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Neoadjuvant PD-1 inhibitor combines with chemotherapy versus neoadjuvant chemotherapy in resectable squamous cell carcinoma of the lung

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Abstract

Background: A single-agent of anti programmed cell death 1/programmed cell death ligand 1 (anti-PD-1/PD-L1) therapy has been explored for resectable lung cancer before surgery. However, the effectiveness and safety of neoadjuvant programmed cell death 1 (PD-1) blockade combined with chemotherapy have not been published.

Methods: Twenty-one consecutive patients with potentially resectable squamous cell carcinoma of the lung who received neoadjuvant therapy followed by surgery in Beijing Cancer Hospital were included in this study. Eight patients received two cycles of neoadjuvant platinum-based doublet chemotherapy combined with anti-programmed cell death 1 (anti-PD-1) therapy, while 13 patients received two cycles of neoadjuvant platinum-based doublet chemotherapy only. Chest computed tomography was repeated before neoadjuvant treatment and surgery. Adverse events were monitored. The major pathological response (MPR) rate was determined after surgery. Selected specimens were sent for immunohistochemical and multiplex immunofluorescence analyses, and T-cell receptor DNA sequencing.

Results: Compared with neoadjuvant chemotherapy alone, the combination of PD-1 blockade and chemotherapy increased the pathological complete response rate (37.5% vs. 7.69%) and MPR rate (50% vs. 38.46%). The pathological and radiological evaluations are not consistent. No unknown adverse effects were reported for all the patients. More tumor infiltrating lymphocytes were observed in patients who received PD-1 blockade. No unknown pathological features associated with PD-1 blockade were found. Immune suppression in the peritumoral spaces around the residual tumor cells

Yuan Feng, Wei Sun, and Jie Zhang contributed equally to this research.

was observed. The amino acid sequences of the T-cell receptors are not significantly shared among the patients.

Conclusions: The combination of neoadjuvant chemotherapy and PD-1 blockade is safe and feasible, and might indicate an increased MPR and pathological complete response rate. More investigations are needed for the best combination of the neoadjuvant therapy.

KEYWORDS

major pathological response, neoadjuvant anti-PD-1 antibody with chemotherapy, squamous cell non-small-cell lung cancer

BACKGROUND

Over the past two decades, the wide application of targeted therapy has greatly improved the overall survival of patients with lung cancers, especially adenocarcinoma with driver gene mutations. However, driver gene mutation-based treatment has not significantly benefited patients with squamous cell lung cancer, which closely relates to smoking and contributes to around 30% of lung cancer cases. Classically, lung squamous cell carcinomas are central airway tumors while about 75% of these patients are diagnosed stage III or later. Since complete dissection (R0 dissection) is extremely difficult, the choice of treatment is usually limited to chemotherapy and radiotherapy, and the outcome remains unsatisfactory.

Immunotherapy based on programmed cell death 1 (PD-1) blockade has greatly improved the treatment of late-stage squamous cell carcinoma of the lung.[1] Pembrolizumab combined with traditional platinum-based doublet chemotherapy has been recommended as the first-line therapy for these patients by National Comprehensive Cancer Network guidelines (NCCN guidelines). Recently, single-agent anti-PD-1 or programmed cell death ligand 1 (anti-PD-L1) antibody has also been explored for resectable patients before surgery and has shown impressive effects.[2,3] Concerning the combination of PD-1 inhibitor and chemotherapy as neoadjuvant treatment, two single-arm, phase II clinical trials published in 2020[4,5] have proved the effectiveness and safety of this treatment strategy.

On the other hand, the phase III clinical trials were also designed to compare neoadjuvant PD-1 inhibitor plus chemotherapy versus neoadjuvant chemotherapy. Although some results of relevant phase II and phase III trials have been reported at international conferences, and revealed the safety and feasibility of the combination of neoadjuvant chemotherapy and PD-1 blockade, time is still needed to prove the disease-free or overall survival benefit. In addition, further exploration on the mechanism of immunotherapy, such as neoadjuvant treatment-induced pathological changes and the immune response of the body, is still needed. Therefore, this study aimed to compare the clinical outcome between neoadjuvant chemotherapy and neoadjuvant PD-1 inhibitor combined with chemotherapy, as well as to try to explore the mechanism of this neoadjuvant therapy combination on resectable squamous cell carcinoma of the lung.

METHODS

Patients and follow-ups

From October 2018 through to June 2019, 21 consecutive patients with squamous cell lung cancer received neoadjuvant therapy followed by surgery after discussion and approval of the multiple discipline team (MDT) of the Center of Thoracic Cancer, Peking University Cancer Hospital (Beijing Cancer Hospital) based on NCCN guidelines. All the patients were scheduled to undergo surgery 5–7 weeks after the administration of the second dose of neoadjuvant chemotherapy or anti-PD-1 antibody by the same surgeon.

All the patients were treatment naïve. Baseline tumor staging includes pretreatment bronchoscopy or computed tomography (CT)-guided fine-needle biopsy, positron-emission tomography-computed tomography (PET-CT), and contrast-enhanced CT or magnetic resonance imaging of the brain and chest. The pre-treatment tumor-node-metastasis (TNM) staging was evaluated according to the criteria of the American Joint Committee on Cancer (8th edition). The CT of the chest was repeated 2–3 weeks before surgery to evaluate the clinical outcome of the neoadjuvant therapy. Changes in tumor size were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1·1.[6] Resection of the primary tumor and lymph nodes was completed according to institutional standards. Adverse events were monitored and graded (Common Terminology Criteria for Adverse Events, Version 5.0, 27 November 2017). Peripheral lymphocyte count was performed before and during treatment. Postoperative adjuvant chemotherapy or radiotherapy was offered as indicated. The clinical and pathological responses were observed during the entire treatment.

