, we employed a multivariable Cox proportional hazards regression analysis with propensity score adjustment. Propensity scores were calculated using logistic regression, where the dependent variable was defined as ICI-PFS ≥300 days based on the median ICI-PFS of approximately 300 days observed in our cohort. Covariates included in the propensity score model were those that showed statistical significance ($p < 0.05$) in a univariate analysis using the log-rank test to minimize overfitting. In the subsequent multivariable Cox regression, propensity scores were used as adjustment covariates, and the primary variable of interest was not included in the propensity score model to avoid overadjustment and ensure independent evaluation [22]. SPSS (ver. 26, SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Patient Characteristics

The characteristics of the patients included in this study are shown in Table 1. There were 23 men and 7 women, with a median age of 71 years (range: 53–82 years), and most of the patients (27 patients, 90%) were smokers. The most common surgical procedure was lobectomy in 20 patients (66.7%), and the median number of DLNs was 14 (range, 0–39). The histological type of the resected primary tumor was adenocarcinoma in 21 patients (70%) and squamous cell carcinoma in 8 patients (26.7%). The PD-L1 expression in surgical specimens was high in 13 patients (43.3%) and low in 10 (33.3%). Thirteen patients (43.3%) had extrathoracic recurrent lesions at the time of initiation. The drugs used in ICI monotherapy were nivolumab (n=17, 56.7%), pembrolizumab (n=10, 33.3%), and atezolizumab (n=3, 10.0%). The median number of lines of ICI monotherapy after recurrence was 2.1, with the most common being second-line ICI monotherapy (16 patients; 53.3%). The median time from surgery to the initiation of ICI monotherapy was 27.7 months (range: 8.4-69.4 months).

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Table 1. Patients' characteristics.

Gender	Male	23 (76.7%)
	Female	7 (23.3%)
Age	Median (range)	71years (53-82)
Smoking	Ever	27 (90.0%)
	Never	3 (10.0%)
Brinkman Index	Median (range)	970 (0-3600)
Surgical Procedure	Wedge resection	3 (10.0%)
	Segmentectomy	2 (6.7%)
	Lobectomy	20 (66.7%)
	Bi-lobectomy	2 (6.7%)
	Pneumonectomy	3 (10.0%)
Number of DLN	Median (range)	14 (0-39)
Histlogical type	Adenocarcinoma	21 (70.0%)
	Squamous cell carcinoma	8 (26.7%)
	Large cell carcinoma	1 (3.3%)
PD-L1 expression in surgical specimens	no expression	7 (23.3%)
	Low expression	10 (33.3%)
	High expression	13 (43.3%)
Extrathoracic lesions	presence	13 (43.3%)
	absence	17 (56.7%)
Generic name of ICIs	Nivolumab	17 (56.7%)
	Pembrolizumab	10 (33.3%)
	Atezolizumab	3 (10.0%)
Therapeutic Lines of ICIs	First	7 (23.3%)
	Second	16 (53.3%)
	Third	5 (16.7%)
	More	2 (6.7%)
Duration from surgery to initiation of ICIs	Median (range)	27.7 months (8.4-69.4 months)

DLN: Dissected lymph Nodes, PD-L1: Programmed Death-Ligand 1, ICI: Immune Checkpoint Inhibitor

3.2. Radiological Effect of ICI Monotherapy

The best radiological effect was a CR in 5 patients, PR in 6 patients, SD in 8 patients, and PD in 14 patients, with an ORR of 36.7% and a DCR of 63.3%. Table 2 shows the correlation between the

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ORR and DCR with ICI monotherapy and clinicopathological factors. The ORR was significantly higher in the high PD-L1 expression group than in the low PD-L1 expression group in surgical specimens (p=0.018). Figure 1 shows the radiological response according to PD-L1 expression in surgical specimens, the number of DLNs, and the presence or absence of extrathoracic lesions. Regarding the number of DLNs, the DCR in the group with ≥15 DLNs was 50.0%, which was lower than that in the group with <15 DLNs (75.0%); however, this difference was not statistically significant (p=0.257). Regarding the presence or absence of extrathoracic lesions, the DCR in the presence group was 46.2%, which was lower than the 76.5% in the absence group; however, this difference was not statistically significant (p=0.132).

Table 2. Relationship between response rate of ICIs and clinicopathological factors.

