Neoadjuvant PD-1 Inhibitor combines with Chemotherapy in Resectable Squamous Cell Non-Small Cell Lung Cancer

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Research

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Abstract

Background

Single agent of PD-1 or PD-L1 inhibitor has been explored recently for resectable patients before surgery. However, the effectiveness and safety of neoadjuvant PD-1 blockade combine with platinum-based doublet chemotherapy has not been fully investigated.

Methods

21 patients with squamous cell lung cancer accepted neoadjuvant therapy followed by surgery in Beijing Cancer Hospital were involved. 8 patients accepted two cycles of neoadjuvant platinum-based doublet chemotherapy combine with anti-PD-1 therapy, while 13 patients accepted two cycles of neoadjuvant platinum doublet chemotherapy. Besides baseline tumor staging, chest CT was repeated two to three weeks before surgery. Adverse events were monitored. Peripheral lymphocytes counting was tested during whole treatment. The residual viable tumor cells were counted after surgery to decide a major pathological response (MPR) rate. Selected specimen was sent for immunohistochemical, multiplex immunofluorescence analyses, and T-cell receptor DNA sequencing.

Results

Comparing with neoadjuvant chemotherapy alone, combination with 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 evaluation is not consistent with that of radiological evaluation. Although peripheral lymphocyte counting was influenced by neoadjuvant therapy, no unknown adverse effects were reported for all the patients. The tumor infiltrating lymphocytes were observed more in patients accepted PD-1 blockade, and seem infiltrated more in relative “non-responders”. No special pathological features associated with PD-1 blockade were found. Multiplexed immunofluorescence analyses revealed potential immune suppression status in the peritumoral spaces around the residual tumor cells. T-cell receptor DNA sequencing found although some amino acids were shared in primary tumor and lymph nodes in single patients, they are hardly shared among different patients.

Conclusions

Neoadjuvant chemotherapy combine with PD-1 blockade is safe and feasible for patients with potentially resectable squamous cell lung cancer, to improve the clinical and pathological outcome. Even with PD-1 blockade, the immune suppressive status around the residual tumor cells still exists. The squamous cell lung cancers, and corresponding immune responses are extremely highly individualized. The treatment

needs to be designed accordingly. The combination strategy of traditional neoadjuvant chemotherapy with current anti-PD-1 inhibitor are still need further investigation.

Background

The past two decades witnessed the rapid progress on treatment of lung cancers. The widely use of targeted therapy greatly improved the overall survival of patients with lung cancers, especially adenocarcinoma with driver gene mutations. However, the driver gene mutation-based treatment is still difficult to help patients with lung squamous cell carcinoma which closely relates to smoking and contributes around 30% cases of lung cancer. Since 75% of these patients are diagnosed relatively late and the squamous carcinoma often locates in proximal bronchus, the complete dissection (R0 dissection) is very hard to be achieved. Thus, the choice of treatment is usually limited to chemotherapy and radiotherapy, although the outcome is still unsatisfactory.

The PD-1 blockade immunotherapy has made great improvement on treatment of late stage squamous non-small cell lung cancer ¹. Pembrolizumab combine with traditional platinum-base doublet chemotherapy has been recommended as first line therapy for late stage squamous cell lung cancer. Single agent of PD-1 or PD-L1 inhibitor has also been explored recently for resectable patients before surgery. Nivolumab and atezolizumab have shown impressive effect as single agent neoadjuvant therapy regimen ^{2,3}. However, the effectiveness and safety of PD-1 blockade combine with platinum-based doublet chemotherapy have not been fully investigated.

Methods

Patients

From October 2018 through June 2019, 21 consecutive patients with squamous cell lung cancer accepted neoadjuvant therapy followed by surgery after the discussion and approval of multiple discipline team (MDT) of Center of Thoracic Tumor, Beijing Cancer hospital, including 8 patients with stage IIB or IIIA squamous cell lung cancer who accepted two cycles of neoadjuvant platinum-based doublet chemotherapy combine with anti-PD-1 therapy (patient No. IM-1 to IM-8) and 13 patients accepted two cycles of neoadjuvant platinum doublet chemotherapy (patient No. C-1 to C-13). All the patients were current or former smokers. Informed consent was received from all patients before treatment. All the patients planned to accepted surgery five to seven weeks after the second cycle of neoadjuvant therapy by same surgeon. The characteristics of the involved patients are shown in Table 1.

