Neoadjuvant Chemoimmunotherapy in Resectable IIIA/IIIB Non-small Cell Lung Cancer

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Research

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Abstract

Background: A small portion of patients experience objective clinical benefit after neoadjuvant PD-1 blockade in NSCLC. The combining checkpoint blockade and chemotherapy as neoadjuvant chemoimmunotherapy therapeutic regimen might be more effective but has not been tested in resectable stage IIIA/IIIB NSCLC.

Methods: This is a retrospective study of 35 patients with resectable stage IIIA and IIIB NSCLC who were treated with neoadjuvant chemoimmunotherapy (NCIO). The pathological complete response (pCR), major pathologic response(MPR), the safety and feasibility were evaluated. The correlation between the pathology response and some clinical factors was studied to identify some predictors.

Results: NCIO was associated with few immediate adverse events. The NCIO did not delay planned surgery and led to a complete pathological response(pCR) in 51.43% patients and a major pathological response in 74.29% patients in the primary tumor. No association was observed between PD-L1 expression before the treatment and pathological response to the NCIO (Pearson's r=-0.071; P=0.685). However, significant difference was observed between invasion status of the bronchus(ISB) and pathological response (P<0.05). The patients with Invade status were with higher pCR and MPR rates as compared with No-Invade status, with 76.47% pCR and 100% MPR rate *vs.* 31.58% pCR and 50.00% MPR rate(Pearson's r=0.7280; P=0.0009).

Conclusions: NCIO was associated with few side effects, did not delay surgery, and induced a complete pathological response in 51.43%% of resected tumors. No significant correlation between the pathological response and PD-L1 expression. While the ISB was predictive of the pathological response to NCIO.

Background

The majority of the treatment methods have focused on the establishment of new treatment options for non-small cell lung cancer (NSCLC). Programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) immune checkpoint inhibitors (ICIs) are currently the most investigated immunotherapies [1]. Therapeutically blocking this inhibitory molecular axis using specific monoclonal antibodies targeted to PD-1 and PD-L1 activates the immune system to recognize and target the cancer cells via a T cell-mediated immune response [2]. Recently, the neoadjuvant PD-1 blockade has also been investigated in resectable NSCLC but only a small percentage of patients got pathological complete response(pCR). Clinical trials LCMC3 (ClinicalTrials.gov numbers: NCT02927301) has reported that only 21% of the patients present major pathological response (MPR), while 5% exhibited pathological complete response (pCR) of the resected tumors [3]. Thus, the combination of checkpoint blockade and chemotherapy explores the potential for synergistic immune activation. Several clinical studies have focused on neoadjuvant immunotherapy prior to tumor resection for advanced non-small cell lung cancer (NSCLC). In the 2019 World Conference on Lung Cancer, it has been reported that neoadjuvant anti-programmed

death-1 (PD-1) immunotherapy does not delay surgery and achieves a major pathological response in 83% of patients who undergo tumor resection [4]. While in the 2020 ASCO Annual Meeting, Ralph Zinner reported that only 6/13 (46%) and 5/13 (38%) having an MPR and pCR respectively[5]. Recently, Catherine A Shu also reported a phase II clinical trail about the neoadjuvant atezolizumab and chemotherapy in patients with resectable non-small-cell lung cancer. It reported that there are 17 (57%; 95% CI 37–75) of 30 patients had a major pathological response and 10 (33%; 17–53) of 30 patients had a pathological complete response[6]. However, there still no reported which systematically and fully discussed this topic in any journal and develop a high-performance predictive factor which is critical and of tremendous assistance to avoid non-essential surgery. Herein, we examined the safety and feasibility of the use of neoadjuvant PD-1 antibody plus chemotherapy in a small group of patients with resectable stage IIIA/IIIB NSCLC. In addition, the correlation between the pathology response and some clinical factors was established to find some predictors, including PD-L1 expression, CD4 + and CD8 + T cells and Treg cell, and invasion status of the bronchus.

Methods

Patients

This is a retrospective study of 35 patients with resectable stage IIIA and IIIB non-NSCLC who were treated with Pembrolizumab plus chemotherapy as neoadjuvant chemoimmunotherapy at Tianjin Medical University Cancer Institute and Hospital (TJMUCH), Tianjin, China. The clinical stage was predicted according to the American Joint Committee on Cancer (AJCC) 8th edition.

The effect, safety, adverse events , and radiological and pathological responses to treatment was studied. The adverse events are according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Changes in the tumor size were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [7]. All patients underwent R0 resection including lobectomy, sleeve lobectomy, completely pneumonectomy, pulmonary arterioplasty, and radical dissection of lymphonde. Feasibility was defined as any delay in the planned surgery of <30 days. Informed consent is signed by all study participants and this study was approved by the institutional review boards at the Tianjin Medical University Cancer Institute and Hospital.

Immunohistochemistry (IHC)

PD-L1 was analyzed by IHC at the Pathology Department of TJMUCH using the Monoclonal Mouse Anti-human PD-L1 clone 22C3 (LOT10145059, Dako, Carpentaria, CA, USA). Tumor proportion score (TPS) and combined positivity score (CPS) were calculated as the percentage of at least 100 viable cells with complete or partial membrane staining. However, in patients with no residual tumor after the treatment, only CPS was calculated. The interpretation of TPS or CPS was provided by the pathologist of TJMUCH.

Flow cytometry

Fresh tumor tissue was obtained during the surgery and were mechanically and enzymatically disaggregated into a single cell suspension. CD45, CD3, CD4, CD8, CD127, and CD25 were purchased from BioLegend (San Diego, CA, USA). The antibodies were as follows: FITC anti-human CD4 317408; PE anti-human CD25 302606; PE anti-human CD163 326506; PerCP/Cy5.5 anti-human CD127 (IL-7Rα) 351322; APC anti-human CD68 333810; Brilliant Violet 421 anti-human CD3 300434; Brilliant Violet 510 anti-human CD45 304036. The cells were stained with optimal amounts of each antibody for 30 min at 4 ℃ in the staining buffer, according to the manufacturer's protocol. Data (10,000 events) were acquired on BD-Aria II flow cytometer (BD Biosciences, San Diego, CA, USA) and analyzed using FlowJo software (FlowJo LLC, Ashland, OR, USA).