Factors		RECIST				ORR	ORR	DCR	DCR
		CR	PR	SD	PD	(%)	p-value	(%)	p-value
Gender	Male	3	5	5	10	34.8	0.515	56.5	0.215
	Female	2	1	3	1	42.9		85.7	
Age	>= 70	3	4	4	5	43.8	0.371	68.8	0.707
	< 70	2	2	4	6	28.6		57.1	
Brinkman Index	>= 600	3	4	5	9	33.3	0.429	57.1	0.419
	< 600	2	2	3	2	44.4		77.8	
Number of DLN	>= 15	2	2	3	7	28.6	0.317	50.0	0.257
	< 15	3	4	5	4	43.8		75.0	
Histlogical type	AD	3	5	6	7	38.1	0.571	66.7	0.687
	Others	2	1	2	4	33.3		55.6	
PD-L1 expression in surgical specimens	>= 50%	5	3	2	3	61.5	0.018*	76.9	0.259
	< 50%	0	3	6	8	17.6		52.9	
Extrathoracic lesions	Presence	1	4	1	7	38.5	0.721	46.2	0.132
	Absence	4	2	7	4	35.3		76.5	
Therapeutic Lines of ICIs	>= 3rd line	1	1	3	2	28.6	0.485	71.4	1.000
	< 3rd line	4	5	5	9	39.1		60.9	
Duration from surgery to initiation of ICIs	>= 2 years	1	4	3	4	41.7	0.61	66.7	1.000
	< 2 years	4	2	5	7	33.3		61.1	

ICI: Immune Checkpoint Inhibitor, RECIST: Response Evaluation Criteria in Solid Tumors, CR: Complete Response, PR: Partial Response, SD: Stable Disease, PD: Progressive Disease, ORR: Objective Response Rate, DCR: Disease Control Rate, DLN: Dissected Lymph Nodes, AD: Adenocarcinoma, PD-L1: Programmed Death-Ligand 1

*: p<0.05

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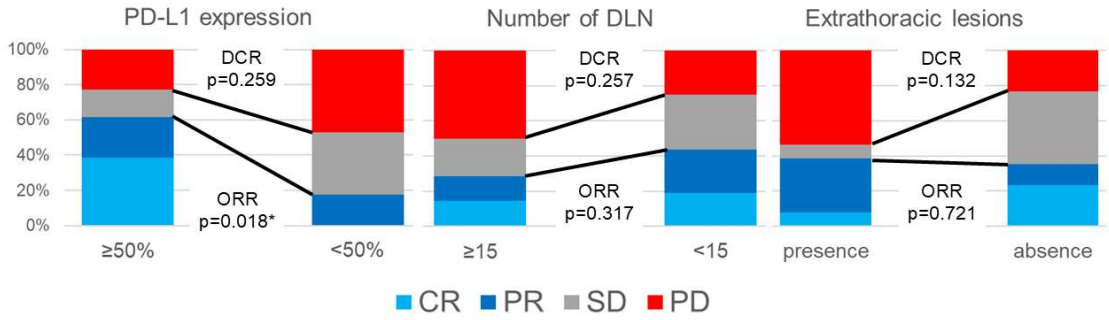

Figure 1. Radiological response to ICI therapy according to the PD-L1 expression in surgical specimens, number of DLNs, and the presence or absence of extrathoracic lesions. ICI, immune checkpoint inhibitor; PD-L1, programmed death ligand 1; DLN, dissected lymph node; DCR, disease control rate; OR, objective response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; * p<0.05.