Table 1
Characteristics of the Patients at Baseline

Characteristics	All Patients (N = 21) (%)	Neoadjuvant Immunotherapy with Chemotherapy (N = 8) (%)	Neoadjuvant Chemotherapy Only (N = 13) (%)
Age - yr			
Mean ± SD	62.90 ± 5.59	62.38 ± 5.48	63.23 ± 5.85
Median(range)	64.00 (51–70)	64 (52–68)	65 (51–70)
Sex – no (%)			
Female	1 (4.76)	0 (0)	1 (7.69)
Male	20 (95.34)	8 (100)	12 (92.31)
Histologica diagnosis – no (%)			
Squamous-cell carcinoma	19 (90.48)	7 (87.5)	12 (92.31)
SCC with other type	2 (9.52)	1 (12.5)	1 (7.69)
Clinical disease stage – (%)			
II	8 (38.10)	3 (37.5)	5 (38.46)
IIIA	13 (61.90)	5 (62.5)	8 (61.54)
Smoking status – no (%)			
Never	1 (4.76)	0 (0)	1 (7.69)
Former or current	20 (95.34)	8 (100)	12 (92.31)

All the patients were treatment naïve and underwent baseline tumor staging, including pretreatment bronchoscopy or CT guided fine needle biopsy, positron-emission tomography-computed tomography (PET-CT), and contrast enhanced CT or magnetic resonance imaging of brain and chest. Chest CT was repeated two to three weeks before surgery to evaluate the outcome of neoadjuvant therapy. Changes in tumor size was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1⁴. Resection of primary tumor and lymph nodes was completed according to institutional standards. After the removal of the lobe, station No. 12 and 13 lymph nodes were further harvested. Adverse events

were monitored for all the patients. Peripheral lymphocytes counting was tested before and during whole treatment. Postoperative adjuvant chemotherapy or radiotherapy was offered, if such therapy was indicated according to the clinical and pathological staging.

Neoadjuvant therapy regimen

Neoadjuvant chemotherapy was given every three weeks (21 days) for two cycles. PD-1 inhibitor (Pembrolizumab or Toripalimab) was given one dose before infusion of chemotherapy agents on first day of each cycle. Detailed information on neoadjuvant therapy regimen for each patient are shown in Table S1 in Supplementary Appendix.

Pathology

Pathological assessments

The size of primary lung cancer, involved lymph nodes, and metastases were evaluated according to the criteria of the American Joint Committee on Cancer (Eight Edition). Primary tumors were further assessed for the percentage of residual viable tumor cells that was identified on routine hematoxylin and eosin (H&E) staining. We also examined the presence of histological features of dissected primary tumors referring to the past studies⁵⁻⁷: (1) feature of cell death: coagulation necrosis, cholesterol clefts, foam cell infiltration; (2) tissue repair/wound healing; (3) immune infiltrates with features of activation: tertiary lymphoid structures (TLS), tumor infiltrating lymphocytes (TIL), plasma cells infiltrates, collections of fused macrophages (giant cell) and granuloma formation.

Evaluation of Residual Viable Tumor Cells

The residual viable tumor cells were evaluated by two pathologists (W. S. and XY. L.) by (1) measuring the gross maximum diameter, (2) then take hematoxylin and eosin-stained slides of at least one section per greatest tumor diameter, (3) measure percentage of viable tumor cells in each slide, and (4) sum the percentage of viable tumor cells in each slide and divide by number of slides examined. The number of each tumor was recorded, and major pathological response (MPR) is defined as no more than 10% residual viable tumor cells⁽⁸⁾.

Immunologic analysis and T-Cell Receptor Sequencing

The immunohistochemical and multiplex immunofluorescence analyses of tumors are described in the Methods section in Supplementary Appendix 1.

The T-cell receptor DNA sequencing was used to define T-cell clonal distribution and functional specificity for mutant tumor antigens. The detailed methods were described in Supplementary Appendix 1.