Pathological assessments

Major pathological response was assessed by local pathologists who measured the percentage of residual viable tumour in resected primary tumours from each patient at the time of surgery, using previously reported methods[1,6,8,9].All tumour bed samples that were less than 6 cm in their greatest diameter were submitted in their entirety. For tumour bed samples that were 6 cm or more in greatest diameter, a minimum of one section per cm of the greatest tumour bed dimension was assessed for major pathological response. Tumour tissue samples were sectioned, and the percentage of viable tumour tissue was recorded for each tumour slide. The average percentage of viable tumour tissue for each patient was then calculated. And tumors with <10% viable tumor cells were considered to have a major pathological response (MPR), and no viable tumor cells were deemed as pCR.

Statistical analysis

Mann-Whitney test was used to compare the pathological response between Invasion and no Invasion status and TPS score. Independent sample t-test was used to compare the proportion of CD4, CD8, and Treg cells between genotype cohorts. PFS and OS was estimated using Kaplan-Meier curve and log-rank test. Pearson's correlation coefficient were used to analyze the correlation between TPS score and pathological response. All P-values were based on a two-sided hypothesis, and data were analyzed using SPSS 22.0.

Results

From January 2019 to May 2020, 35 patients with resectable stage IIIA and IIIB non-NSCLC were treated with neoadjuvant chemoimmunotherapy at TJMUCH. The median age of the cohort was 62.17 ± 5.99 (43–72) years, 82.86% were men, 71.43% had squamous carcinoma, 88.57% were stage IIIA, and 48.57% were PD-L1 positive(≥ 50%). For the clinical TNM stage, 12 patients are T1N2M0; 8 are T2N2M0;10 are T3N1M0; 5 is T3N2M0 and 1 are T4N1M0. All the patients underwent baseline tumor staging, including pretreatment biopsy, pathological evaluation of mediastinal lymph nodes (if indicated) by bronchoscopy or mediastinoscopy, positron-emission tomography-computed tomography (PET-CT), and contrast-enhanced CT or magnetic resonance imaging of the brain and chest. Patients with squamous carcinoma

received 2 courses of Pembrolizumab 2 mg/kg IV q3w added to cisplatin 75 mg/m^2 IV q3w plus paclitaxel liposome 135 mg/m^2 q3w; and patients with non-squamous carcinoma received Pemetrexed 500 mg/m^2 IV q3w instead of paclitaxel liposome. (Table 1).

Table 1
Characteristics of the patients at baseline according to the pathological response

		All patients	pCR	no PCR	P value
Age					
	>=65	12	6	6	0.903
	< 65	23	12	11	
Sex					
	Male	29	16	13	0.33
	Female	6	2	4	
cTNM					0.33
	IIIA	29	16	13	
	IIIB	6	2	4	
Smoking status					0.186
	Never	8	2	6	
	Former	4	3	1	
	Current	33	13	10	
Histology					0.275
	Adenocarcinoma	7	2	5	
	Squamous carcinoma	26	15	11	
	Large cell carcinoma	1	0	1	
	Sarcomatoid carcinoma	1	1	0	
PD-L1					0.625
	≥ 50%	20	11	9	

§Video-assisted thoracoscopic surgery

Does not include pneumonectomy, bilobectomy, or complex lobectomy

$ Does not include pneumonectomy

*Complex lobectomy includes sleeve resection and sleeve or partial resection of the pulmonary artery or surgery for pancoast tumors

		All patients	pCR	no PCR	P value
	< 50%	15	7	8	
Invasion status of the bronchus					0.004
	Yes	17	13	4	
	No	18	5	13	
RECIST status					0.404
	PR		11	8	
	SD		7	9	
Surgical approach					0.296
	Open	34	18	16	
	Completed VATS§	1	0	1	
Type of resection					0.846
	Single Lobectomy #	9	5	4	
	Pneumonectomy	3	1	2	
	Bilobectomy$	9	4	5	
	Complex Lobectomy*	14	8	6	

§Video-assisted thoracoscopic surgery

Does not include pneumonectomy, bilobectomy, or complex lobectomy

$ Does not include pneumonectomy

*Complex lobectomy includes sleeve resection and sleeve or partial resection of the pulmonary artery or surgery for pancoast tumors

Safety and feasibility

Neoadjuvant Pembrolizumab plus paclitaxel liposome or pemetrexed combined with cisplatin was not associated with any previously unreported toxic effects. Pre-surgical grade 3 toxicity, i.e. rash, occurred in 1/35 patients. All the 35 eligible patients (100%) underwent complete tumor resection and the median interval between the administration of the second dose of NCIO and surgery was 33.4 (range, 28–35) days, and. No treatment-related surgical delays occurred as defined in the protocol. 18/35 (51.43%)

patients achieved pCR, and 26/35 (74.29%) exhibited MPR. And there are 1 patients had no residual tumor in the primary tumor but had a residual tumor in hilar lymph nodes, which we considered as 0% pathological regression . According to the pathological responses, all patients were dived into pCR group and non-pCR group. In the pCR group, the median age was 61.5±6.88 years, 88.89% were men, 83.33% had squamous carcinoma, 88.89% were stage IIIA, and 61.10% were PD-L1 TPS >50%, while in the non-pCR group, the median age was 62.88±5.01 years, 76.47% were men, 64.71% had squamous carcinoma, 76.47% were stage IIIA, and 52.94 % were PD-L1 TPS >50%. (Table 1, Figure 1).