3.3. Prognosis After Initiation of ICI Monotherapy

The median follow-up period after the initiation of ICI monotherapy was 21.2 months, during which 24 patients developed PD and 26 deaths occurred. Patients whose surgical specimens showed high PD-L1 expression levels had significantly better ICI-PFS and ICI-OS than those with low PD-L1 expression levels (Figure 2A; p=0.003 and Figure 2B; 0.029, respectively). A significant difference was observed only in the PD-L1 expression in ICI-OS patients. The ICI-PFS in the group with ≥15 DLNs was significantly worse than that in the group with <15 DLNs (Figure 3A; p=0.047), but the ICI-OS did not differ to a statistically significant extent (Figure 3B; p=0.228). Furthermore, in patients with extrathoracic recurrent lesions, there was no significant difference in ICI-PFS between the group with ≥15 DLNs and the group with <15 DLNs (Figure 3C; p=0.798); however, in patients without extrathoracic recurrent lesions, the ICI-PFS of the group with ≥15 DLNs was significantly worse than that of the group with <15 DLNs (Figure 3D; p=0.003). The results of the multivariate analysis for ICI-PFS are shown in Table 3. In the multivariate analysis, the number of DLNs (hazard ratio [HR], 2.702; 95% confidence interval [CI], 1.064 – 6.849; p=0.037), PD-L1 expression in surgical specimens (HR, 0.161; 95% CI, 0.044 – 0.598; p=0.006), and the presence of extrathoracic recurrent lesions were identified as independent prognostic factors (HR, 3.521; 95% CI, 1.307 – 9.524; p=0.013).

 doi:10.20944/preprints202505.1530.v1

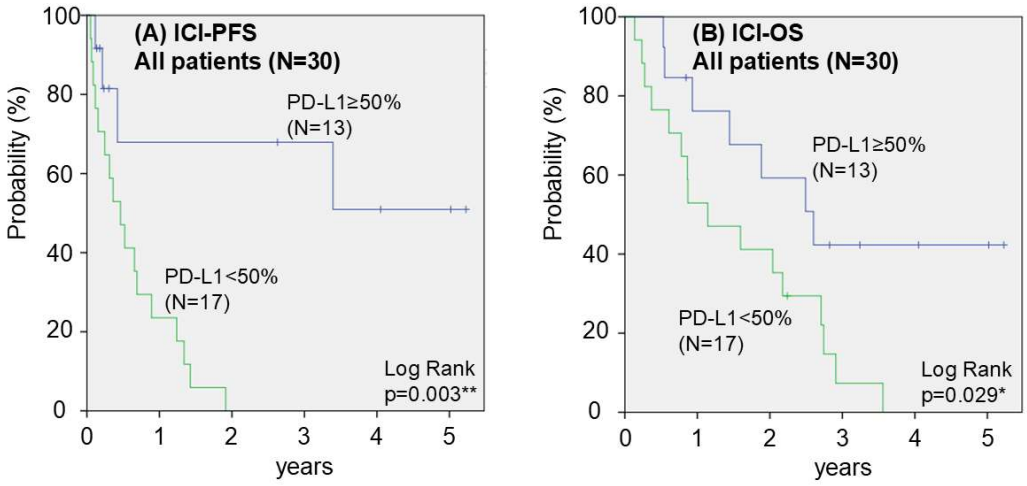

Figure 2. Association of the PD-L1 expression with the prognosis. (A) The association between the PD-L1 expression and ICI-PFS in all patients. (B) The association between the PD-L1 expression and ICI-OS in all patients. PD-L1, programmed death ligand 1; ICI, immune checkpoint inhibitor; ICI-PFS, progression-free survival after the initiation of ICI therapy; ICI-OS, overall survival after the initiation of ICI therapy; *p<0.05, **p<0.01.