Statistical Analysis

The institutional review board at the Peking University Cancer Hospital approved this observational study. The requirement of patient consent was waived because of the retrospective nature of this study.

Side effects, adverse events were continuously monitored. The response was evaluated by RECIST 1·1 and pathologically. The difference of response rate was compared by Chi-square test. P value for differences were calculated with a significance level of $P \leq 0.05$. SPSS software was used for all analysis.

Results

Safety and Feasibility

Neoadjuvant PD-1 inhibitor combines 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 most common adverse events of chemotherapy, instead of anti-PD-1 therapy. However, one patient (IM-3) was diagnosed grade 3 immune-related pneumonia after surgery (on Postoperative Day 11, POD 11) and was cured after management of prednisolone.

Patients accepted neoadjuvant platinum-based doublet chemotherapy (without anti-PD-1 therapy, C group) reported similar grade 1–2 adverse events (nine of 13 patients). No grade 3 or higher treatment related adverse events were reported (Table S2 in Supplementary Appendix).

There were no treatment-related surgical delays, as defined in Methods. The median interval between the administration of the second dose of chemotherapy or PD-1 inhibitor was 44.50 days (range, 30·00 to 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

The response was accessed by RECIST 1.1 criteria. For patients who accepted neoadjuvant PD-1 inhibitor combine with chemotherapy (IM group), partial response (PR) was achieved in seven (87·5%) of eight patients, while one (12·5%) patient had stable disease (SD). Of the 13 patients who accepted neoadjuvant chemotherapy only (C group), six patients had a partial response (46·15%) while seven patients had stable disease (53·85%). Among these 21 patients, pathological down-staging from the pretreatment clinical stage occurred in 6 patients (75%) in IM group while 9 patients (69·23%) in C group (Table S3 in Supplementary Appendix). However, pathological up-staging happened in one patient in C group. This patient was diagnosed T2aN1M0 before treatment while diagnosed T2cN2M0 histologically after surgery.

At a median of 4·53 (range, 2·17 – 8·53) months follow-ups, all the patients were disease free.

Pathological Assessment

Of the 8 patients who accepted neoadjuvant PD-1 inhibitor combine with chemotherapy, three patients (37.5%) had complete response (CR) pathologically, and the rest 5 (62.5%) patients had partial response, including four patients with less than 30% residual tumor cells and 1 patient with less than 50% tumor cells. The median degree of pathological regression in the primary tumor was – 85.05% (rang, -100 to -55.50, mean – 84.69%). One patient (IM-2) was diagnosed with squamous cell carcinoma with neuroendocrinal component after surgery (Table S3 in Supplementary Appendix). Although there were 13.4% residual tumor cells, the squamous carcinoma component is less than 10%. Thus, in this group, a major pathological response occurred in four patients (50%).

Of the 13 patients who accepted neoadjuvant chemotherapy only, a major pathological response occurred in five patients (38.46%), including one patient (7.69%) with complete response. The median degree of pathological regression in the primary tumor was – 69.30% (rang, -100 to -8.55, mean – 63.28%). (Fig. 1)

Then comparison between RECIST and pathological assessment indicates the discordance of these two methods. The result of image test is not the indicate of pathological regression. (Table 2)

Table 2
RECIST 1.1 and Pathological evaluation on response to neoadjuvant therapy

Response	All Patients (N = 21) (%)	Neoadjuvant Immunotherapy with Chemotherapy (N = 8) (%)	Neoadjuvant Chemotherapy Only (N = 13) (%)	P Value
RECIST 1.1				
PR	13 (61.90)	7 (87.5)	6 (46.15)	0.058
SD	8(38.10)	1 (12.5)	7 (53.85)	
Pathological evaluation				
CR	4 (19.05)	3 (37.5)	1 (7.69)	0.098
PR	9 (42.86)	5 (62.5)	10 (76.93)	
SD	8 (38.09)	0	2 (15.38)	
Major Pathologic Response	5 (23.81)	4 (50)	5 (38.46)	0.604

Peripheral Lymphocytes counting

Peripheral lymphocytes counting was recorded before and around day-7 of both cycle of neoadjuvant therapy, as well as in seven days before surgery. Figure 2 shows the change of the median of peripheral lymphocytes counting during the treatment. After neoadjuvant agents infusion of each cycle, the lymphocytes counting dropped, and then restored before next cycle or before surgery.