All patients were suggested regular outpatient clinic follow-up after surgery at the first month postoperatively, then every 3 months during the first 2 years, every 6 months between years 2 and 5, and once a year after year 5. The follow-up tests include chest CT, brain MRI, and ultrasonic for abdomen, neck, and supraclavicular area. The last follow-up was performed in January 2021. Disease-free survival (DFS) is defined as the time from surgery to detection of recurrence or metastasis (confirmed by image tests or biopsy). Overall survival (OS) is defined as the time from surgery to death or last follow-up.

Neoadjuvant therapy regimen

Neoadjuvant chemotherapy (gemcitabine, paclitaxel or nab-paclitaxel plus cisplatin or carboplatin) was administered every 3 weeks (21 days) for two cycles. One dose of anti-PD-1 antibody (pembrolizumab or toripalimab) was administered before the infusion of chemotherapy agents on the first day of each cycle. Detailed information on the neoadjuvant therapy regimen for each patient is shown in Supporting Information Table S1.

Pathology

Pathological assessments

The sizes of the primary lung tumor, involved lymph nodes, and metastases were evaluated according to the criteria of the American Joint Committee on Cancer (8th edition). Primary tumors were further assessed for the percentage of residual viable tumor cells identifiable by routine hematoxylin and eosin (H&E) staining. Referring to past studies,[7–9] we also examined the dissected primary tumors for the presence of the following pathological features: (1) feature of cell death (coagulation necrosis, cholesterol clefts, and foam cell infiltration); (2) tissue repair/wound healing; and (3) immune infiltrates with features of activation, including tertiary lymphoid structures (TLS), tumor-infiltrating lymphocytes (TILs), plasma cells infiltrate, collections of fused macrophages (giant cells), and granuloma formation.

Evaluation of residual viable tumor cells

The residual viable tumor cells were evaluated by two pathologists (W.S. and X.Y.L.) who (1) measured the gross maximum diameter, (2) obtained H&E-stained slides of at least one section per greatest tumor diameter, (3) measured the percentage of viable tumor cells in each slide, and (4) summed the percentage of viable tumor cells in each slide and divided that total by the number of slides examined. The number of each tumor was recorded, and a major pathological response (MPR) was defined as ≤10% residual viable tumor cells.[10]

Immunologic analysis

Selected specimens of primary tumors and lymph nodes (normal or metastasized) were assessed with multiplexed immunofluorescence staining for simultaneous detection of cytokeratin (tumor cells), CD8 (cytotoxic T cells), FoxP3 (regulatory T cells), CD68 (macrophages), and CD56 (natural killer cells).

The information of the specimen and detailed immunohistochemical and multiplex immunofluorescence analyses of tumors are described in Table S4 of Appendix S1.

T-cell receptor sequencing

A total of 15 samples, as shown in Supporting Information - Table S4, were collected from primary cancer tissue, normal lymph nodes (nLNs), and metastasized nodes (LNMs) during the surgery. All the samples were formalin-fixed and paraffin-embedded fixed and stored until further analysis.

The detailed DNA extraction, T-cell receptor (TCR) sequencing, and data analysis procedure are described in the "Methods" section of Appendix S1.

Statistical analysis

Side effects and adverse events were continuously monitored. The difference in response rates was compared by the chi-square test. The Student's *t*-test and Mann–Whitney *U* test were used for the analysis of normally and non-normally distributed data, respectively. The p values were calculated with a significance level of $p < 0.05$. IBM SPSS Statistics 19 software was used for analysis.

RESULTS

Patients characteristics

Among the 21 patients involved in this study, eight patients with stage clinical IIA or IIIA squamous cell lung cancer received two cycles of neoadjuvant platinum-based doublet chemotherapy combined with anti-PD-1 therapy (patients IM-1 to IM-8) and 13 patients with clinical IIB to IIIB received two cycles of neoadjuvant platinum doublet chemotherapy (patients C-1 to C-13). The detailed information of the pre-treatment TNM stage is shown in Supportign Information Table S3. Informed consent was received from all patients before treatment. One patient was a nonsmoker while the rest of the patients were current or former smokers. The characteristics of the enrolled patients are shown in Table 1.

Safety and feasibility of neoadjuvant therapy

Neoadjuvant anti-PD-1 antibody combined with platinum-based doublet chemotherapy (IM group) was not associated with any previously unreported toxic effects. Preoperatively, treatment-related adverse events of any grade occurred in five of eight patients. Besides one case of grade 3 leukopenia, all the adverse events were grade 1 or 2 and were considered as the most common adverse events due to chemotherapy, not anti-PD-1 therapy. However, one patient (IM-3) was diagnosed with grade 3 immune-related pneumonia after surgery (on postoperative day 11, POD 11) and was cured after management with prednisolone.