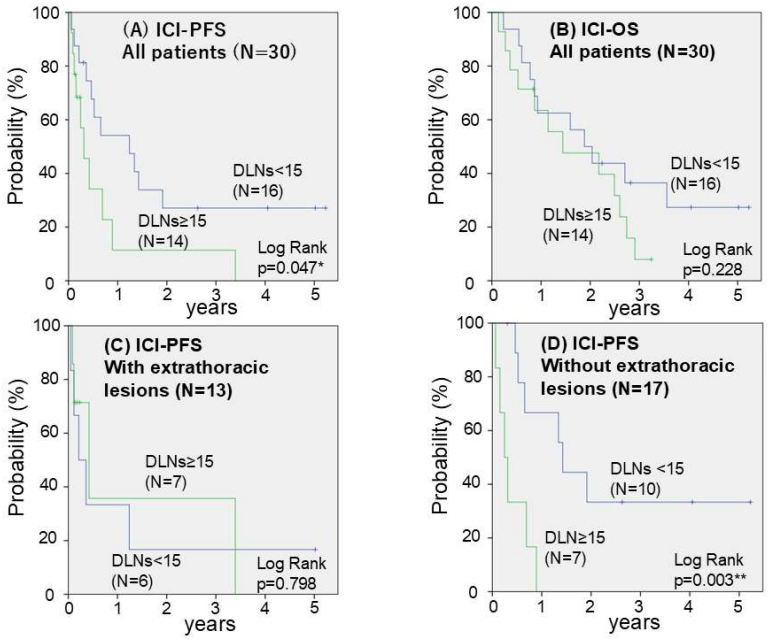

Figure 3. Association of the number of DLNs with the prognosis. (A) Association between the number of DLNs and ICI-PFS in all patients. (B) Association between the number of DLNs and ICI-OS in all patients. (C) Association between the number of DLNs and ICI-PFS in patients with extrathoracic recurrent lesions. (D) Association between the number of DLNs and ICI-PFS in patients without extrathoracic recurrent lesions. DLN, dissected lymph node; ICI, immune checkpoint inhibitor; ICI-PFS, progression-free survival after the initiation of ICI therapy; ICI-OS, overall survival after the initiation of ICI therapy; *p<0.05, **p<0.01.

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Table 3. Univariate and multivariate analyses of ICI-PFS.

Factors	Univariate p-value (log-rank test)	Multivariate		
		HR	95% CI	p-value
Gender (Male vs Female)	0.596	1.019	0.324 - 3.204	0.974
Age (>= 70 vs < 70)	0.228	0.846	0.345 - 2.074	0.714
Brinkmann Index (>= 600 vs < 600)	0.425	0.985	0.364 - 2.668	0.977
Number of DLN (>= 15 vs < 15)	0.047*	2.702	1.064 - 6.849	0.037*
Histlogical type (AD vs others)	0.476	0.843	0.322 - 2.207	0.728
PD-L1 expression in surgical specimens (>= 50% vs < 50%)	0.003**	0.161	0.044 - 0.598	0.006**
Extrathoracic lesions (Presence vs Absence)	0.335	3.521	1.307 - 9.524	0.013*
Therapeutic Lines of ICIs (>= 3rd vs < 3rd)	0.342	1.295	0.477 - 3.516	0.612
Duration from surgery to initiation of ICIs	0.745	–		

ICI: Immune Checkpoint Inhibitor, ICI-PFS: Progression Free Survival after initiation of ICI, HR: Hazard Ratio, CI: Confidence Interval, DLN: Dissected Lymph Nodes, AD: adenocarcinoma, PD-L1: Programmed Death-Ligand 1

*: $p<0.05$, **: $p<0.01$

4. Discussion

In this study, we investigated the factors influencing the efficacy of ICI monotherapy for postoperative recurrence of NSCLC. It was revealed that patients with a large number of lymph nodes dissected at surgery had significantly worse ICI-PFS than those with a small number of lymph nodes dissected at surgery, and that patients whose surgical specimens had high PD-L1 expression levels had significantly better ICI-PFS and ICI-OS than those with low PD-L1 expression levels. Furthermore, in the multivariate analysis of ICI-PFS, the number of DLNs, PD-L1 expression rate in surgical specimens, and presence or absence of extrathoracic recurrent lesions were identified as independent prognostic predictors.

Several preclinical models have shown that tumor-draining lymph nodes (TDLNs) play a central role in cancer immune responses. Fransen et al. showed that PD-1 blockade by ICIs activates CD8+ T cells in TDLNs and induces the influx of these effector cells into the tumor microenvironment [23]. Dammeijer et al. showed that TDLNs, rather than the tumor microenvironment, are important

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regulators of ICI efficacy [24]. Furthermore, Saddawi-Konefka et al. used a lymphadenectomy mouse model to show that lymphadenectomy markedly reduced the response to ICI therapy [25]. They attributed this to the loss of conventional type I dendritic cells and type I interferon signaling, which are upregulated in TDLNs.

In addition, a retrospective clinical study was conducted to examine whether there was a difference in the therapeutic effect of ICIs depending on the extent of lymph node dissection in 26 patients with recurrent NSCLC. As a result, patients who underwent systematic lymph node dissection had significantly worse PFS than those who underwent selective lymph node dissection [18], which was partly in accordance with our current study results. However, more than half of the patients in this study were administered ICIs in combination with chemotherapy; therefore, the results should be interpreted with caution.