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

The patients underwent neoadjuvant chemotherapy combine with anti-PD-1 inhibitor or not share similar gross morphological changes of the tumor. The features described in “Pathology-Pathological assessment” present in patients of both groups. (Fig. 3A and 3B).

The presence of immune infiltrates in the patients accepted neoadjuvant chemotherapy only indicates the traditional platinum-base doublet chemotherapy may also induce some degree of immune response to the tumor cells. The lymphocytes seem more densely infiltrated in the primary/residual tumor (or regression bed) in IM group than that in the C group, which may indicate that the combination with PD-1 blockade may induce somehow more lymphocytes proliferation and recruitment. However, the tumor infiltrating lymphocytes are not dense as expected or as reported in former study ⁶, and we also found the tumor infiltrates are more likely be found in the non-responders (SD), rather than responders (PR or CR) (Fig. 3B).

Besides the features observed above, in the specimen of pathological partial responders, immune exclusion happens in both groups. The immune exclusion is defined as immune cells in the immediate peritumoral stroma, but not infiltrate into the tumor parenchyma. Even in the patients who accepted anti-PD-1 inhibitor, the T cells are still can not penetrate the “barrier” of the residual tumor cells. On immunohistochemical staining, the immune cells infiltrate in the peritumoral stroma are mainly composed by CD4 + T cells and CD20 + B cells, but not CD8 + T cells (Figure S1 and S2 in Supplementary Appendix). In the multispectral immunofluorescence on the metastased lymph node, the infiltrated cells in or around the tumor cells including dense FOXP3 + Treg cells. However, in the normal lymph node (subcarinal lymph node), Treg cells can be seen with much less density (Fig. 4).

T-Cell Receptor Sequencing

Specimen from six patients (three patients for each group, detailed information is show in Supplementary Appendix) were tested through T-cell receptor sequencing.

In the amino acid clonotype and Shannon entropy analysis, the diversity of T-cell receptors is different between patients and specimen. However, in each single patients, the number of amino acid clonotype consistently presents with primary tumor→metastased lymph node→normal lymph node (Figure S3 in Supplementary Appendix). The diversity of T cells in primary tumor is statistically less than that in normal lymph node ($p = 0.003$). (Fig. 5)

Regarding to the shared amino acid clonotype, we analyzed the top 100 used amino acid of each specimen. The results revealed that in single patient, some amino acids were shared among primary tumor, normal lymph node and metastased lymph node. Nevertheless, different patients share almost no same amino acids (Figure S4 and S5 in Supplementary Appendix).

Discussion

Clinical Analysis

In this research, comparing with platinum-based doublet neoadjuvant chemotherapy, we observed that the combination of neoadjuvant chemotherapy and anti-PD-1 immunotherapy was associated with few additional adverse events, did not delay the planned surgery, and led to the improvement of both radiological and pathological evaluation (Table 2). Although the safety of neoadjuvant PD-1 blockade have been reported, we still observe one patient with immune-related pneumonia after surgery. Surgeons still need to be aware of the serious adverse events, especially those potentially lead to cancellation of the planned surgery, that could radically change the treatment of the resectable lung cancers.

The relationship between pathological response and long-term survival has been explored by many studies. Researches have investigated and indicated the correlation between complete pathological response and overall survival⁹⁻¹². Other researches also have proved the validity of major pathological response as a surrogate of survival¹³⁻¹⁶. In this study, the complete response is different between IM and C group (37.5% vs. 7.69%). The small *P*-value (0.098) may suggest potential statistical different with increasing of sample size. However, the rate of major pathological response in the chemotherapy only group is 38.46%, which is much higher than reported before (26%)¹⁷, while the rate of MPR in IM group reached 50%, same as reported in clinical trial Impower030 (NCT 03456063, Neoadjuvant atezolizumab plus chemotherapy versus placebo plus chemotherapy in patients with non-small cell lung cancer). Although the difference is not statistically obvious, we are confident in the improvement of the rate of MPR in clinical trials with larger sample size.