Patients who received neoadjuvant platinum-based doublet chemotherapy (without anti-PD-1 therapy, C group)

TABLE 1 Characteristics of the patients at baseline

Characteristics	All patients ($N = 21$)	Neoadjuvant immunotherapy with chemotherapy (IM group) ($N = 8$)	Neoadjuvant chemotherapy (C group) ($N = 13$)
Age ($p = 0.74$)			
Mean ± SD	62.90 ± 5.59	62.38 ± 5.48	63.23 ± 5.85
Median (range)	64 (51–70)	64 (52–68)	65 (51–70)
Sex ($p = 0.42$)			
Female	1	0 (0)	1
Male	20	8 (100)	12
Histological diagnosis ($p = 0.72$)			
Squamous-cell carcinoma	19	7	12
Non-squamous-cell carcinoma	2	1[a]	1[b]
Clinical disease stage ($p = 0.28$)			
IIA-IIB	10	5	5
IIIA-IIIB	11	3	8
Smoking status ($p = 0.42$)			
Never	1	0	1
Former or current (median pack-year)	20 (36.25, range 15–90)	8 (32.50, range 15–60)	12 (40, range 20–90)

[a]This patient is diagnosed as squamous cell carcinoma with neuroendocrinal component.
[b]This patient is diagnosed with squamous cell carcinoma on the left lower lobe and adenocarcinoma on the left upper lobe of the lung.

reported similar grade 1–2 adverse events (nine of 13 patients). No grade 3 or higher treatment-related adverse events were reported (Supporting Information Table S2). No statistical difference was found for each observed adverse event ($p > 0.05$, respectively).

There were no treatment-related surgical delays. The median interval between the administration of the second dose of chemotherapy or anti-PD-1 antibody was 44.50 days (range 30.00–77.00). All the patients underwent complete tumor resection (according to NCCN guidelines criteria).

Response of lung squamous cell cancer to neoadjuvant therapy

Clinical assessment

For patients of the IM group, partial response (PR) was achieved in seven (87.50%) of eight patients, while one (12.50%) patient had stable disease (SD). Of the 13 patients of the C group, six had a PR (46.15%) while seven had SD (53.85%). Although a higher PR rate were observed in IM group, no statistical difference was concluded between the two groups ($p = 0.058$). Among these 21 patients, pathological down-staging from the pretreatment clinical stage occurred in six patients (75%) in the IM group and nine patients (69.23%) in the C group (Supporting Information - Table S3). However, pathological up-staging occurred in one patient in the C group. This patient was diagnosed with T2aN1MO before treatment and T2cN2M0 histologically after surgery.

Follow-ups

The median follow-up is 20.53 (range 11.40–24.80) months. One patient of the C group was diagnosed with lung metastasis 8.90 months after surgery and overall survived was 11.40 months. One patient of the IM group was diagnosed with brain metastasis 3.77 months after surgery and was still alive at the last follow-up. The rest of the patients of both groups were disease-free.

Pathological assessment

Of the eight patients in the IM group, three patients (37.50%) had pathological CR (pCR) and the other five (62.50%) patients had a PR, including four patients with ≤30% residual tumor cells and one patient with ≤50% residual tumor cells. The mean residual viable cells were 15.31 ± 15.75%. The median degree of pathological regression in the primary tumor was 85.05% (range −100 to −55.50, mean −84.69%). One patient (IM-2) was diagnosed with squamous cell carcinoma with a neuroendocrinal component after surgery (Supporting Information Table S3). Although the ratio of residual tumor cells was 13.40%, the squamous carcinoma component was less than 10%. Thus, in this group, an MPR occurred in four patients (50%).

Compared with the IM group, of the 13 patients in the C group, an MPR occurred in five (38.46% vs. 50%, $p = 0.673$), including one patient with CR (7.69% vs. 37.50%, $p = 0.09$). The mean residual viable cells were 36.72 ± 32.49%, while the median degree of pathological

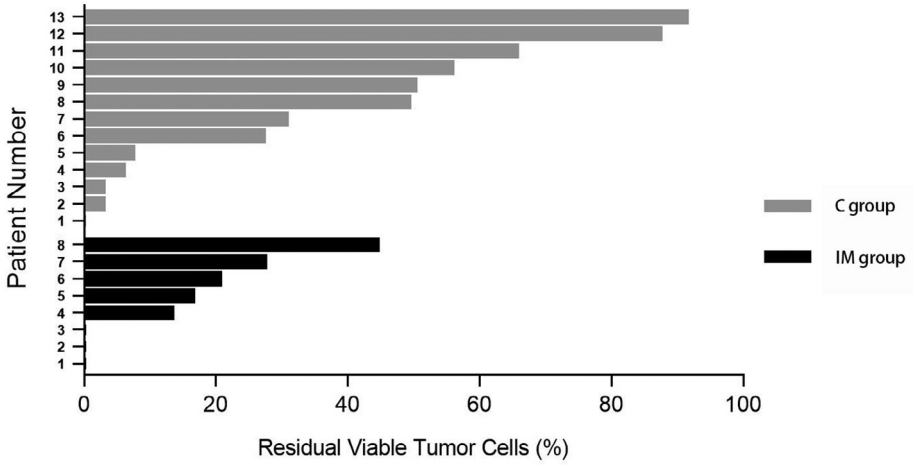

FIGURE 1 The residual viable tumor cell after surgery in each patient

TABLE 2 RECIST 1.1 and pathological evaluation on response to neoadjuvant therapy

Response	All patients ($N = 21$) (%)	IM group ($N = 8$) (%)	C group ($N = 13$) (%)	*p* value
RECIST 1.1				
PR	13 (61.90)	7 (87.50)	6 (46.15)	0.058
SD	8 (38.10)	1 (12.50)	7 (53.85)	
Pathological evaluation				
CR	4 (19.05)	3 (37.50)	1 (7.69)	0.098
PR	15 (71.43)	5 (62.50)	10 (76.93)	
SD	2 (9.52)	0	2 (15.38)	
Major pathologic response	9 (42.86)	4 (50)	5 (38.46)	0.673
Residual viable cells (%)		15.31% ± 15.75%	36.72% ± 32.49%	0.058

regression in the primary tumor was −69.30% (range −100 to −8.55, mean −63.28%) (Figure 1).

