18 F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography Predicts the Function of Tumor Immune Microenvironment in Early Triple-Negative Breast Cancer

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Research article

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Abstract

Background

The maximum standardized uptake value (SUVmax) on $^{18}$F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) is presumed to visualize not only tumor cells but also active immune cells in the tumor microenvironment based on their glycolysis activity. This study aimed to investigate the relationship between SUVmax on FDG PET/CT and tumor-infiltrating lymphocyte (TIL) subsets.

Methods

Fifty-four patients with invasive triple-negative breast cancer (TNBC) underwent FDG PET/CT before neoadjuvant chemotherapy, and pre-treatment biopsy specimens were pathologically evaluated. The expression status of CD8, forkhead box P3 (FOXP3), programmed cell death-1 (PD-1), and programmed cell death-ligand 1 (PD-L1) were assessed by immunohistochemistry. The relationship between TIL subsets and SUVmax or pathological complete response (pCR) was investigated.

Results

TILs, CD8, FOXP3, PD-1, and PD-L1 were high in 15 (27.8%), 15 (27.8%), 39 (72.2%), 18 (33.3%), and 26 (48.2%) patients, respectively. SUVmax was significantly correlated with tumor size, Ki-67 labeling index, and CD8/FOXP3 ratio ($P = 0.003$, $P = 0.043$, and $P = 0.017$, respectively). In multiple linear regression analysis, tumor size and CD8/FOXP3 ratio predicted SUVmax ($P < 0.001$ and $P = 0.045$, respectively). Seventeen patients (31.5%) achieved a pCR. TILs, CD8/FOXP3 ratio, PD-1, and PD-L1 were significantly correlated with the pCR rate. In multivariate analysis, the CD8/FOXP3 ratio was the only independent predictive factor for pCR ($P = 0.010$).

Conclusion

SUVmax on FDG PET/CT was related to tumor biological factors and the immune microenvironment after adjusting for confounding factors in TNBC. FDG uptake was influenced by the CD8/FOXP3 ratio, which predicts pCR after neoadjuvant chemotherapy.

Background

Breast cancer consists of tumor cells and components of the tumor microenvironment, and the relationship between the immune system and breast cancer is complex [1, 2]. A recent study showed that tumor-infiltrating lymphocytes (TILs), one of the major components of the tumor microenvironment, are greatly related to tumor progression and the response to chemotherapy in breast cancer [3, 4]. In triple-negative breast cancer (TNBC), TILs have been reported as favorable prognostic factors and predictive biomarkers for a pathological complete response (pCR) following neoadjuvant chemotherapy [3–10].
Since $^{18}$F-fluorodeoxyglucose (FDG) is taken into cells via active glucose metabolism, the maximum standardized uptake value (SUVmax) in FDG positron emission tomography/computed tomography (FDG PET/CT) is presumed to be related not only to tumor cells but also to TILs, which are also a part of the tumor microenvironment. A previous study reported that SUVmax on FDG PET/CT was related to tumor size, Ki-67 labeling index, and TIL concentration in invasive breast cancers [11]. There are two types of TILs: functional TILs that have immune functions, such as cytotoxic T lymphocytes (CTL) and regulatory T cells (T-reg), and bystander TILs, which do not have immune functions [12]. CD8 and forkhead box P3 (FOXP3) are immune-functional markers of TILs, and programmed cell death-1 (PD-1) is expressed on TILs activated in the tumor microenvironment [13, 14]. Programmed cell death-ligand 1 (PD-L1) is a biomarker of immune checkpoint inhibitors and reportedly correlates with the response to neoadjuvant chemotherapy in breast cancer [15–17]. In particular, a high CD8/FOXP3 ratio has been related to a high pCR rate following neoadjuvant chemotherapy and a favorable prognosis in patients with TNBC [18, 19].

The correlation between SUVmax on FDG PET/CT and the tumor microenvironment factors has been associated with TILs and FOXP3 in gastric cancer, while TILs, CD8, FOXP3, PD-1, and PD-L1 are related to non-small cell lung cancer [20, 21]. However, although the association between SUVmax on FDG PET/CT and PD-L1 has been reported in breast cancer, other tumor microenvironment factors have not been considered sufficiently [22]. In addition, these reports did not adjust for tumor factors affecting FDG uptake and might not adequately reflect the characteristics of the microenvironment.