At a median of 13.29 (range, 3–24) months of postoperative follow-up, 33/35 (94.29%) patients who had undergone surgical resection were alive and recurrence-free. One patient died 3 months after the surgery because of cerebral ischemic stroke (patient 3), and the other died after 10 months post-surgery due to mass N2 lymph nodes metastasis (patient 1). One patient was diagnosed with brain metastasis (patient 25) 12 months after the surgery. The median duration of recurrence-free survival had not been reached at the time of data analysis. Hence, based on the available data, no significant differences were detected in the progression-free survival (PFS) and overall survival (OS) between the pCR and non-pCR groups (Figure 2).

Pathological findings after neoadjuvant PD-1 blockade

Representative radiological and pathological responses after two preoperative NCIO are shown in Figure 3. MPR occurred in 26/35 (74.29%) patients; 18 patients (51.43%) had pCR in the primary tumor. Despite apparent tumor enlargement on preoperative CT (possibly because of the infiltration of immune cells into the tumor), one patient exhibited pCR, that is pseudoprogression . In primary tumors with MPR, we observed a large number of infiltrating lymphocytes and macrophages. This finding was compatible with an immunological mechanism of response along with the phenomenon that necrotic tumor was associated with fibrotic tissue repair (Figure 3).

Invasion status of the bronchus with pathological response

We also analyzed the invasion status of the bronchus (ISB)by bronchoscope based on CT or PET-CT for all the patients. If the tumor invades the bronchus, a neoplasm is seen in the bronchus by the bronchoscope. If the tumor has not invaded the bronchus, we can only observe the red color and narrowing near the opening of the bronchus due to the pressure from the tumor (Figure 4a). Interestingly, 17/35 (48.57%) patients were evaluated as Invade and 18/35 (51.43%) as no-Invade. Of these, 13 (76.47%) patients in the Invade group presented pCR response, and all the 17(100%) patients showed the MPR response. On the other hand, only 5 (27.78%) patients in the no-Invade group showed pCR response, and 9 (50.00%) patients did not demonstrate MPR . The Pearson’s rho is 0.7280 and there is significant difference between the two groups (P=0.0009; Figure 4b).

Expression of PD-L1 with pathological response

The expression of PD-L1 could be evaluated in pretreatment biopsy samples in all patients (Figure 5A). A pCR or MPR response occurred in both PD-L1-positive and PD-L1-negative tumors. The correlation between the TPS scores of pretreatment biopsy samples and pathological regression was analyzed. Figures 5B shows that there is no association between PD-L1 expression before the treatment and the pathological response to NCIO (Pearson's r=-0.071; P=0.685).

Immune proofing of T cells to NCIO

To further explore the T-cell proofing of these patients, flow cytometry analysis was performed. CD4+ and CD8+ T cells and Treg cells were evaluated in the post-surgery samples in 17 available patients (Figure 7). The ratio of the CD4+, CD8+ T cells, and Treg cells to T cells and all cells of the sample were analyzed in the pCR and MPR groups. As shown in Figure 7b, the proportion of CD 4+ T cells to T cells was 36.93±12.34 and 43.67±14.61 in the pCR group and non-PCR group, respectively (P>0.05), while the percentage of CD 4+ T cells to all cells in the sample was 7.42±4.94 and 10.39±11.90 in the pCR and non-PCR groups, respectively (P>0.05). Moreover, the percentage of CD8+ T cells to T cells was 50.74±12.78 and 43.90±19.70 in the pCR and non-PCR groups, respectively (P>0.05). Furthermore, the percentage of CD 8+ T cells to all cells in the sample was respectively 10.43±7.97 and 9.71±6.97 in the pCR and non-PCR groups, respectively (P>0.05). The percentage of Treg cells to T cells was 1.55±1.25 and 3.57±2.69 in the pCR and non-PCR groups, respectively (P>0.05), while that of Treg cells to all cells in the sample was 0.41±0.57 and 1.04±1.27 in the pCR and non-PCR groups, respectively (P>0.05).

Discussion

The combination of checkpoint blockade and chemotherapy explores the potential for synergistic immune activation and was found to be impressive. Several clinical studies have been reported that neoadjuvant anti-programmed death-1 (PD-1) immunotherapy achieves a major pathological response in 46–83% of patients and 38%-56% got a pathological complete response who undergo tumor resection [3–6]. Dur to these impressive results, there are more and more stage IIIA or IIIB NSCLC patients in China who take neoadjuvant chemoimmunotherapy before surgery. And according to the related reports and our experience, the patients with squamous carcinomas appear with better pathological respons. Therefor there are much more patients with squamous carcinomas than adenocarcinoma.

In our study, we observed that neoadjuvant administration of two doses of Pembrolizumab plus paclitaxel liposome or pemetrexed combined with cisplatin in patients with IIIA-IIIB resectable stage NSCLC was associated with few immediate adverse events. NCIO did not delay the planned surgery and led to pCR in 51.43% patients and MPR in 74.29% patients in the primary tumor. Interestingly, there are 2 patients had no residual tumor in the primary tumor but had a residual tumor in hilar lymph nodes. Also, it was not associated with any previously unreported toxic effects [1–4, 10]. The NADIM study reported that 13% of the patients encountered G3–5 TRAEs, and the most common postsurgical complication was respiratory infections [10]. In the current study, only one patient encountered G3–5 TRAE, which is rash, and none of the patients experienced postsurgical complications, including respiratory infections.

Immune-checkpoint blockade derived long-term OS in a subset of cancer patients [11–12]. Strikingly, only a subset of patients with advanced solid tumors responded to the treatment. Therefore, developing a method to identify patients who are most likely to respond to immunotherapy is essential. Several predictors, such as PD-L1 [13–15], tumor mutational burden (TMB) [16], and radiomics [17], were explored to help in the prediction of clinical outcomes. However, there is yet no reliable predictor to aid in clinical decision-making. Based on our findings, no significant correlation could be established between the PD-L1 expression and pathological regression. Also, the pathological regression did not differ significantly between the TPS scores ≥ 50% and < 50% (Pearson's r=-0.071 and P = 0.685). The pCR or MPR occurred in both patients with high or low TPS. The PD-L1 expression is not a good predictor of the pathological response. Hence, we also studied the dynamic changes in squamous cell carcinoma (SCC), including the changes in the tumor size with respect to NCIO. These factors did not show any significant difference between the pCR and non-pCR groups. Surprisingly, ISB was detected as the pathological response. 76.47% patients in the Invade group showed pCR and 100% patients exhibited MPR, while only 31.58% patients in the no-Invade group presented pCR response, 50% patients showed MPR, and 50% patients did not exhibit MPR($P < 0.001$). And the Pearson's rho is 0.7280 which indicates that this might be a valuable parameter for the prediction of the pathological response to NCIO.