The comparison between RECIST and pathological assessment indicates the discordance of these two methods. The pathological regression may not always be found by imaging analysis (Table 2).

Peripheral lymphocyte count

The peripheral lymphocyte count was recorded before and at around day 7 of both cycles of neoadjuvant therapy, as well as 7 days before surgery. Figure 2 shows the change in the mean peripheral lymphocyte count during the treatment. In general, after the neoadjuvant agent infusion of each cycle, the lymphocyte count dropped, then was restored before the next cycle or before surgery.

Pathological features of lung squamous cell carcinoma treated by neoadjuvant chemotherapy with or without anti-PD-1 antibody

The tumors of patients who underwent neoadjuvant chemotherapy both with and without anti-PD-1 antibody, demonstrated similar gross morphological changes. The features present in the patients of both groups are described in the pathology-pathological assessment (Figure 3(a),(b)).

The TILs appeared to be denser in the primary/residual tumors (or regression bed) in the IM group than in the C group (Figure 3). The results of multiplexed immunofluorescence on selected specimens also support that the addition of PD-1 blockade recruited more CD8+ T cells to the primary tumor or tumor bed (Figure 4). Nevertheless, in most of the specimens the TILs were not as dense as expected or as reported in the former study (Figure 3(a)).[6]

Besides the features observed above, in the specimen of pathological partial responders, immune exclusion, defined as immune cells present in the immediate peritumoral stroma but not infiltrating into the tumor parenchyma, occurred in both groups. Even in the patients who received the anti-PD-1 antibody, the T cells were not able to penetrate the "barrier" of the residual tumor cells. Based on the immunohistochemical staining, the immune cell infiltrates in the peritumoral stroma were mainly composed of CD4+ T cells and CD20+ B cells, but not CD8+ T cells (Supporting Information Figures S1 and S2). In the multispectral immunofluorescence staining of the lymph node with metastasis, dense FoxP3+ Treg cells were present among or around the tumor cells. However, in the normal lymph node (subcarinal lymph node), Treg cells are seen with much lower density (Figure 5).

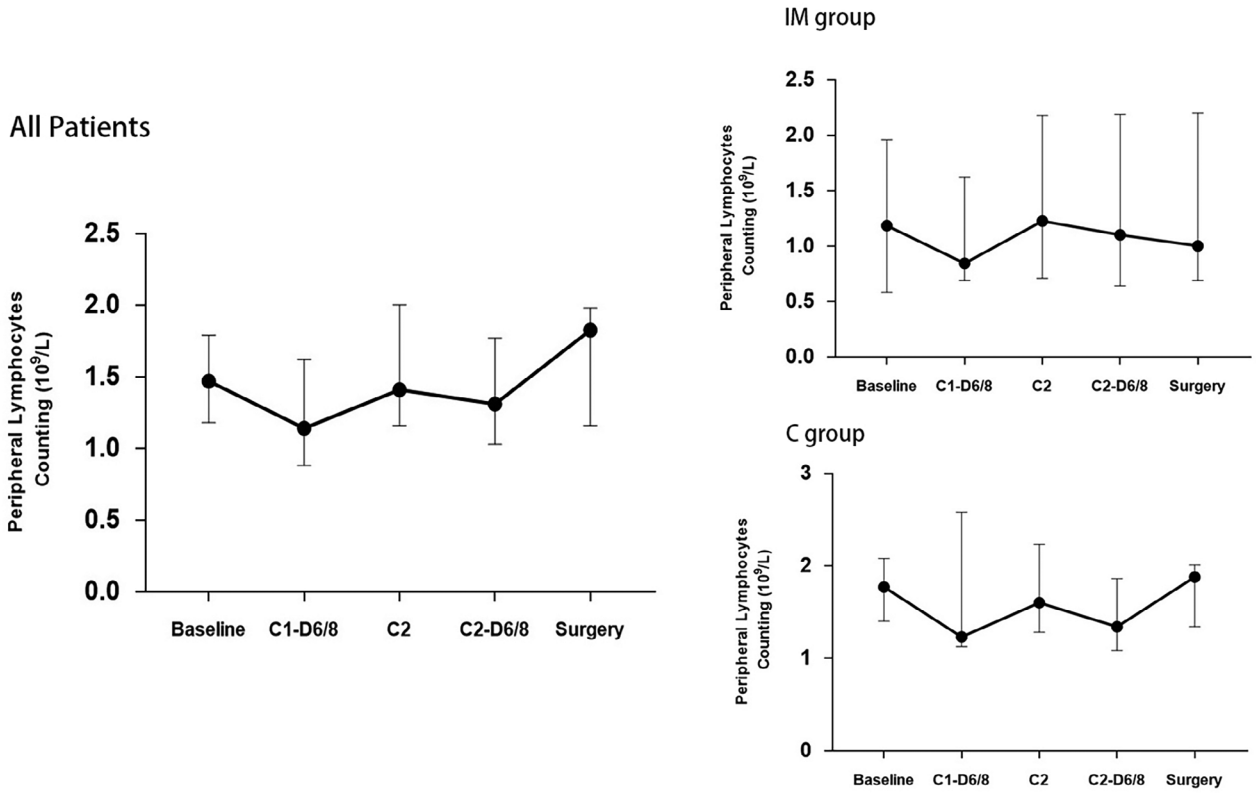

FIGURE 2 Change of the peripheral lymphocyte count during neoadjuvant therapy. The mean value of the peripheral lymphocyte count of all patients before treatment (baseline) and on days 6–8 of the first cycle (C1-D6/8) of neoadjuvant therapy (1.45 ± 0.53 vs. 1.31 ± 0.63, $p = 0.083$), before the second cycle of (C2), and on days 6–8 of the second cycle (C2-D6/8) of neoadjuvant therapy (1.58 ± 0.50 vs. 1.39 ± 0.52, $p = 0.039$)