We hypothesized that FDG uptake in FDG PET/CT reflects both the biology of tumor cells and the functionality of TILs in TNBC. We investigated the relationship between SUVmax and the functional parameters of TILs after adjusting for confounding factors. Additionally, the correlation between the tumor immune microenvironment and pCR was assessed in patients with TNBC.

**Methods**

**Study population**

Fifty-five consecutive patients with primary TNBC who received neoadjuvant chemotherapy and pre-treatment FDG PET/CT between August 2008 and May 2019 at Hiroshima University Hospital were included in this study. All patients were diagnosed by core needle biopsy before neoadjuvant chemotherapy, which consisted of anthracycline- and taxane-based chemotherapies. Among them, one was excluded because of insufficient sample volume for immunostaining, and a total of 54 patients were retrospectively assessed. The institutional review board approved this study (E-559).

**Pathological assessment and immunohistochemistry**

A pathological evaluation was performed using pre-treatment biopsy specimens, excluding the assessment of pathological responses in the surgical specimens. Hormonal receptors and HER2 were assessed using the American Society of Clinical Oncology/College of American Pathologists Guidelines [23, 24]. Based on the recommendations of the international TILs working group, the proportion of stromal TILs was determined by two pathologists blinded to the remaining clinical information, and
patients were divided into high and low groups using a cut-off of 50% [25]. A pCR was defined as no residual carcinoma at the primary site and regional lymph nodes (ypT0N0).

The following antibodies were used to assess the TIL subsets: anti-CD8 antibody (SP57, prediluted; Roche, Basel, Switzerland), anti-FOXP3 antibody (D2W8E, 1:50 dilution; Cell Signaling Technology, Danvers, MA, USA), and anti-PD-1 antibody (NAT105, prediluted; Cell Marque, Rocklin, CA, USA). According to the median of positive cells infiltrating stromal compartments, these markers were subdivided into high and low groups. Anti-PD-L1 antibody (28–8, 1:400 dilution; Abcam, Cambridge, UK) was used to perform immunohistochemistry for tumor-cell membrane staining. Cases with a PD-L1 expression status of 1% or more in the tumor-cell membrane were defined as high.

FDG PET/CT examination

Patients fasted for at least 4 h before the intravenous injection of FDG (3–3.7 MBq/kg of body weight). PET/CT scanning was performed 1 h after FDG administration using a Discovery ST16 PET/CT scanner (GE Healthcare, Little Chalfont, UK). Low-dose non-enhanced CT images (3- to 4-mm slice thickness) for attenuation correction and localization of lesions identified using PET were obtained from the head to the pelvic floor of each patient according to a standard protocol. Immediately after CT, the identical axial field of view (FOV) (154 mm) was scanned using PET for 2–3 min per table position depending on the patient condition and the scanner performance. The acquired data were reconstructed as 128 × 128 matrix images (pixel size, 4.7 × 3.25 mm) using Fourier rebinning and ordered-subset expectation maximization algorithms. Both PET and CT studies were performed on patients under normal tidal breathing.

PET image evaluation and quantification of the SUVmax were performed using the Xeleris workstation version 1.1452 (GE Healthcare, Little Chalfont, UK). Regions of interest were first delineated within the primary tumor on attenuation-corrected FDG PET images and within the ipsilateral normal breast tissue for the background uptake, and SUVmax was then measured. All PET images of the subjects were read by two professionals: a nuclear medicine radiologist and a breast cancer specialist.

Statistical analysis

The summarized data are presented as the median and interquartile range (IQR) for continuous variables and number (%) for categorical variables. Frequencies were compared using Fisher's exact test for categorical variables. Correlation analyses were performed using Spearman's rank correlation coefficients. Linear regression analysis was used to predict the SUVmax, and logistic regression analysis was used to predict the pCR. The cut-off for the Ki-67 labeling index was defined based on the median value. Statistical significance was set at p < 0.05. All statistical analyses were performed using JMP 14 (SAS Institute Inc., Cary, NC, USA).

