

Pathologic Responses to Neoadjuvant Chemoimmunotherapy in Primary Limited-Stage Small Cell Lung Cancer

Meng Lu (✉ sdlumeng@163.com)

Tianjin Cancer Institute: Tianjin Tumor Hospital <https://orcid.org/0000-0001-7990-2100>

Ran Zhang

Tianjin Medical University General Hospital Tianjin Lung Cancer Institute

Lisha Qi

Tianjin Cancer Institute: Tianjin Tumor Hospital

Yalei Wang

Tianjin Cancer Institute: Tianjin Tumor Hospital

Xiaoxuan Sun

Tianjin Medical University General Hospital Tianjin Lung Cancer Institute

Jian You

Tianjin Medical University Cancer Institute and Hospital: Tianjin Tumor Hospital

Research Article

Keywords: Neoadjuvant chemoimmunotherapy, Limited-stage small cell lung cancer (LS-SCLC), PD-L1, Surgery, Patial pathologic response.

Posted Date: March 2nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1288378/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Immunotherapy has been proved its gigantic influence in extensive-stage small cell lung cancer (ES-SCLC), however, the role of immunotherapy in limited-stage small cell lung cancer (LS-SCLC) is still unknown.

Methods

A retrospective study of 6 patients with LS-SCLC who were treated with neoadjuvant chemoimmunotherapy (durvalumab plus etoposide combined with cisplatin) was performed. Patients were evaluated by the safety, feasibility and pathologic responses of neoadjuvant chemoimmunotherapy.

Results

Neoadjuvant chemoimmunotherapy was associated with few immediate adverse events and did not delay planned surgery. All patients achieved partial pathologic response (pPR) instead of major pathologic response (mPR) or pathologic complete response (PCR). No association was observed between programmed death-ligand 1 (PD-L1) expression in tumor specimens and the pathologic response. However, tumors with high expression of FoxP3 + regulatory T cells (Tregs) had a better pathologic response than tumors with low expression of FoxP3 + Tregs (Pearson's $r = 0.7280$; $P = 0.04$).

Conclusions

Neoadjuvant chemoimmunotherapy achieved pPR with few side effects in all resected tumors with LS-SCLC. The FoxP3 + Tregs in tumor microenvironment might play an important role in the chemoimmunotherapy in LS-SCLC.

Background

Small cell lung cancer (SCLC) is a highly malignant tumor, accounts for 10–15% of all lung cancer pathologic types, and is divided into limited-stage (LS-SCLC) and extensive-stage (ES-SCLC) according to the existence of extrathoracic metastasis^[1, 2]. In addition to regular radiochemotherapy, the immunotherapies that block the immune inhibition of programmed death 1 (PD-1) protein or programmed death-ligand 1 (PD-L1) has played a huge effect in ES-SCLC^[3–5], furthermore, the neoadjuvant chemoimmunotherapy could induce the major pathologic response (mPR) and even pathologic complete response (PCR) in local advanced non-small cell lung cancer (NSCLC) in various clinical studies^[6–8]. Therefore, based on its significant effect in ES-SCLC and local advanced NSCLC, the neoadjuvant

chemoimmunotherapy for LS-SCLC might have the advantage of improving prognosis^[3, 9]. Durvalumab is a recombinant humanized anti-PD-L1 monoclonal antibody which blocks interactions between PD-1 and its ligands, and previous clinical trials have shown that durvalumab achieved a good effect in ES-SCLC with low side effects^[9, 10]. Therefore, durvalumab has been approved in China for ES-SCLC by the Chinese Center for Drug Evaluation in 2018, but the role of it in LS-SCLC is still unknown^[11, 12]. The safety and feasibility of neoadjuvant chemoimmunotherapy in local advanced NSCLC patients had also been proved in several studies^[7, 13], however there were no studies reported on neoadjuvant chemoimmunotherapy in LS-SCLC. Herein, we characterized the pathologic features of neoadjuvant chemoimmunotherapy in patients with LS-SCLC, reported the clinical factors that might influence the pathologic response, aimed to provide the basis for the treatment improvement of LS-SCLC.

Methods

Patients selection and data collection

We performed a retrospective study of 6 patients with LS-SCLC in accordance with the Declaration of Helsinki at the Department of Lung Cancer, Tianjin Medical University Cancer Hospital. This study was approved by the Tianjin Medical University Cancer Hospital Institutional Review Board, all patients received 2 circles of neoadjuvant chemoimmunotherapy [ie.intravenous durvalumab plus chemotherapy (etoposide combined with cisplatin, EP) every 3 weeks] followed by R0 resections (4-6weeks after the last dose of chemoimmunotherapy) and adjuvant therapy (2 circles of adjuvant chemoimmunotherapy). The safety was evaluated by the severity of adverse effect, the feasibility was evaluated by the time of surgery delay and post-operative recovery. The treatment effects of tumors were evaluated based on the Response Evaluation Criteria in Solid Tumors, version 1.1^[14, 15].