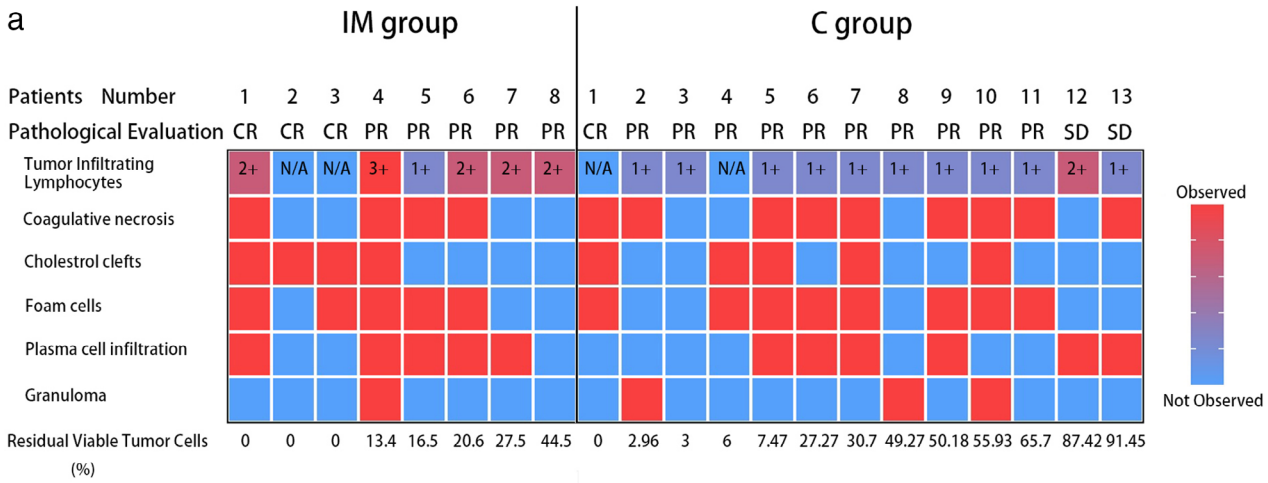

FIGURE 3 Pathological features of the patients. (a) N/A: no obvious tumor infiltration lymphocytes were found. (b) Cholesterol clefts: artifactual crystal-shaped spaced in tissue sections, indicative of insoluble (cell-membrane) lipid accumulation. Proliferative fibrosis: characteristics of tissue repair/wound healing early stage when inflammatory cells release cytokines and growth factors that stimulate proliferation of fibroblast foci

T-cell receptor sequencing

Specimens from six patients (three patients from each group; Supporting Information Table S4) were analyzed with T-cell receptor sequencing.

Analysis of the amino acid clonotype (amino acid sequences for the formation of the TCR) and Shannon entropy showed that the degree of T-cell receptor diversity varied among specimens and patients. In patients of both the IM and C groups, the number of amino acid clonotypes