Furthermore, we also studied the immune proofing of T cells to the NCIO and identified CD4 + and CD8 + T cells and Treg cells. Goldberg et al. demonstrated that PD-1 blockade enhances the early stage of T-cell activation in lymph nodes (18). Liu et al. reported that neoadjuvant PD-1 blockade enhances the systemic priming of antitumor T cells, thereby potentially eliminating micrometastatic cancer that might otherwise cause postsurgical relapse [19]. Our findings did not reveal any significant differences in CD4 + and CD8 + T cells and Treg cells between the pCR and non-pCR groups and the MPR and non-MPR groups. The number of CD4 + and CD8 + T cells and Treg cells might be the same between the pCR and non-pCR groups. However, the function might be different as these or other immune cells caused tumor death. Salmon et al. found the importance of dendritic cells to the antitumor effects of PD-1 pathway blockade, indicating that PD-1 blockade not only worked to directly unleash the intratumoral T-cell killing but also enhanced the tumor antigen-driven priming of T cells (20). In the present study, the TPS changes before and after the treatment were also analyzed. The proportion of the TPS increase and decrease was 64.28% and 27.27%, respectively, in the pCR group, while in the non-pCR group, 2/11 patients showed an increase, and 3/11 patients showed a decrease in the score. Strikingly, in the pCR group, no tumor was left but a large number of PD-L1-positive immune cells were detected. How the anti-PD-1 drug influences the cells with PD-L1 antigen might be the key to understanding the antitumor mechanism.

Nevertheless, the present study has some limitations: the small sample size and the short postoperative follow-up period. However, this report, for the first time, demonstrated the safety of surgical resection after treatment with Pembrolizumab plus paclitaxel liposome or pemetrexed combined with cisplatin in stage IIIA to IIIB NSCLC. This study, for the first time, confirms the ISB as a predictor of the pathological response to NCIO which is critical and of tremendous assistance to avoid non-essential surgery. Also, long-term follow-up of these studies will be necessary to decipher whether MPR and pCR rates prolong OS and PFS.

Conclusions

Neoadjuvant PD-1 blockade plus chemotherapy was associated with few side effects, did not delay surgery, and induced a complete pathological response in 51.43% of the resected tumors. The current results supported the widespread use of NCIO to resectable stage IIIA and IIIB non-NSCLC patients. No significant correlation between the pathological response and PD-L1 expression. While the ISB was predictive of the pathological response to NCIO. And long-term follow-up of these studies will be necessary to decipher whether MPR and pCR rates prolong OS and PFS.

Abbreviations

NSCLC: Non-small Cell Lung Cancer

NCIO: neoadjuvant chemoimmunotherapy

pCR: pathological complete response

MPR: major pathologic response

PD-1: programmed cell death 1

PD-L1: programmed cell death ligand 1

IHC: Immunohistochemistry

TPS : tumor proportion score

CPS: combined positivity score

CT：computed tomography

Declarations

DATA SHARING STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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DECLARATION OF INTERESTS

None exist.