We also observed the inconsistency of radiologic and pathologic assessment. One major reason is that 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 in this period. Another reason may be the repaired tissue (fibrosis) may also be shown on CT. Although not observed in our study, pseudo progression should also be considered during the radiological assessment. We advocate the modification of RECIST 1.1 to fit the “golden standard” of pathological assessment.

Histological Findings

The features of tumor cell death and tissue repair are present in both the patients who accepted neoadjuvant chemotherapy combine with PD-1 blockade or not. Presentation of features of immune activation, such as formation of granuloma, tertiary lymphoid structure, plasma cell infiltration and giant cells, in primary tumors after neoadjuvant chemotherapy advise some degree of immune response to

chemo agents induced tumor cell death. Although no unique features were found after PD-1 blockade, more tumor infiltrating lymphocytes in patients accepted PD-1 blockade indicates enhanced T cell activation/reactivation. However, we did not find lymphocytes infiltration as dense as reported in patients accepted neoadjuvant PD-1 blockade only ¹. We hypothesized that the original infiltrated T lymph cells were “energized” by PD-1 blockade, together with chemotherapy, most of the tumor killing happened in early stage of neoadjuvant therapy while the rest of tumors may primarily resistant to chemotherapy or anti-PD-1 inhibitor. Tissue repair could have occupied most of the following time, thus the tumor infiltration lymphocytes were as dense as before, and the matured fibrosis no longer recruit more lymphocytes to the regression bed.

Another hypothesis bases on the toxicity of chemo agents to lymphocytes. We supposed the peripheral lymphocyte counting would increase in patients accepted PD-1 blockade and chemotherapy. However, as shown in Fig. 2, the peripheral lymphocytes counting decreased after each cycle of neoadjuvant therapy, regardless what neoadjuvant regimen the patients accepted. Then it is reasonable to consider that the immune cells could also be killed by the chemo agents in the tumor where these agents work best. This hypothesis further leads us to consider the current strategy on combination of platinum-based doublet chemotherapy with PD-1 blockade. Vivek Verma and colleagues ¹⁸ have revealed the PD-1 blockade in subprimed CD8 cells induces dysfunctional PD-1⁺CD38^{hi} cells and anti-PD-1 resistance in animal experiments. The PEMBRO-RT phase 2 randomized clinical trial indicated the delayed PD-1 blockade after 3 doses of SBRT (8 Gy) greatly improve the overall response rate, median progression-free survival and overall survival, even in patients with PD-L1-negative tumors ¹⁹. Whether chemotherapy could be trigger of immune response or whether delayed PD-1 blockade could achieve better oncological outcome than current combination regimen deserves more investigation.

In the residual tumor cells, we can still find the feature of lung squamous cell carcinoma growth. The grouped cancer cells are surrounded by a layer of tight connected and deep stained cells. Dense lymphocytes infiltrated in the peritumoral stroma but cannot break the “barrier” cells. These “barrier” cells are dense and alive, it may also a potential mechanism of immune escape of lung squamous carcinoma, most probably independent of PD-1/PD-L1 expression or T cell exhaustion. Besides, the infiltrated lymphocytes are mostly CD4⁺ and CD20⁺. Treg cells (FOXP3⁺ through multiplexed immunofluorescence staining) are densely infiltrated in the circumstance around the residual tumor cells. As immunosuppressive cells, Treg cells were recruited to the microenvironment of cancer cells, to inhibit the cytotoxicity of Tc cells, even with the existence of PD-1 blockade. Thus, we consider Treg cells as a potential target to improve the outcome of current anti-PD-1/PD-L1 therapies.

T-cell receptor sequencing

T cell repertoire is dynamic and directly reflects the diversity of immune responses. T-cell receptors are cell specific and represent a sort of “molecular tag” of T cells and have been widely studied to monitor the dynamics of T cells in terms of clonality and diversity in different diseases, including malignancies²⁰.

The six patients we selected from both groups include pCR, pPR and pSD. Since no SD patients in IM group, we chose the patient with most residual viable tumor cells. The results of the T-cell sequencing did not show difference between both groups with different neoadjuvant therapy. Although small size of samples can not draw solid conclusions, some tendencies are still observed.