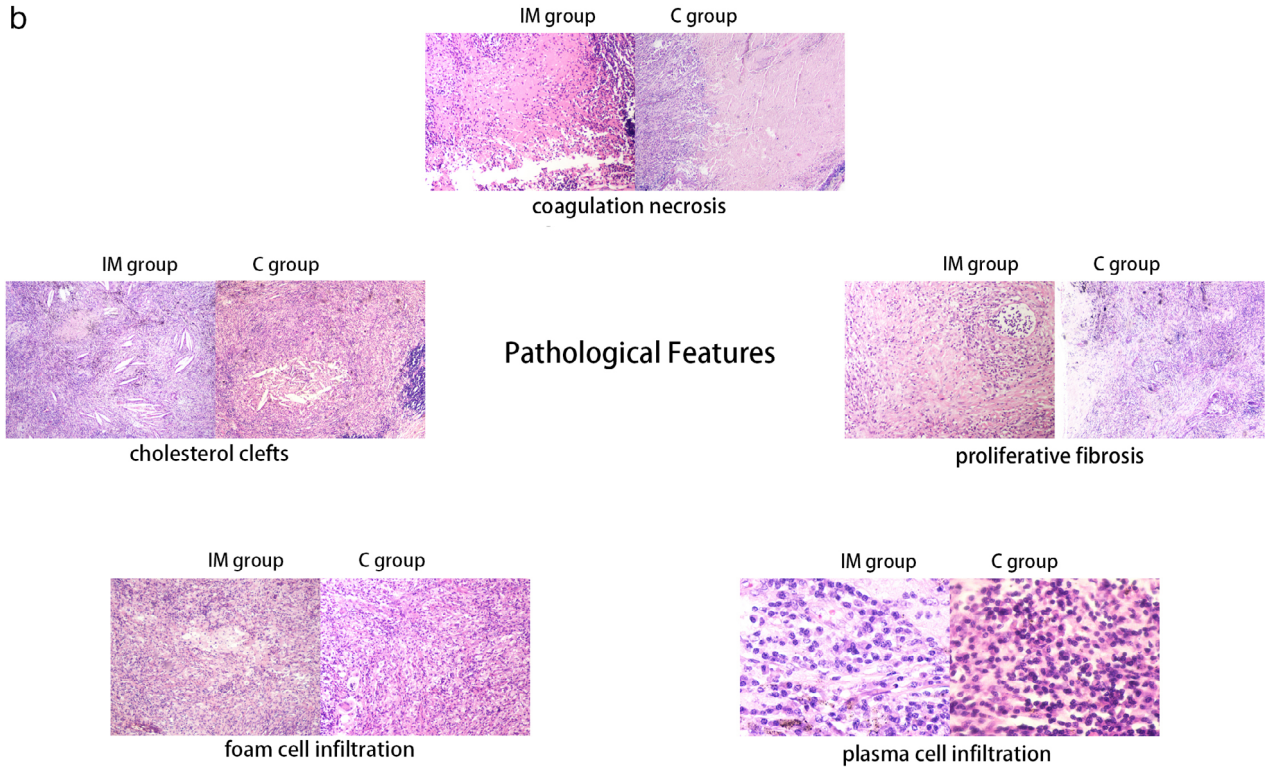

FIGURE 3 (Continued)

in primary tumors was always lower than that in lymph nodes, while the TCR diversity in metastatic lymph nodes was lower than that in normal lymph nodes (Supporting Information Figure S3). Consistently, the diversity of T cell receptors evaluated by the Shannon Index in primary tumors was also statistically lower than that in normal lymph nodes ($p = 0.003$) (Figure 6).

We further extracted the top 100 most frequently detected TCR amino acid clonotypes of each specimen and analyzed the shared amino acid clonotype among them. The results revealed that in the specimen from the same patient, some amino acids were shared among the primary tumor, normal lymph nodes, and lymph nodes with metastasis. Nevertheless, very few TCR amino acid clonotypes were shared by different patients (Supporting Information - Figures S4 and S5).

DISCUSSION

The PD-1 blockade has been proved to be able to improve the disease-free and overall survival for patients with late-stage lung cancer. Whether or not a PD-1 blockade may be utilized as neoadjuvant therapy for resectable lung cancer has aroused the interest of physicians and scientists.

Encouraged by the promising results of phase II clinical trials on neoadjuvant PD-1 blockade monotherapy, researchers are investigating whether the combination of PD-1 blockade with traditional platinum-based chemotherapy would further improve the outcome of treatment on lung cancer. In a single-arm, phase II trial, Shu et al.[4] proved the safety and efficacy of neoadjuvant atezolizumab plus carboplatin plus albumin-bound paclitaxel. The patients received four cycles of neoadjuvant therapy with an MPR rate of 50% and a pCR rate of 21.4%. In the neoadjuvant chemotherapy and nivolumab in resectable non-small-cell lung cancer (NADIM) study,[5] patients who underwent surgery after three cycles of neoadjuvant therapy with nivolumab plus carboplatin plus paclitaxel had an MPR rate of 83%, a pCR rate of 71%, and 90% of the tumor was down-staged, confirming this treatment mode to be safe and feasible. The American Society of Clinical Oncology (ASCO) 2021 released the surgical outcomes of CheckMate-816.[11] After three cycles of neoadjuvant treatment, 24.0% of patients in the nivolumab plus chemotherapy arm achieved a pCR compared with 2.2% in the chemotherapy alone group ($p < 0.0001$). In addition, the nivolumab plus chemotherapy regimen was tolerable and did not lead to more postoperative complications.

Besides the published clinical trials, phase III clinical trials such as IMpower-030 (NCT03456063), KEYNOTE-671 (NCT03425643), AEGEAN (NCT03800134), and CheckMate-77T (NCT04025879)[12] are also under investigation. The comparison between neoadjuvant chemotherapy and immunotherapy plus chemotherapy may help elucidate PD-1 blockade immunotherapy and its benefit to patients with lung cancer.