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Figures

Figure 1

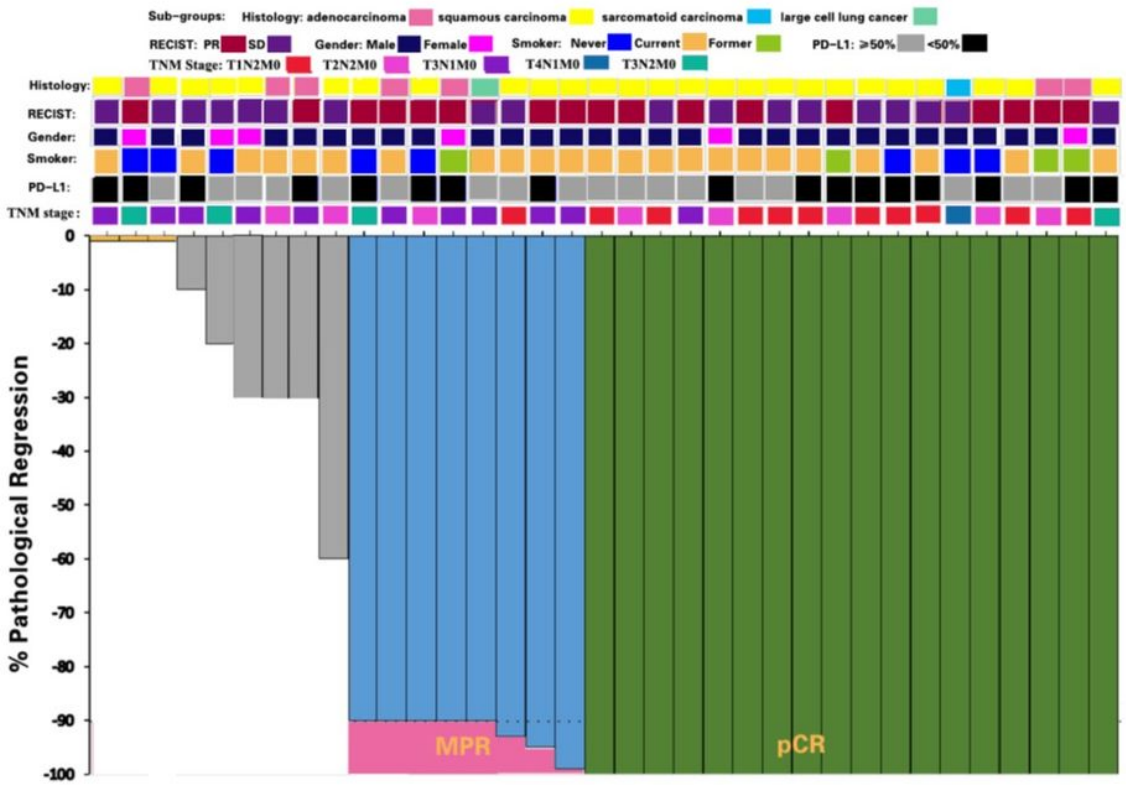

Figure 1

Pathological assessment of response to NCIO Pathological regression in the resected primary lung tumor after NCIO, according to the percentage of remaining viable tumor cells, for each of the 35 patients who underwent surgical resection. The gray horizontal line indicates the threshold for a major pathological response (90% regression). Clinical and pathological features that include the histology, the preoperative radiologic response (according to RECIST), gender, smoker status, PD-L1 expression in the pretreatment biopsy sample and TNM stage are annotated for each patient. PR denotes partial response, SD stable disease, and PD-L1 programmed death-ligand 1.

Figure 2

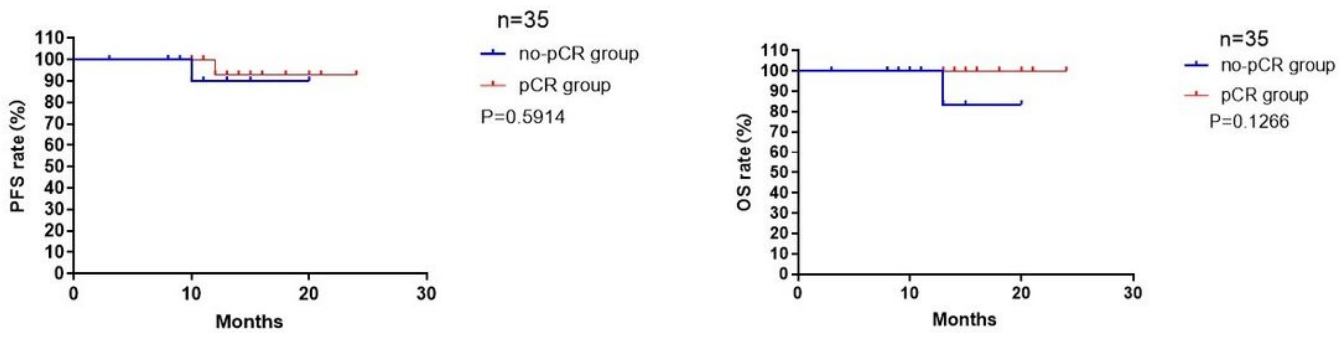

Figure 2

Kaplan–Meier curves of PFS and OS for pCR and non-pCR groups. PFS curve (a) and OS curve (b) for all patients.