Results

Patients’ characteristics
The characteristics of the 54 patients are presented in Table 1. The median age was 56.6 years, and all tumors were infiltrating duct carcinomas not otherwise specified. The median SUVmax was 7.0, and 15 (27.8%) patients were TIL high. Seventeen tumors (31.5%) achieved a pCR.
<table>
<thead>
<tr>
<th></th>
<th>Number (%)</th>
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<tbody>
<tr>
<td><strong>Age (y), median (range)</strong></td>
<td>56.6 (22–76)</td>
</tr>
<tr>
<td><strong>T status</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17 (31.5)</td>
</tr>
<tr>
<td>2</td>
<td>29 (53.7)</td>
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<tr>
<td>3</td>
<td>3 (5.6)</td>
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<tr>
<td>4</td>
<td>5 (9.2)</td>
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<tr>
<td><strong>N status</strong></td>
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<tr>
<td>0</td>
<td>20 (37.0)</td>
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<tr>
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<td>24 (44.4)</td>
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<tr>
<td>2</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td>3</td>
<td>9 (16.7)</td>
</tr>
<tr>
<td><strong>Nuclear grade</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4 (8.9)</td>
</tr>
<tr>
<td>2</td>
<td>7 (15.6)</td>
</tr>
<tr>
<td>3</td>
<td>34 (75.5)</td>
</tr>
<tr>
<td><strong>Pathological response</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Ki-67 labeling index (%)</strong>, <strong>median (IQR)</strong></td>
<td>92.5 (66.5–99.0)</td>
</tr>
<tr>
<td><strong>TILs</strong></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>39 (72.2)</td>
</tr>
<tr>
<td>High</td>
<td>15 (27.8)</td>
</tr>
<tr>
<td><strong>Pathological response</strong></td>
<td></td>
</tr>
<tr>
<td><strong>pCR</strong></td>
<td>17 (31.5)</td>
</tr>
<tr>
<td><strong>Non-pCR</strong></td>
<td>37 (68.5)</td>
</tr>
<tr>
<td><strong>SUVmax, median (IQR)</strong></td>
<td>7.0 (4.5–9.6)</td>
</tr>
</tbody>
</table>

HER2, human epidermal growth factor receptor 2; IQR, interquartile range; pCR, pathological complete response; SUVmax, maximum standardized uptake value; TILs, tumour-infiltrating lymphocytes; T1, tumor ≤ 2 cm; T2, tumor > 2–5 cm; T3, tumor > 5 cm; T4, tumor extension to chest wall or skin.
Figure 1 shows representative images of the TILs and immunohistochemical staining of CD8, FOXP3, PD-1, and PD-L1. The median number of CD8-, FOXP3-, and PD-1- positive cells were 132.5 (78.8–207.3), 28.0 (15.0–38.0), and 31.0 (12.0–56.5), respectively. There were 15 (27.8%), 39 (72.2%), 18 (33.3%), and 26 (48.2%) high cases of CD8, FOXP3, PD-1, and PD-L1, respectively.

Correlation with SUVmax and TILs subsets

The SUVmax was significantly correlated with the tumor size ($r = 0.392$, $P = 0.003$), Ki-67 labeling index ($r = 0.293$, $P = 0.043$), and CD8/FOXP3 ratio ($r = 0.324$, $P = 0.017$) (Fig. 2). Multiple linear regression analysis revealed that tumor size and CD8/FOXP3 ratio were significant factors influencing SUVmax ($P < 0.001$ and $P = 0.045$, respectively) (Table 2). The Ki-67 labeling index, PD-1, and PD-L1 expression were not related to SUVmax.