Lymph nodes are the places where T cell priming and proliferation. They are the “warehouse” of T cells. Since our research found the diversity index (Shannon Index) is statistically different between in primary tumor and in normal lymph node, we consider that the tumor infiltrating T cells are from the lymph nodes that processed the tumor-associated antigens²¹. On the other hand, only small part of the top 100 most frequently utilized amino acid overlapped among primary tumor, normal lymph node and metastased lymph node. Even less, nearly no amino acids were shared among different patients. All the results indicate the extremely high individualized development of squamous cell lung cancer and its corresponding immune response. Different tumor infiltrating T cells may be recruited from different lymph nodes which reflect the antigen presentation, T cell priming, and proliferation may be different in each lymph nodes. This highly individualized tumor needs “real” individualized treatment. Current anti-PD-1/PD-L1 or anti-CTLA-4 immunotherapies are the practice of this conception.

Also, as shown in our research, PD-1 blockade seems not induce unknown mechanisms of immune response other than that induced by chemotherapy. We advocate researches back to the basic mechanism of PD-1 blockade on T cell priming and activation, as to enhance the capability of CD8 + T cells on killing tumor cells. Combination therapy is important because therapies with different mechanisms may help to overcome the resistance of tumor cells on PD-1 blockade, as to release the full potentiality of PD-1 blockade. However, the combination strategy (like the timing of PD-1 blockade), the timing of surgical intervention, and follow-up treatment still need to be further explored.

The limitation of our study includes, 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 specimen is necessary to confirm the histological features of both chemotherapy and immunotherapy. Since pathological assessment is often objective, subjective standard and tools are crucial for comparison between different therapies.

Conclusion

Base on the results of our research, neoadjuvant chemotherapy combine with PD-1 blockade is safe and feasible for patients with potentially resectable squamous cell lung cancer, to improve the clinical and pathological outcome. However, we also find even with PD-1 blockade, the immune suppressive status around the residual tumor cells still exists. The squamous cell lung cancers, and corresponding immune responses are extremely highly individualized, according to the T-cell receptor sequencing. The treatment needs to be designed accordingly. The combination strategy of traditional neoadjuvant chemotherapy with current anti-PD-1 inhibitor are still need further investigation.

List Of Abbreviations

PD-1 Programmed Death-1

PD-L1 Programmed Death-Ligand 1

MDT Multiple Discipline Team

MPR Major Pathological Response

RECIST Response Evaluation Criteria in Solid Tumors

PR Partial Response

CR Complete Response

SD Stabel Disease

TLS tertiary lymphoid structures

TIL tumor infiltrating lymphocytes

Declarations

Ethics approval: The institutional review board at the Peking University Cancer Hospital approved this observational study.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its supplementary appendix.

Competing interests

All authors declare no competing interests.

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Authors' Contributions

JF, DM-L and YY conceived the study, YF, WS and JZ contributed to study design and analysis, and wrote the first draft of the manuscript. YW, JF-C, XY-L, LW, SL-L, CL, FL-L, JZ-Z, YY-M, YH, SS-X, TW and RJ contributed to data collection and interpretation of the results. YF and WS contributed to data preparation and analysis. ZP-W, LP-Q and NL contributed to the study design, interpretation of the results, and

finalising the report. JF, DM-L and YY critically reviewed the manuscript. All authors agreed with the decision to submit for publication.

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Figures

Figure 1

The residual viable tumor cell after surgery in each patient

Figure 3

Change of the peripheral lymphocyte counting during neoadjuvant therapy

Figure 5

Pathological features of the patients.

Figure 7

Multiplexed immunofluorescence. A. In the lymph node with metastasis, immune infiltrating cells can be observed in the center of the field. CD8+ T cells (yellow) can be seen scattered in and surround the tumor cells. Relatively dense FOXP3+ cells (green) accumulates in the peritumoral space. Small amount of macrophages (red) and CD20+ cells (purple) also can be seen. B. In normal lymph node, FOXP3+ cells (green) can be found with much less density.

Figure 9

The diversity (Shannon Index) analysis of the specimen

Supplementary Files

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