Figure 3a

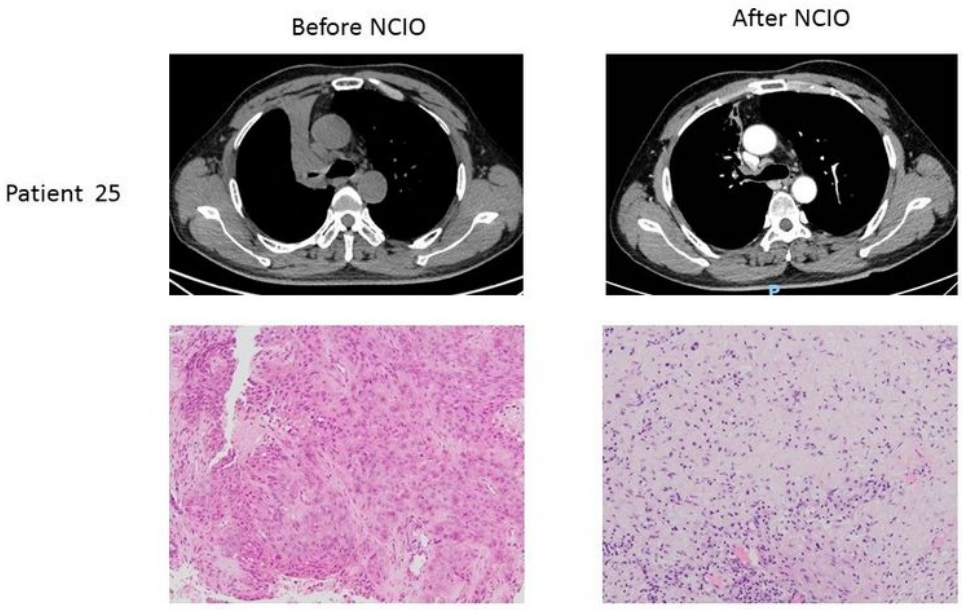

Figure 3b

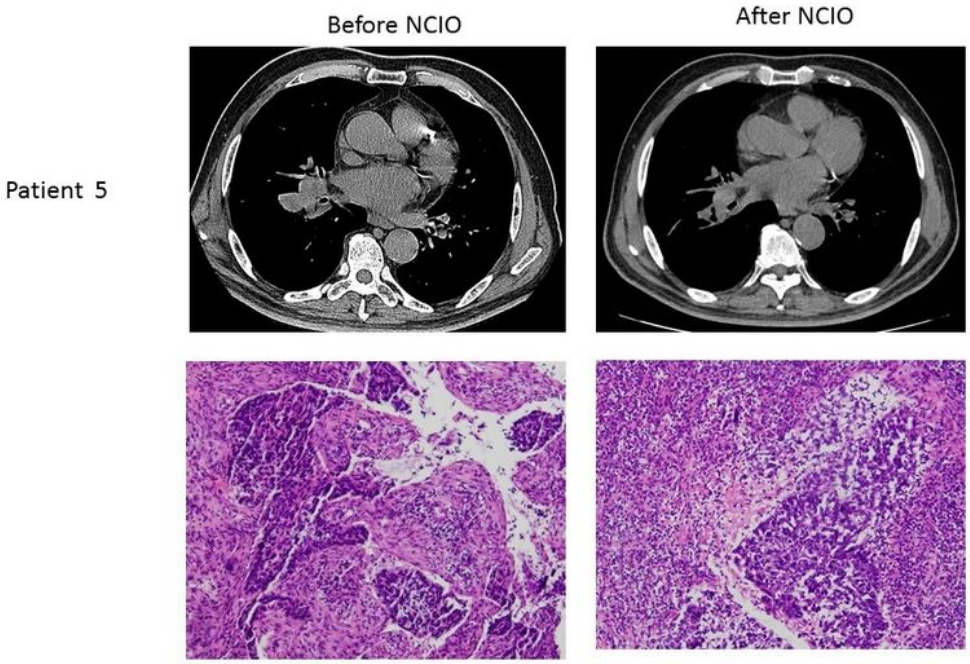

Figure 3

Patterns of radiological and pathological response to NCIO. 3A. CT imaging or PET-CT of the chest of a 56-year-old male never smoker (Patient 25) with stage IIIA squamous lung cancer before and after the administration of NCIO. In the upper panel, the pretreatment scan shows a primary tumor mass with pulmonary atelectasis in the upper lobe of the right lung. A scan performed before the surgery shows 90% shrinkage with associated tumor cavitation. The lower panel consists of the representative sections of

tumor specimens obtained from this patient before the administration of NCIO and after (right) HE staining. This patient had 100% pathological regression of the large primary lung tumor and had no lymph-node metastases in the resection specimen. 3B. CT or PET-CT of the chest of a 63-year-old male smoker (Patient 5) with stage IIIA lung adenocarcinoma. In the upper panel, the tumor is stable on imaging before and after the NCIO. In the lower panel, representative sections of tumor specimens obtained from this patient before (left) and after (right) HE. Neoplastic cells are present throughout the pretreatment specimen, whereas in the posttreatment specimen, 60% tumor regression was detected.

Figure 4a

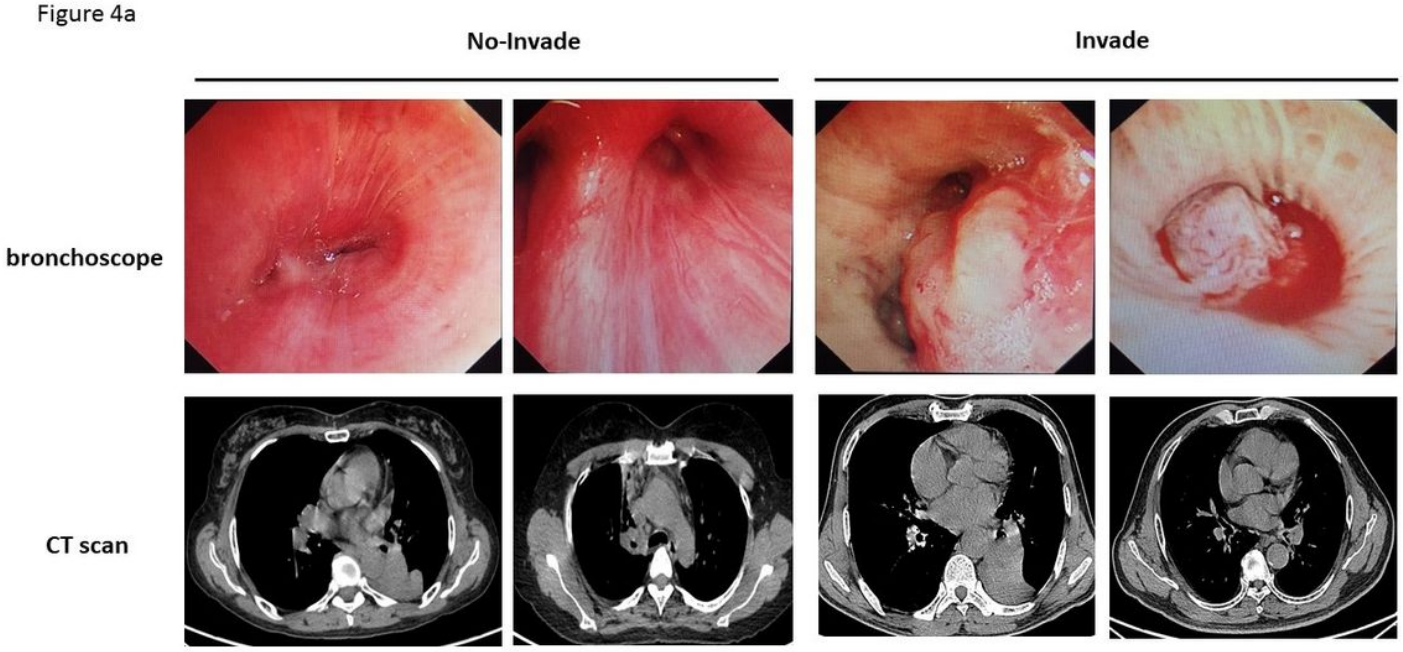

Figure 4b

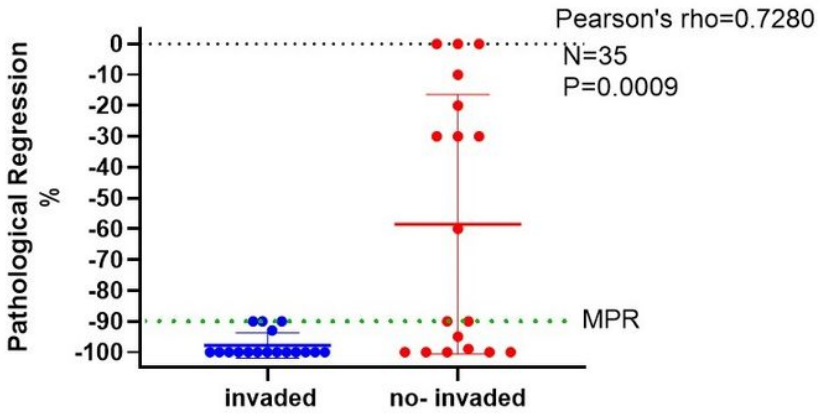

Figure 4

Association between invasion status of the bronchus and pathological response to NCIO Figure 4a Representative images of bronchoscopy and CT scan of Invade and No-Invade status. The Invade status