Gross pathologic examination and histologic assessments

All tumor tissues were sectioned, and each tumor slide was assessed. Two pathologists evaluated the average percentage of residual viable tumor cells (RVT), which was determined by the ratio of tumor area to tumor bed area in all slides. Hematoxylin and eosin (HE) stained slides from tumors were assessed histologically based on the immune-related pathologic response criteria (irPRC)^[16], which defined the tumor regression bed as a major feature of pathologic response, specifically accompanied by the fibrosis with neovascularization and the immune cell proliferation. In this system, the tumor bed is defined as the regression bed, the RVT and the necrosis. Tumors were grouped as having a PCR (absence of any viable invasive tumor cells), mPR (%RVT ≤ 10%), pPR (partial pathologic response, 10%<%RVT < 90%) and pNR (no pathologic response, %RVT ≥ 90%) according to the %RVT.

Immunohistochemistry

The primary tumors were made into consecutive slides of 5um thickness, and all tissue sections were deparaffinized, rehydrated, pretreated for antigen retrieval. PD-L1 was analyzed by immunohistochemistry using the Monoclonal Rabbit Anti-Human PD-L1 clone SP263 (Ventana, Roche). Furthermore, the

fluorescence staining was conducted on immune cells with primary antibodies (CD4 [Clone EPR6588, ab133616, abcam, UK], CD8[Clone EPR22483-288, ab245118, abcam, UK], FoxP3[Clone 236A/E7, ab20034, abcam, UK].

Statistical analysis

All data were analyzed using SPSS 23.0 (IBM Corporation, New York, USA). The correlation between clinicopathological factors and pathologic response was conducted by the Pearson's correlation coefficient test, all P-values were based on a two-sided hypothesis, and $P < 0.05$ was considered statistically significant.

Results

Safety and feasibility

Six patients who were diagnosed with resectable local advanced LS-SCLC received neoadjuvant chemoimmunotherapy and R0 resections in our department from April 2020 to April 2021. All patients underwent baseline tumor staging and were clinically staging IIIA-IIIB (resectable IIIB, T3 or T4) preoperatively. The clinicopathological characteristics of all patients were listed in Table 1. Of the patients, the median age was 52.17 ± 7.91 (40–60) years, 66.7% (4/6) were male and long-term smokers. Neoadjuvant durvalumab plus EP didn't induce any severe toxic effects in patients, whereas all patients were discharged from hospital within one week after surgery, without severe surgical complications. The median time between the last administration of chemoimmunotherapy and radical resection was 35.2 (range, 30–44) days, there were no surgical delays occurred. Until December 2021, after a median of 10.8 (range, 7–15) months of postoperative follow-up, 83.3% (5/6) of patients were alive. One patient died 7 months after surgery because of severe pneumonia induced by the bacterial infection (patient 1). No patients were diagnosed with any tumor relapses during the follow-up time.

Table 1
Clinicopathological characteristics of all patients

Patients No.	1	2	3	4	5	6
Age/Sex	58/M	45/F	60/M	56/M	40/F	54/M
Smoking	+	-	+	+	-	+
Clinical stage	IIIA	IIIB	IIIA	IIIB	IIIB	IIIA
Pathological stage	IIB	IIIA	IA2	IIIA	IIIA	IIIA
Recist status	PR	PR	SD	PR	PR	SD
Pre-neoadjuvant radiographic size	2.4cm	5.6cm	1.5cm	2.6cm	5.4cm	3.1cm
Post-neoadjuvant radiographic size	0.6cm	1.0cm	1.3cm	0.5cm	1.3cm	2.4cm
Gross pathologic size	1.5cm	1.0cm	2.0cm	1.0cm	1.8cm	3.0cm
%RVT in primary tumor	25%	58%	72%	54%	30%	80%
Pathologic response	pPR	pPR	pPR	pPR	pPR	pPR
%PD-L1 in tumor cells	5	6	0	3	0	0
%PD-L1 in immune cells	42	40	25	20	20	3
Type of resection	Single lobectomy	Complex Lobectomy	Single lobectomy	Single lobectomy	Complex Lobectomy	Bilobectomy
Surgical approach	OPEN	VATS	OPEN	VATS	OPEN	OPEN
Time between neoadjuvant therapy and surgery (days)	35	33	44	31	30	38
Postoperative hospital stay (days)	4	4	5	3	6	5

OPEN: Open thoracotomy; VATS=Video-asisted thoracic surgery.

Patients No.	1	2	3	4	5	6
Follow-up time (months)	7	15	14	11	9	9
Survival status	No	Yes	Yes	Yes	Yes	Yes
Relapse status	No	No	No	No	No	No
OPEN: Open thoracotomy; VATS□Video-asisted thoracic surgery.						

Features of pathologic response in primary tumors

The RVT differed in various cases. The %RVT rised from 25% (patient 1) to 80% (patient 6), with a median percentage of $(53 \pm 22)\%$. Although there were no PCR or mPR occurred in primary tumors, all achieved pPR in postoperative tumor specimens, infiltrating lymphocytes and macrophages were also widely distrubuted in tumors' microenvironments (Fig. 1). Although the radiographic tumor sizes before neoadjuvant chemoimmunotherapy had no relationship with %RVT in resected tumors(Fig. 2A), tumors with radiographic partial response (PR) had a better pathologic response (%RVT: $42 \pm 17\%$) than tumors with radiographic stable disease (SD) (%RVT: $76 \pm 6\%$) ($P = 0.006$) (Fig. 3). Both PD-L1-positive and PD-L1-negative tumors achieved pPR, there were no associations between PD-L1 expression and %RVT (Pearson's test; $P \geq 0.05$) (Fig. 2B, Fig. 2C).