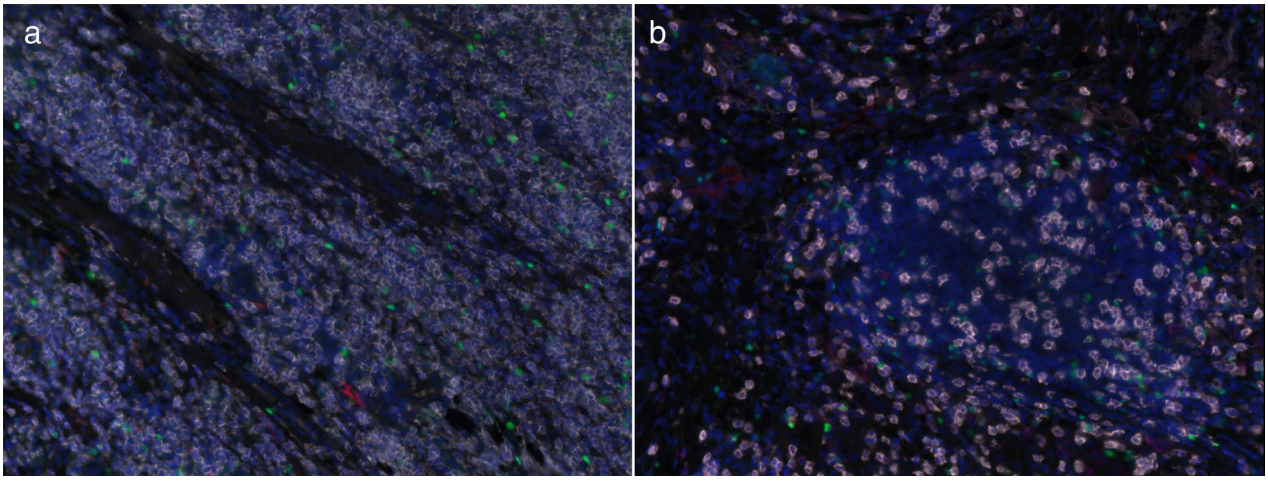

FIGURE 4 Multiplexed immunofluorescence. Comparison of immune cells on the tumor bed of pCR patients. (a) The tumor bed of IM group PN-2 (patient number 2). (b) The tumor bed of C group PN-1 (patient number 1). Both patients were evaluated as pCR (pathological complete response, without viable residual tumor cells). However, with PD-1 blockade, denser CD8+ T cells (pink) were infiltrated in the tumor bed. Other types of scattered immune cells were also found on the tumor bed. Green, regulatory T cells (FoxP3+); red, natural killer cells (CD56+); purple, macrophages (CD68+)

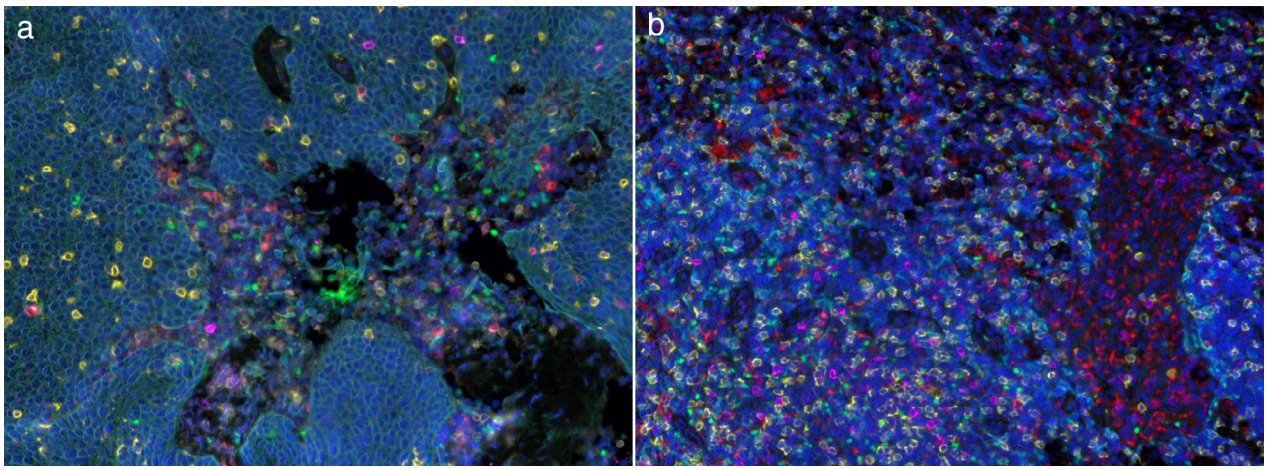

FIGURE 5 Multiplexed immunofluorescence. (a) In the lymph node with metastasis, infiltrating immune cells can be observed in the center of the field. CD8+ T cells (yellow) are seen scattered among and surrounding the tumor cells. Relatively dense FoxP3+ cells (green) accumulate in the peritumoral space. Small amounts of macrophages (red) and CD20+ cells (purple) are also present. (b) In a normal lymph node, FoxP3+ cells (green) can be found with much less density

Clinical analysis

In this study, we observed that the combination of neoadjuvant chemotherapy and anti-PD-1 immunotherapy was associated with few additional adverse events (Supporting Information Figure S2), without delaying the planned surgery, and indicated a tendency to improve both the radiological and pathological evaluation (Table 2). Nevertheless, one patient with immune-related pneumonia after surgery warned us that surgeons still need to be aware of the serious adverse events of the immunotherapy that may lead to the cancellation of the planned surgery.

Studies have demonstrated the correlation between complete pathological response and overall survival,[13–16] and proved the validity of the MPR as a surrogate of survival.[17–20] In this study, the pathological response is different between the IM and C groups ($p = 0.098$; Table 2) and the degree of residual viable cells is 15.31 ± 15.75% versus 36.72 ± 32.49% ($p = 0.058$; Figure 1 and Table 2). However, the rate of the MPR in the chemotherapy-only group was 38.46%, which is much higher than that previously reported (around 26%),[21] while the rate of the MPR in the IM group reached 50%, the same as reported by Shu et al.[4] and Impower-030. Although the difference was not statistically significant, we expect that an improved sample size would verify the benefit of PD-1 on the MPR rate.

We also observed inconsistency between the radiologic and pathological assessment, especially that some pCR