means that a neoplasm could be seen in the bronchus and bleeding easily by bronchoscope touch. The CT scan shows the tumor invasion into the bronchus. The No-Invade status means that only the red color and narrowing near the opening of the bronchus could be seen due to pressure from the tumor. The CT scan shows no tumor invasion into the bronchus. Figure 4b The association between the Invade status of the bronchus and pathological response to the NCIO were analyzed. The patients with Invade status were with higher pCR and MPR rates as compared with No-Invade status, with 76.47% pCR and 100% MPR rate vs. 31.58% pCR and 50.00% MPR rate. The Pearson's rho is 0.7280 and there is significant difference between the two groups (P=0.0009).

Figure 5a

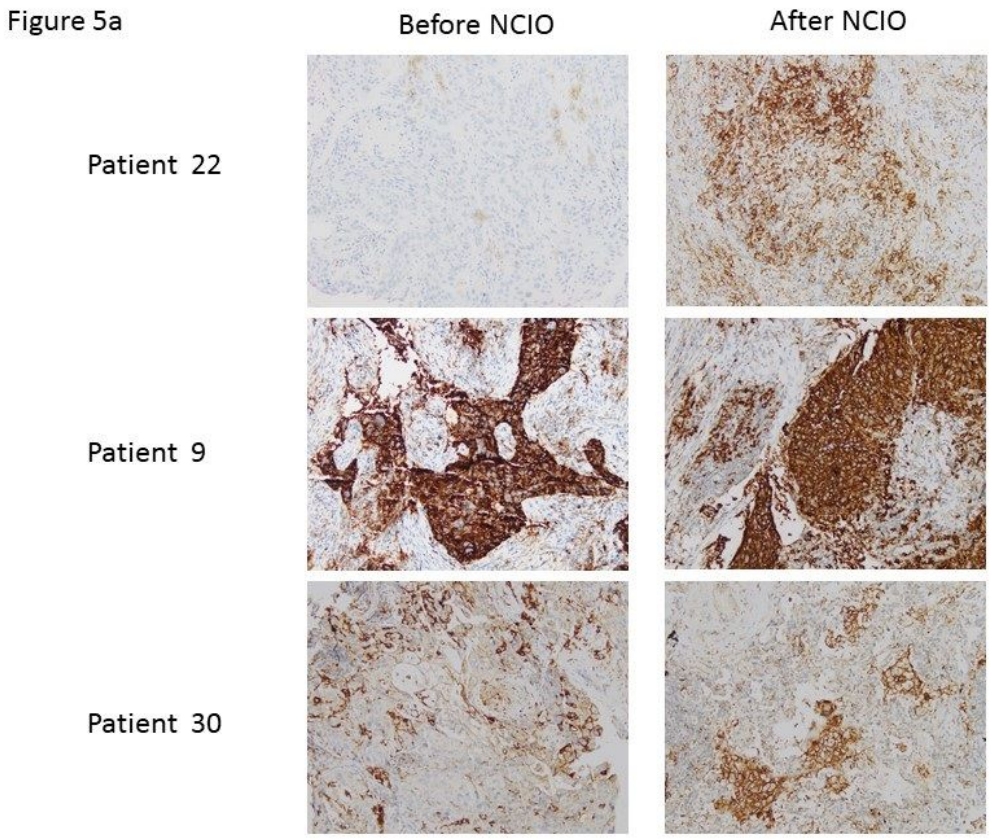

Figure 5b

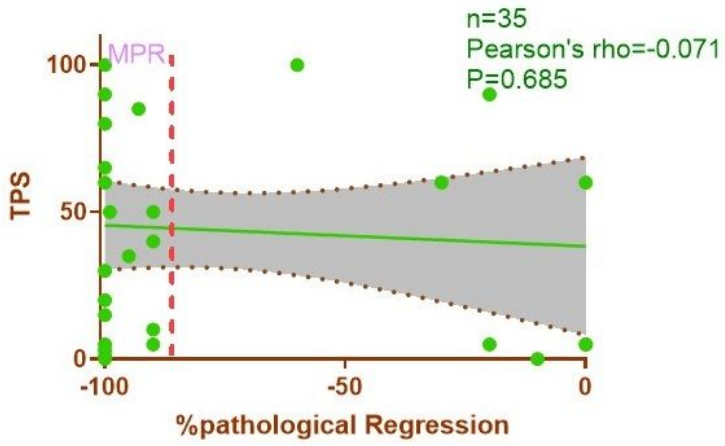

Figure 5

Association between PD-L1 and pathological response to NCIO Figures 5a PD-L1 expression changes before and after NCIO. The pretreatment biopsy sample and post-surgery sample are obtained, and PD-L1 testing was performed using the Monoclonal Mouse Anti-Human PD-L1 clone 22C3. In the upper panel, the TPS score before NCIO of the patient 26 who had 100% pathological regression was 0%, and the CPS score after NCIO was 60%. In the middle panel, the TPS score before and after NCIO of the patient 5 who

had 60% pathological regression were both 100%. In the lower panel, the TPS score before NCIO of the patient 30 who had 100% pathological regression was 30%, and the CPS score after NCIO was 50%. Figures 5B No association was observed between PD-L1 expression before the treatment and pathological response to the NCIO (Pearson's r=-0.071; P=0.685). The dashed black line indicates the linear regression line, and the dashed gray lines indicate the upper and lower boundaries of the 95% confidence interval.