Fluorescence staining of immune cells

The fluorecence staining was performed to explore the variations of immune cells (CD4 + T cells, CD8 + T cells and FoxP3 + Tregs) after neoadjuvant chemoimmunotherapy, the ratios of these immune cells to all cells in tumor microenvironments were analyzed (Fig. 4). The ratios of CD4 + T cells and CD8 + T cells had no relationships with %RVT ($P > 0.05$), whereas the ratio of FoxP3 + Tregs was associated with %RVT ($P = 0.04$). The tumor with a higher ratio of FoxP3 + Tregs tended to have a lower %RVT after neoadjuvant chemoimmunotherapy (Fig. 5).

Discussion

SCLC is almost the highest malignant type of lung cancer, and it's not sensitive to conventional treatment such as the chemoradiotherapy^[17, 18]. Single chemoradiotherapy had a low effectiveness in ES-SCLC, whereas the combination of immunotherapy significantly improved the survival rate in ES-SCLC^[17, 19-21]. Radical surgery plus adjuvant chemotherapy was the routine treatment for LS-SCLC, but the long-term prognosis was still unsatisfactory^[19, 22, 23]. Nowadays the immunotherapy combined with chemotherapy provided the synergistic effect in local-advanced NSCLC^[6], and thus providing a basis for the application of immunotherapy in LS-SCLC.

As reported before, neoadjuvant chemotherapy promoted the earlier elimination of micrometastatic diseases, reduced the surgery risks and improved the tolerability with the therapy in NSCLC^[8, 24]. In this study, the neoadjuvant durvalumab plus chemotherapy (EP) in patients with staging IIIA–IIIB LS-SCLC resulted few adverse events, and didn't delay the anticipated surgery. Only 1 patient encountered severe pneumonia induced by the bacteria infection 7 months after surgery, which had no direct relationship with the chemoimmunotherapy. The evaluation of pathologic response ratio after neoadjuvant therapy allowed the early estimation on curative efficacy, and potentially predicts disease-free (DFS) and overall survival (OS)^[25]. Clinical trials had reported that neoadjuvant chemoimmunotherapy achieved a mPR in 46–83% and a PCR in 38–56% of patients with NSCLC^[6, 13], but the chemoimmunotherapy could hardly achieve mPR in LS-SCLC as shown in our study. However, all patients had lesser than 90% RVT in tumor bed and achieved pPR, consistent with the phenomena of immunologic activation and tumor necrosis. No tumor relapses occurred during the follow-up period. The prognosis statistics and the efficacy of neoadjuvant chemoimmunotherapy for LS-SCLC needs to be further assessed in the future.

We also studied the dynamic changes in response to neoadjuvant chemoimmunotherapy including changes in tumor size, carcinoembryonic antigen (CEA) and neuron specific enolase (NSE). Of these 6 patients, 4 achieved radiographic PR, 2 were radiographic SD, tumors with PR had a better pathologic response than tumors with SD ($P = 0.006$), which indicated that the pathologic regression after chemoimmunotherapy was consistent with the radiographic changes. The PD-L1 expression levels in tumor cells were extremely low in all patients, and the PD-L1 expression had no significant relationship with the pathologic response (%RVT), indicating that PD-L1 expression might not be a good predictor for pathologic response in LS-SCLC.

As reported in previous research, The therapeutic effect of immunotherapy was closely related to the tumor's immune microenvironment, the immune checkpoint inhibitors could hardly come into play if the immune cells in the state of extreme deficiency^[26, 27]. Our study revealed that the ratios of CD4 + and CD8 + T cells had no significant relationships with the pathologic response, whereas the FoxP3 + Tregs could influence the pathologic response. Tumors with higher expression of FoxP3 + Tregs presented a lower %RVT, indicating that the FoxP3 + Tregs increased the pathologic response and play a key role in the immunotherapy in LS-SCLC.

The study still had some drawbacks. Firstly, it was a small sample size, the statistical data might be influenced. Secondly, only a short postoperative follow-up period was conducted due to the time limitation, the prognosis of all patients needed to be evaluated in the future. However, this study has preliminarily confirmed the the safety and feasibility of radical surgery after neoadjuvant durvalumab plus chemotherapy (EP) in IIIA–IIIB LS-SCLC for the first time, and also confirmed that tumors with radiographic PR presented a better pathologic response to neoadjuvant chemoimmunotherapy, which will be of great value in screening out the patients who were not suitable for surgery in the future. It is necessary to continue long-term studies to evaluate whether or not the pPR could translate into prolonged DFS or OS and the relationship between the %RVT with the prognosis.

Conclusion

In summary, this study found that neoadjuvant chemoimmunotherapy achieved pPR with few side effects in all resected tumors with LS-SCLC. More significantly, we confirmed that the high expression of FoxP3 + Tregs in tumor microenvironment was associated with the lower %RVT in primary tumors, indicating that the FoxP3 + Tregs might play an important role in the chemoimmunotherapy in LS-SCLC. These findings will help surgeons to recognize patients who are sensitive to the neoadjuvant chemoimmunotherapy, therefore develop a personalized treatment plan for resectable LS-SCLC in the future.