Table 2
Single and multiple linear regression analyses for factors influencing SUVmax.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Single linear regression analysis</th>
<th>Multiple linear regression analysis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Regression coefficient Standard error $t$ $P$</td>
<td>Regression coefficient Standard error $t$ $P$</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.858 1.82 0.47 0.640</td>
<td></td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>0.104 0.034 3.07 0.003</td>
<td>0.132 0.034 3.93 &lt; 0.001</td>
</tr>
<tr>
<td>Ki-67 labeling index (%)</td>
<td>0.040 0.019 2.08 0.043</td>
<td>0.026 0.017 1.51 0.139</td>
</tr>
<tr>
<td>CD8/FOXP3 ratio</td>
<td>0.166 0.067 2.47 0.017</td>
<td>0.149 0.072 2.06 0.045</td>
</tr>
<tr>
<td>PD-1</td>
<td>-0.008 0.017 -0.49 0.630</td>
<td>-0.029 0.017 -1.76 0.085</td>
</tr>
<tr>
<td>PD-L1</td>
<td>0.020 0.033 0.61 0.546</td>
<td>0.059 0.033 1.75 0.087</td>
</tr>
</tbody>
</table>

FOXP3, forkhead box P3; PD-1, programmed cell death-1; PD-L1, programmed death-ligand 1; SUVmax, maximum standardized uptake value

Figure 3 shows representative images of FDG PET/CT and pathological findings according to the CD8/FOXP3 ratio in breast cancers with similar tumor biology. When the CD8/FOXP3 ratio was high, the SUVmax was also high and a pCR was obtained (Fig. 3a). However, when the CD8/FOXP3 ratio was low, the SUVmax was also low and a pCR was not achieved (Fig. 3b).

Relationship between pCR rate and TILs subsets

In Fisher's exact test, the pCR rate was significantly higher in the high groups of TILs, CD8/FOXP3 ratio, PD-1, and PD-L1, respectively (odds ratio [OR] 5.81; 95% confidence interval [CI] 1.60–21.2; $P = 0.009$, OR
17.7; 95% CI 3.46–90.9; \( P < 0.001 \), OR 7.86; 95% CI 2.16–28.6; \( P = 0.002 \), and OR 6.00; 95% CI 1.62–22.2; \( P = 0.008 \), respectively) (Fig. 4). Multivariate logistic regression analysis revealed that the CD8/FOXP3 ratio was an independent predictor for a pCR (OR 32.2; 95% CI 2.26–458.2; \( P = 0.010 \)), whereas tumor size (\( \geq T2 \)), Ki-67 labeling index, PD-1, and PD-L1 were not associated with pCR (Table 3).

### Table 3

<table>
<thead>
<tr>
<th>Factors</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>( P )</td>
</tr>
<tr>
<td>T2–4</td>
<td>0.36 (0.11–1.22)</td>
<td>0.100</td>
</tr>
<tr>
<td>Ki-67 labeling index_high</td>
<td>2.28 (0.63–8.25)</td>
<td>0.209</td>
</tr>
<tr>
<td>CD8/FOXP3 ratio_high</td>
<td>17.7 (3.45–90.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PD-1_high</td>
<td>7.86 (2.16–28.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>PD-L1_high</td>
<td>6.00 (1.62–22.2)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

CI, confidence interval; FOXP3, forkhead box P3; OR, odds ratio; pCR, pathological complete response; PD-1, programmed cell death-1; PD-L1, programmed death-ligand 1

### Discussion

This study demonstrated that SUVmax on FDG PET/CT was related to the CD8/FOXP3 ratio, representing the functionality of the tumor microenvironment, even after adjusting for tumor factors. To our knowledge, this is the first report to consider the relationship between FDG PET/CT and TIL subsets in TNBC after adjusting for confounding factors.

TNBC is an aggressive biological subtype associated with poor prognosis and a high risk of early recurrence [26, 27]. It is essential to predict a pCR after neoadjuvant chemotherapy because a pCR is a surrogate marker of prognosis in TNBC [10, 9]. TNBC is a subtype that contains the most abundant TILs in breast cancer [3]. Previous randomized trials have reported that the abundance of TILs leads to better prognosis and the therapeutic effect of neoadjuvant chemotherapy in TNBC because of high tumor immune cytolytic activity [5–8].