Figure 6a

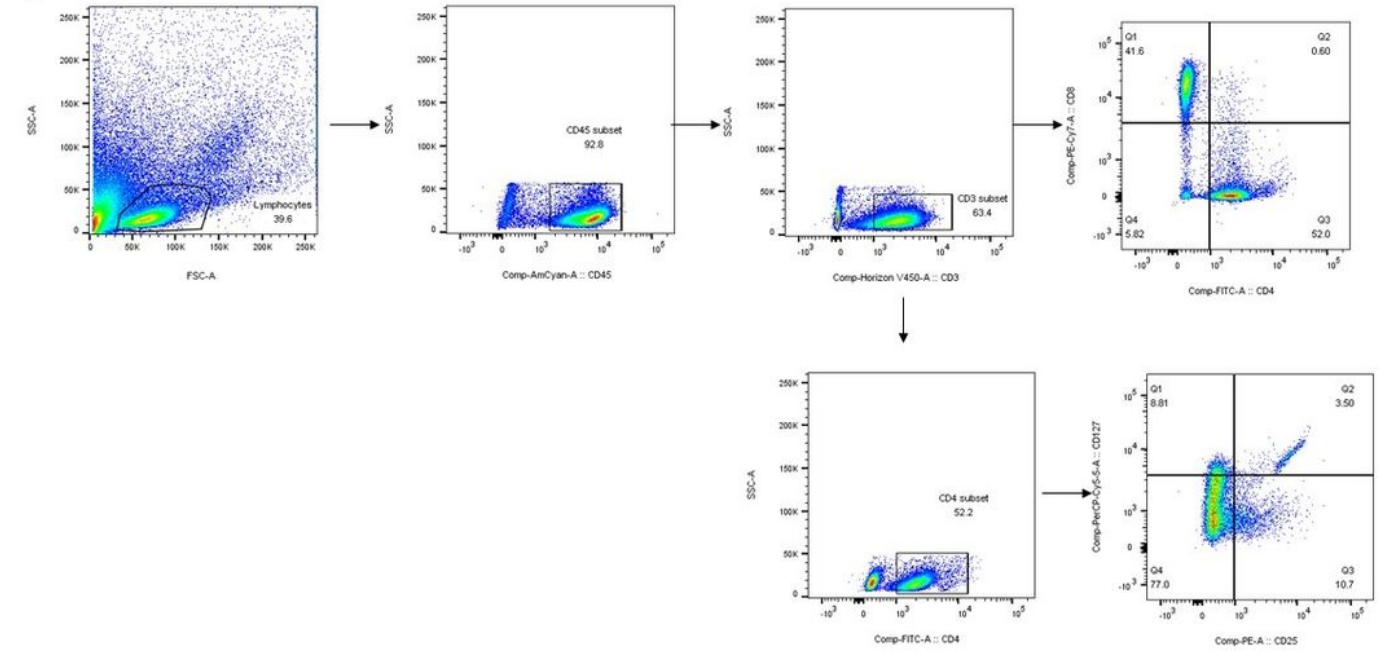

Figure 6b

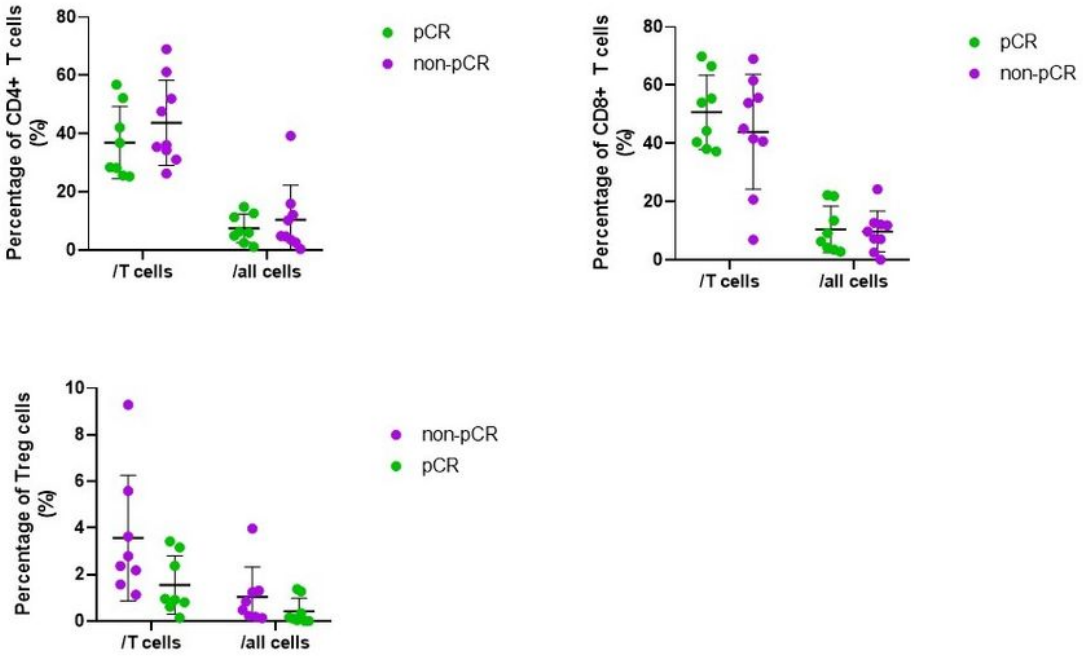

Figure 6

Immune proofing of T cells in the post-surgery sample to the NCIO Figure 6a Gating scheme for CD4+ and CD8+ T-cells and Treg cell intracellular cytokine cytometry. FSC-A, forward scatter A; SS, side scatter. Black polygons indicate gated cell subsets. Figure 6b Proportion of CD4+ andCD8+ T-cell and Treg cells to the T cells, and all cells of the sample in the pCR group showed no significant difference as compared to the non-PCR group (P>0.05).