Abbreviations

ES-SCLC☐Extensive-stage small cell lung cancer

LS-SCLC☐Limited-stage small cell lung cancer

SCLC☐Small cell lung cancer

pPR☐Partial pathologic response

mPR☐Major pathologic response

PCR☐Pathologic complete response

PD-L1☐Programmed death-ligand 1

Tregs☐Regulatory T cells

PD-1☐Programmed death 1

NSCLC☐Non-small cell lung cancer

EP☐Etoposide combined with cisplatin

RVT☐Residual viable tumor cells

HE☐Hematoxylin and eosin

irPRC☐immune-related pathologic response criteria

pNR☐No pathologic response

PR☐Partial response

SD☐Stable disease

CEA☐Carcinoembryonic antigen

NSE☐Neuron specific enolase

DFS☐Disease free survival

OS☐Overall survival

Declarations

Ethics approval and consent to participate

The study was in accordance with the Declaration of Helsinki and approved by the Tianjin Medical University Cancer Hospital Institutional Review Board. Before enrollment in this current study, all participants handed in informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

LM, YJ conceived and designed the present study. QL,WY performed the HE staining and immunohistochemistry. LM,QL,SX analyzed the results of the immunohistochemical experiment. LM,ZR performed statistical analysis and data interpretation. ZR,SX performed clinical data collection and samples collection. LM,ZR,YJ wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

- [1] Torre L A, Siegel R L, Jemal A. Lung Cancer Statistics[J]. *Adv Exp Med Biol*,2016,893:1-19.
- [2] Abdelhamid K, Kakourou A, Degrauwe N, et al. [Small-cell lung cancer: management and novelties][J]. *Rev Med Suisse*,2020,16(695):1079-1085.
- [3] Calles A, Aguado G, Sandoval C, et al. The role of immunotherapy in small cell lung cancer[J]. *Clinical and Translational Oncology*,2019,21(8):961-976.
- [4] Iams W T, Porter J, Horn L. Immunotherapeutic approaches for small-cell lung cancer[J]. *Nat Rev Clin Oncol*,2020,17(5):300-312.
- [5] Paz-Ares L, Dvorkin M, Chen Y, et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial[J]. *Lancet*,2019,394(10212):1929-1939.
- [6] Gentzler R D, Riley D O, Martin L W. Striving toward Improved Outcomes for Surgically Resectable Non-Small Cell Lung Cancer: the Promise and Challenges of Neoadjuvant Immunotherapy[J]. *Current Oncology Reports*,2020,22(11).
- [7] Chen Y, Yan B, Xu F, et al. Neoadjuvant chemoimmunotherapy in resectable stage IIIA/IIIB non-small cell lung cancer[J]. *Translational Lung Cancer Research*,2021,10(5):2193-2204.
- [8] Ahern E, Solomon B J, Hui R, et al. Neoadjuvant immunotherapy for non-small cell lung cancer: right drugs, right patient, right time?[J]. *Journal for ImmunoTherapy of Cancer*,2021,9(6):e2248.
- [9] Esposito G, Palumbo G, Carillio G, et al. Immunotherapy in Small Cell Lung Cancer[J]. *Cancers*,2020,12(9):2522.
- [10] Yang S, Zhang Z, Wang Q. Emerging therapies for small cell lung cancer[J]. *Journal of Hematology & Oncology*,2019,12(1).
- [11] Constantinidou A, Aliferis C, Trafalis D T. Targeting Programmed Cell Death -1 (PD-1) and Ligand (PD-L1): A new era in cancer active immunotherapy[J]. *Pharmacology & Therapeutics*,2019,194:84-106.
- [12] Paz-Ares L, Dvorkin M, Chen Y, et al. Durvalumab plus platinum–etoposide versus platinum–etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial[J]. *The Lancet*,2019,394(10212):1929-1939.
- [13] Shukla N, Hanna N. Neoadjuvant and Adjuvant Immunotherapy in Early-Stage Non-Small Cell Lung Cancer[J]. *Lung cancer (Auckland)*,2021,12:51-60.
- [14] Eisenhauer E A, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1)[J]. *Eur J Cancer*,2009,45(2):228-247.