Central memory T cells and stem cell-like memory T cells in the bone marrow maintain long-term antigen-specific immune memory and are rapidly reactivated when the antigen reappears [26]. In the current study, the presence of extrathoracic recurrent lesions was an independent prognostic factor for ICI-PFS. Furthermore, in patients with extrathoracic recurrent lesions, the number of LNDs did not affect ICI-PFS, whereas in patients with only intrathoracic disease, the number of DLNs was significantly lower than that of the small number of DLNs. This suggests that in cases of intrathoracic recurrence, in addition to reactivated memory T cells, the antitumor immune response of the remaining TDLNs is strongly involved in the efficacy of ICIs.

Sentinel lymph nodes close to tumors are susceptible to cancer and have suppressed immune activity [27,28]. Our previous studies have also shown that the closer the lymph node is to the tumor, the lower the immune activity of monocytic cells [29,30]. To maximize the efficacy of ICI therapy, it is important to reactivate the immune system of the suppressed lymph nodes. Therefore, it is thought that administering ICI before resection of sentinel lymph nodes in an immune-tolerant state, that is, before surgery, will enable more effective induction of antitumor immunity. Liu et al. showed that the therapeutic effect of neoadjuvant ICI therapy was significantly stronger than that of adjuvant ICI therapy in a mouse model of metastatic breast cancer [31]. In advanced melanoma, the group that received three courses of pembrolizumab before surgery and 15 courses after surgery showed significantly better event-free survival than the group that received 18 courses of pembrolizumab only after surgery [32]. A large-scale cohort study of stage II-IIIB NSCLC patients using data from the National Cancer Database in the United States showed that neoadjuvant immunochemotherapy was associated with significantly better OS than adjuvant immunochemotherapy [33]. The results of the current study suggest that dissection of many lymph nodes downregulates antitumor immune activity; therefore, using ICIs before lymph node dissection may maximize the effect of ICI therapy.

The high expression of PD-L1 is a favorable prognostic factor for ICI monotherapy [17]. In the present study, the high expression of PD-L1 in surgical specimens was strongly associated with the response rate of patients receiving ICI therapy, even for recurrent lesions relatively long after surgery, and was a favorable factor for ICI-PFS and OS. Although the immune environment in tumors is heterogeneous, Kitazono et al. showed a good agreement between the PD-L1 expression in biopsy and surgical specimens [34]. In addition, Cho et al. compared the PD-L1 expression in biopsy or surgical specimens separated by an average of 20 months and showed a significant positive correlation between paired samples [35]. However, it has been reported that chemotherapy changes the PD-L1 expression both *in vitro* and in clinical practice [36–38], and it is therefore important to reassess the PD-L1 expression before treatment with ICIs.

The present study was associated with several limitations. First, it was a single-center retrospective study and the number of subjects was limited. Currently, ICI therapy for NSCLC is mainly combined with chemotherapy, which makes it difficult to study ICI monotherapy. Therefore, it is desirable to collect and analyze large-scale data from more institutions. Another limitation was that the factors examined as predictors of recurrence were limited. The high response rate of patients receiving ICI therapy has been examined in various cancers, and in addition to the expression of PD-

 doi:10.20944/preprints202505.1530.v1

L1, there have been reports of tumor mutation burden and oncogenic virus infection status [39–42], Future studies should also examine the relationship with other biological factors.

5. Conclusion

Minimal lymph node dissection may be beneficial in achieving better outcomes after ICI therapy for postoperative intrathoracic recurrence after the resection of NSCLC, providing a rationale to recommend preoperative ICI treatment when the lymphatic immune system is intact.

Author Contributions: Conceptualization, M.A.; Methodology, M.A.; Software, M.A.; Validation, M.A. and G.K.; Investigation, M.A., G.K., Y.T., S.M. T.T., A.H-T., T.N. K.K. and K.U.; Data Curation, M.A.; Writing – Original Draft Preparation, M.A.; Writing – Review & Editing, K.U. and G.K.; Visualization, M.A.; Supervision, K.U. All authors read and approved the final manuscript.

Funding: This research received no external funding.

Ethics Statement: The use of the information was approved by the Ethics Committee of Kagoshima University Graduate School of Medicine and Dentistry (Approval No. 220178epi) and conformed to the principles outlined in the Declaration of Helsinki. Research participants and their relatives could opt out by viewing the research content hosted online.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s).

Conflicts of Interest: The authors declare no conflicts of interest in association with the present study.

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