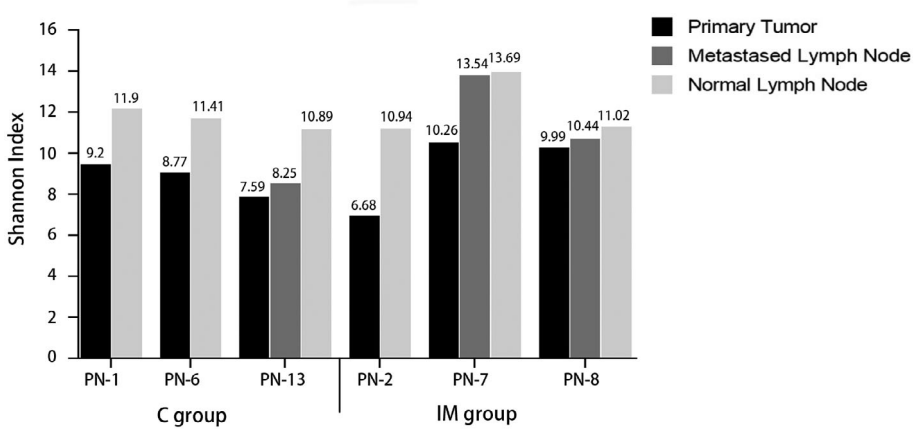

FIGURE 6 The diversity (Shannon Index) analysis of the specimen

patients may be considered as PR radiologically before surgery. One of the reasons may be that since the interval between the second CT scan and surgery was usually more than 10 days, the continued shrinkage of tumors after neoadjuvant therapy could have happened during this period. Furthermore, the repaired tissue (fibrosis) be considered as residual tumor cells. Although not observed in our study, pseudoprogression should also be considered during the radiological assessment.

Histological findings

The features of tumor cell death and tissue repair were present in patients in both the IM and C groups. The presence of features of immune activation, such as the formation of granuloma, tertiary lymphoid structure, plasma cell infiltration, and giant cells, in primary tumors after neoadjuvant chemotherapy suggests some degree of immune response to chemo-induced tumor cell death. Although no unique features were found after PD-1 blockade (Figure 3(b)), an increase in TILs in patients who received PD-1 blockade indicates enhanced T-cell activation/reactivation.

We originally expected that the peripheral lymphocyte count would increase in patients who received PD-1 blockade and chemotherapy. However, as shown in Figure 2, the peripheral lymphocyte count decreased after each cycle of neoadjuvant therapy, regardless of the neoadjuvant regimen. It is reasonable to consider that the immune cells were also killed by the chemo agents in the tumors where these agents worked the best. This hypothesis further leads us to re-evaluate the current strategy of combining platinum-based doublet chemotherapy with PD-1 blockade. Vivek Verma and colleagues[22] revealed the PD-1 blockade in subprimed CD8 cells induced dysfunctional PD-$1^{+}$$CD38^{hi}$ cells and anti-PD-1 resistance in animal experiments. The PEMBRO-RT phase 2 randomized clinical trial[23] showed that the delayed PD-1 blockade after three doses of SBRT (8 Gy) greatly improved the overall response rate, median progression-free survival, and overall survival, even in patients with PD-L1-negative tumors. Whether delayed PD-1 blockade could achieve a better oncological outcome than the current combination regimen deserves more investigation.

In the residual tumor cells, we were still able to observe that the grouped cancer cells were surrounded by a layer of tightly connected and deeply stained cells. Dense lymphocytes infiltrated the peritumoral stroma but could not break the "barrier" cells (Supporting Information Figures S1 and S2). This may be a potential mechanism of immune escape of lung squamous carcinoma, probably independent of programmed cell death 1/programmed cell death ligand 1 (PD-1/PD-L1) expression or T-cell exhaustion, as the infiltrating lymphocytes were mostly CD4+ and CD20+ cells.

T-cell receptor sequencing

The T-cell repertoire is dynamic and directly reflects the diversity of immune responses. T-cell receptors are cell-specific, representing a sort of T-cell "molecular tag", and have been widely studied to monitor the dynamics of T cells in terms of clonality and diversity in different diseases, including malignancies.[24]

We used TCR sequencing technology to investigate the TCR clonotypes. The six patients we selected from both groups demonstrated pCR, pPR, and pSD. As there were no SD patients in the IM group, we chose the patients with the most residual viable tumor cells.

Our study identified that the diversity index (Shannon Index) is statistically different between the primary tumor and normal lymph node, thus we speculate that the tumor-infiltrating T cells were from the lymph nodes that process the tumor-associated antigens.[25] On the other hand, only a small part of the top 100 most frequently detected TCR clonotypes overlapped among the primary tumor, normal lymph nodes, and lymph nodes with metastasis. Furthermore, very few clonotypes were shared among different patients. However, no significant differences were found between these two treatment groups. All the results suggest the extremely individualized pathogenesis and immune response profile of squamous cell lung cancer. Different tumor-infiltrating T cells may be recruited from different lymph nodes with a varying

status of antigen presentation, T cell priming, and proliferation. Therefore, these highly heterogenous tumors require highly individualized treatment strategies.

Furthermore, as shown in our research, the PD-1 blockade did not induce unknown mechanisms of the immune response. Combination therapy is important because therapies with different mechanisms may help to overcome the resistance of tumor cells to PD-1 blockade, thereby releasing the full potentiality of PD-1 blockade. However, combination strategies (like the timing of PD-1 blockade), surgical intervention timing, and follow-up treatments still need to be further explored.

The limitations of our study include, but are not limited to, the small number of patients, the short postoperative follow-up period, and the innate characteristics of retrospective research. Larger prospective randomized studies are needed to confirm the clinical results of our study, while more dissected specimens are necessary to confirm the histological features of both chemotherapy and immunotherapy. Since pathological assessment is often subjective, objective standards and tools are crucial for comparison between different therapies.

CONCLUSION

Based on the results of our research, neoadjuvant chemotherapy combined with PD-1 blockade is safe and feasible for patients with potentially resectable squamous cell lung cancer and may improve the clinical and pathological outcome. However, even with PD-1 blockade, immune suppression within the residual tumor cells still exists. Squamous cell lung cancers and the corresponding immune responses are extremely heterogeneous. The treatment needs to be designed accordingly. The combination strategy of traditional neoadjuvant chemotherapy with current anti-PD-1 antibody needs further investigation.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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