- [15] Schwartz L H, Litiere S, de Vries E, et al. RECIST 1.1-Update and clarification: From the RECIST committee[J]. *Eur J Cancer*,2016,62:132-137.
- [16] Cottrell T R, Thompson E D, Forde P M, et al. Pathologic features of response to neoadjuvant anti-PD-1 in resected non-small-cell lung carcinoma: a proposal for quantitative immune-related pathologic response criteria (irPRC)[J]. *Ann Oncol*,2018,29(8):1853-1860.
- [17] The Role of Surgical Resection and the Effects of Neo-Adjuvant Therapy in the Management of Small Cell Lung Cancer[J]. 1994.
- [18] Wada H, Yokomise H, Tanaka F, et al. Surgical treatment of small cell carcinoma of the lung: advantage of preoperative chemotherapy[J]. *Lung Cancer*,1995,13(1):45-56.
- [19] Li S, Jin K, Pan Y, et al. Role of surgery in a case-control study of patients with clinical stage IIIA small cell lung cancer[J]. *Journal of Thoracic Disease*,2021,13(5):2738-2745.
- [20] Tsiouprou I, Zaharias A, Spyrtos D. The Role of Immunotherapy in Extensive Stage Small-Cell Lung Cancer: A Review of the Literature[J]. *Can Respir J*,2019,2019:6860432.
- [21] Konala V M, Madhira B R, Ashraf S, et al. Use of Immunotherapy in Extensive-Stage Small Cell Lung Cancer[J]. *Oncology*,2020,98(11):749-754.
- [22] Xu Y, Zheng H, Gao W, et al. Is neoadjuvant chemotherapy mandatory for limited-disease small-cell lung cancer?[J]. *Interactive CardioVascular and Thoracic Surgery*,2014,19(6):887-893.
- [23] Long-term Outcomes and Patterns of Failure Following Surgical Resection of Small[J]. 2015.
- [24] Ling Y, Li N, Li L, et al. Different pathologic responses to neoadjuvant anti-PD-1 in primary squamous lung cancer and regional lymph nodes[J]. *NPJ precision oncology*,2020,4(1):32.
- [25] Weissferdt A, Pataer A, Vaporciyan A A, et al. Agreement on Major Pathological Response in NSCLC Patients Receiving Neoadjuvant Chemotherapy[J]. *Clinical Lung Cancer*,2020,21(4):341-348.
- [26] Rowshanravan B, Halliday N, Sansom D M. CTLA-4: a moving target in immunotherapy[J]. *Blood*,2018,131(1):58-67.
- [27] Koh J, Hur J Y, Lee K Y, et al. Regulatory (FoxP3(+)) T cells and TGF-beta predict the response to anti-PD-1 immunotherapy in patients with non-small cell lung cancer[J]. *Sci Rep*,2020,10(1):18994.

Figures

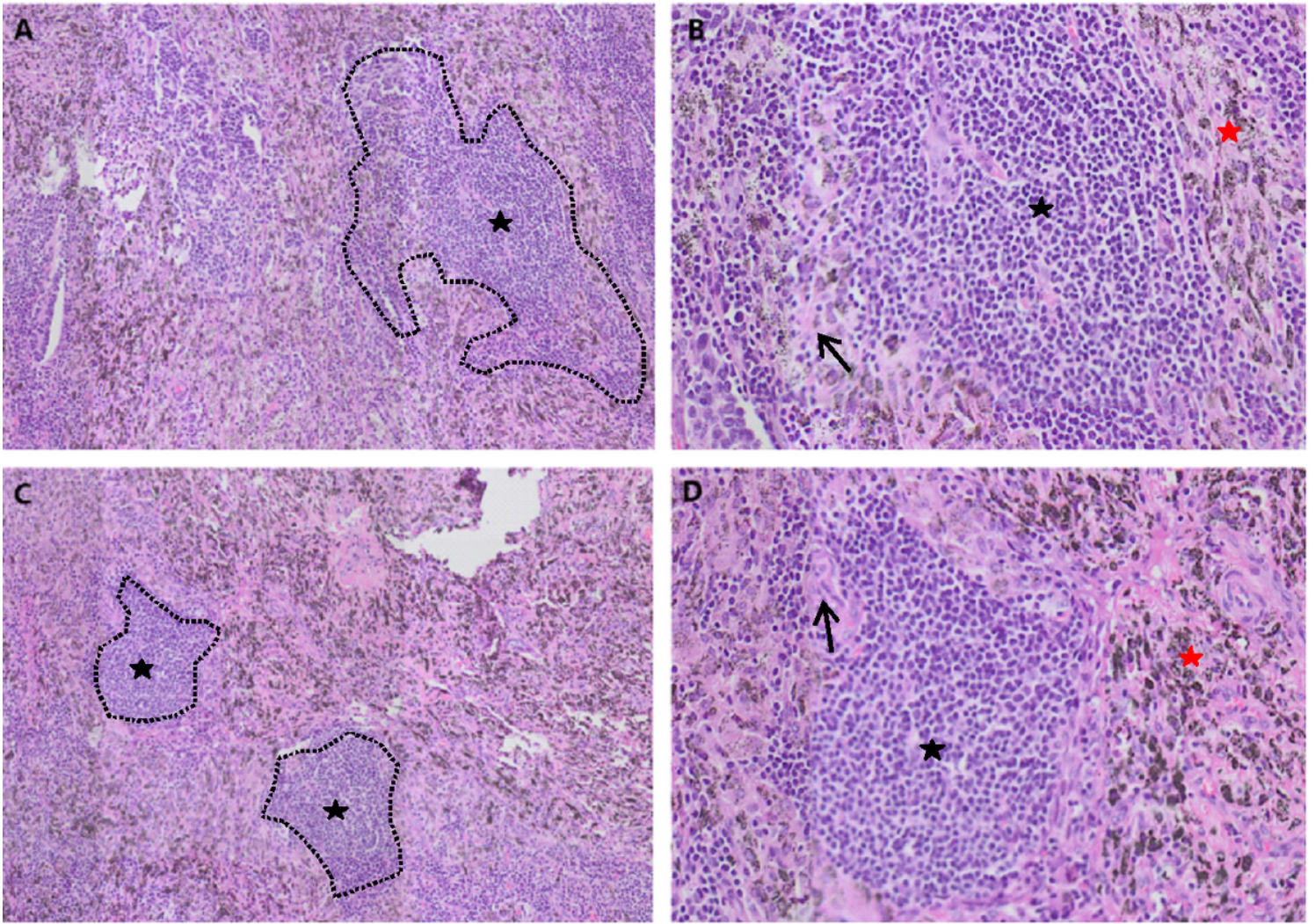


Figure 1

Representative pathologic responses to neoadjuvant anti-PD-L1 in primary tumor specimens of LS-SCLC (pPR). The black circles and stars indicated the residual viable invasive tumor cells in one of the tumor bed of Patient 4 (A,B) and Patient 5 (C,D). The black arrow indicated the lymphocytes infiltrating around the tumor cells in Patient 4 (B) and Patient 5(D). The red stars indicated necrotic tumor cells associated with fibrotic tissue repair. The Original magnifications: A,C×4; B,D×10.

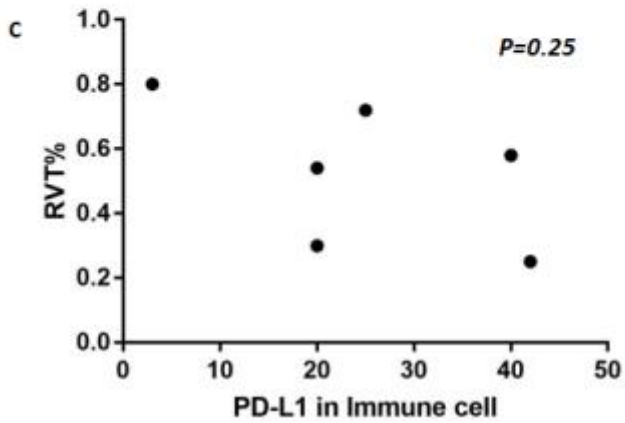
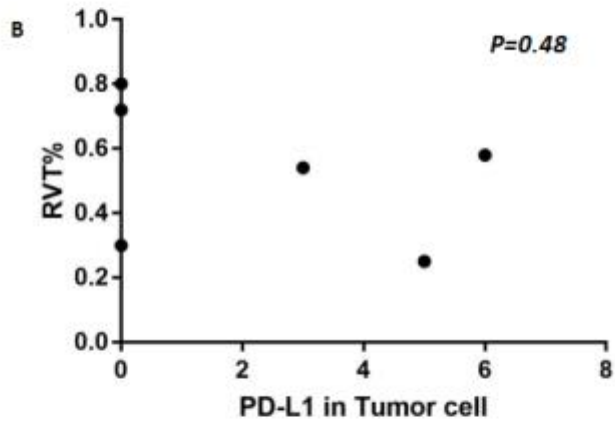
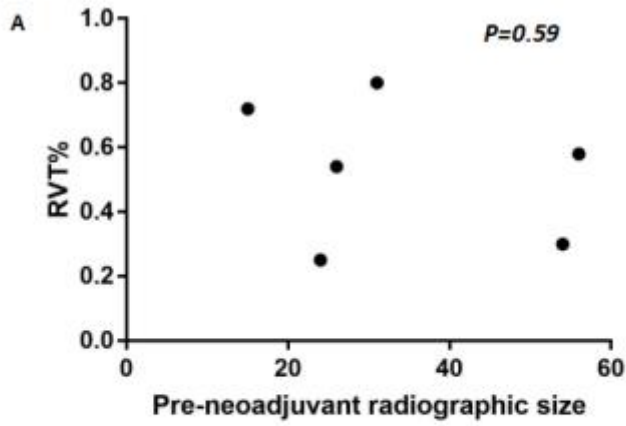


Figure 2

Correlation of pathologic response with pre-neoadjuvant radiographic tumor size (A) and the PD-L1 expressions of primary tumor (B,C). Each dot indicates one patient.

PR(Patient 4)

SD(Patient 6)

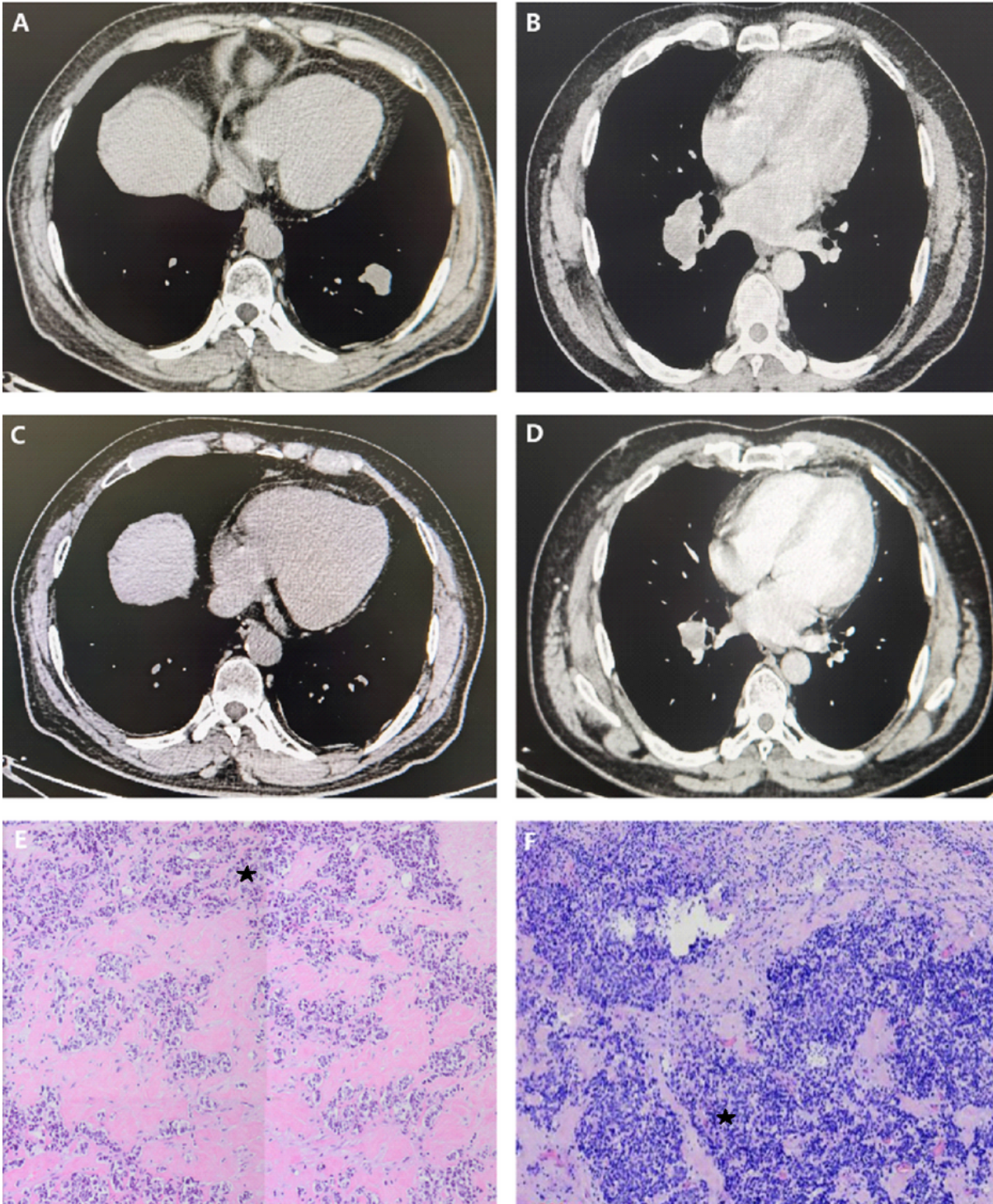


Figure 3

Patterns of radiologic and pathologic response to neoadjuvant chemoimmunotherapy. Left column: Patient 4 (PR); Right column: Patient 6 (SD). A,C presented the chest CT imaging of Patient 4 before and after the administration of neoadjuvant chemoimmunotherapy. E indicated the representative sections of tumor specimens after HE staining. This patient had 54% of RVT in the resected specimen. B,D presented the chest CT imaging of Patient 6 before and after the administration of neoadjuvant

chemoimmunotherapy. F indicated the representative sections of tumor specimens after HE staining. This patient had 80% of RVT in the resected specimen. The black star indicated the RVT.

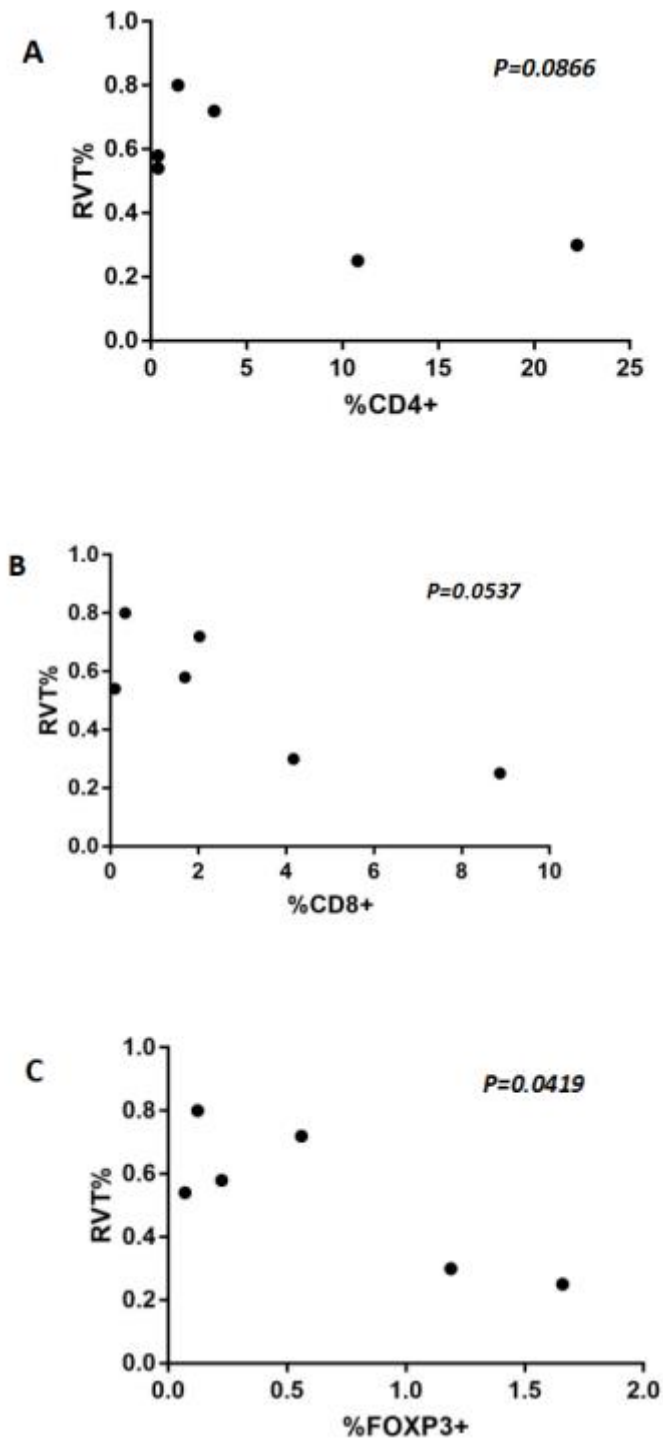


Figure 4

Correlation of immune cells with the %RVT. A. The ratio of CD4+ T cells had no statistical relationship with %RVT ($P=0.0866$). B. The ratio of CD8+ T cells had no statistical relationship with %RVT ($P=0.0537$). C. The ratio of FoxP3+ Tregs had statistical relationship with %RVT ($P=0.0419$).

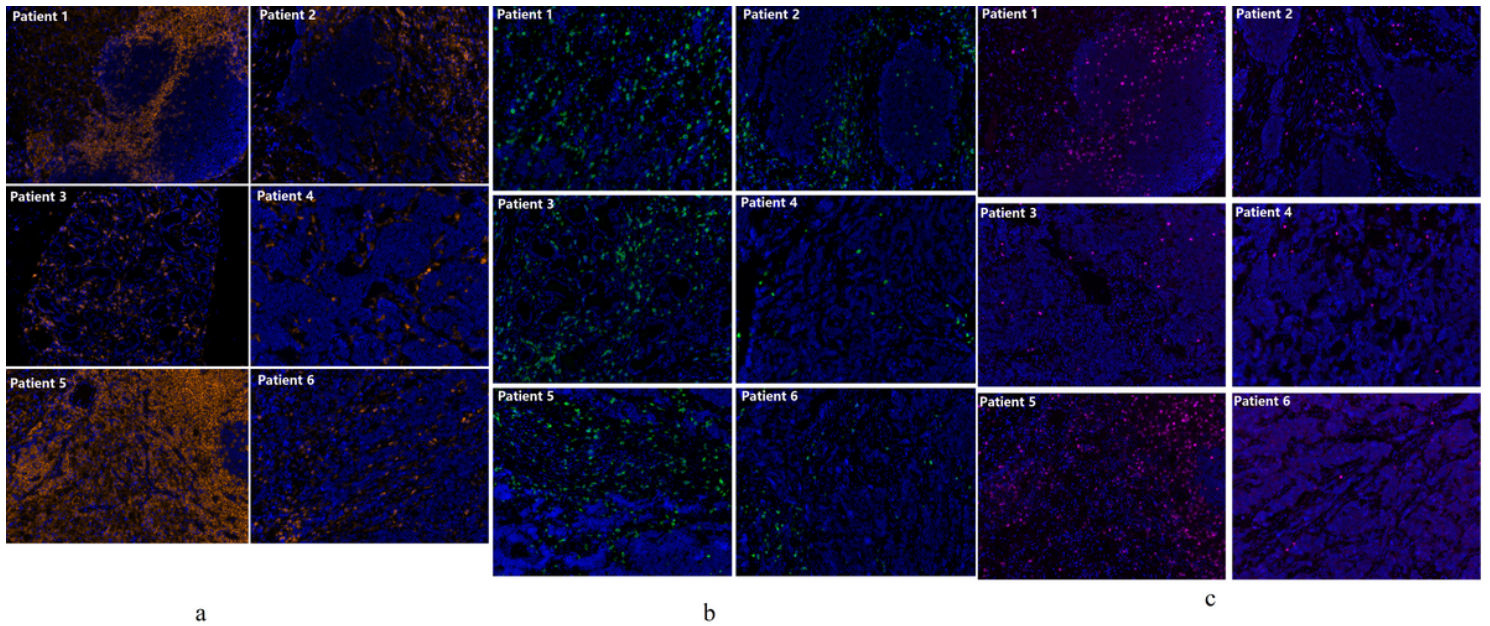


Figure 5

Immune profiling of immune cells to neoadjuvant chemoimmunotherapy in resected primary tumors. A. The fluorescence of CD4+ T cells (orange fluorescence). B. The fluorescence of CD8+ T cells (green fluorescence). C. The fluorescence of FoxP3+ Tregs (pink fluorescence).