In the tumor microenvironment, TILs include CD8-positive T cells, FOXP3-positive T cells, natural killer cells, dendritic cells, and macrophages. There are various regulatory cell groups in the tumor stroma in addition to lymphocytes, such as bone marrow-derived inhibitory cells, tumor-associated macrophages, cancer-associated fibroblasts, and mesenchymal stem cells [4, 14]. In breast cancer, CD8-positive T cells, which are the primary components of TILs, play a critical role in the antitumor immune response. CD8-positive T cells, the so-called CTL, produce interferon gamma (IFN-\( \gamma \)) to attack cancer cells [1, 28]. Recent studies have reported that CTLs are associated with high pCR rates after neoadjuvant chemotherapy and better survival in TNBC [29–31]. FOXP3-positive T cells, the so-called T-reg, act in an immune-suppressive
manner against tumors[13]. T-reg constitutively expresses PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and suppresses the action of effector T cells and dendritic cells [32]. Furthermore, T-reg produces cytotoxic substances (perforin and granzyme) and suppressive cytokines (interleukin-10 and transforming growth factor-β), which suppress CTL [33]. Because CTL and T-reg have paradoxical effects on tumor microenvironmental immunity, the CD8/FOXP3 ratio is considered a reliable biomarker for predicting the prognosis and the effect of neoadjuvant chemotherapy. In particular, it has been reported that a high CD8/FOXP3 ratio is related to improved disease-free survival, overall survival, and pCR rate in TNBC [18, 19, 30]. Our findings also showed a correlation between the CD8/FOXP3 ratio and pCR rate. Previously, we reported that TIL scoring based on FDG PET/CT was related to pCR [34]. Scoring of the CD8/FOXP3 ratio might predict a pCR more accurately. PD-L1 is expressed on cancer cells and tumor-infiltrating macrophages by IFN-γ and suppresses T cell activity via the PD-1/PD-L1 pathway. Immune checkpoint inhibitors that block the PD-1/PD-L1 pathway have been demonstrated to improve progression-free survival and the pCR rate after neoadjuvant chemotherapy in TNBC [35–37].

Previous studies have reported that SUVmax on FDG PET/CT is related to clinicopathological tumor factors, such as tumor size, nuclear grade, and the Ki-67 labeling index [38]. However, SUVmax is different between each tumor, even in those with similar tumor biology, and it might be that other factors are affecting. We demonstrated that SUVmax was influenced by TILs using surgical specimens in early-stage breast cancer after adjustment for tumor factors [11]. Other studies have also reported an association between SUVmax and tumor microenvironment factors [20–22]. However, these analyses did not adjust for tumor factors affecting FDG uptake, and the correlation between SUVmax and TIL subsets is unclear.

The relationship between SUVmax and TILs is explained by glucose metabolism in the tumor microenvironment. In the tumor area, tumor cells and activated immune cells increase glucose metabolism and express glucose transporter 1 (Glut1) [39, 40]. Tumor cells and TILs compete for glucose, and the tumor microenvironment is established by the energy balance due to metabolic competition [41, 42]. FDG PET/CT is a modality that visualizes the metabolic status of glucose and is expected to identify activated TILs.

This study has some limitations. First, it has a potential limitation in that it was a retrospective study design of a small cohort from a single institution. Second, TIL subsets were evaluated using biopsy specimens because the patients received neoadjuvant chemotherapy. The findings of this study may differ from evaluations of whole tumors. Third, the evaluation method of tumor microenvironmental factors is not generalized, and the cut-off values are specific to the present study.

Conclusions

SUVmax on FDG PET/CT reflected the CD8/FOXP3 ratio after adjusting for tumor factors, and the high CD8/FOXP3 ratio was related to pCR in early-stage TNBC. This study suggests that FDG PET/CT, which reflects the tumor immune microenvironment, might predict the effect of neoadjuvant chemotherapy in TNBC.
Abbreviations

TILs: tumor-infiltrating lymphocytes

TNBC: triple-negative breast cancer

pCR: pathological complete response

FDG: $^{18}$F-fluorodeoxyglucose

SUVmax: the maximum standardized uptake value

FDG PET/CT: FDG positron emission tomography/computed tomography

CTL: cytotoxic T lymphocytes

T-reg: regulatory T cells

FOXP3: forkhead box P3

PD-1: programmed cell death-1

PD-L1: Programmed cell death-ligand 1

IQR: interquartile range

OR: odds ratio

95% CI: 95% confidence interval

IFN-γ: interferon gamma

CTLA-4: cytotoxic T-lymphocyte-associated protein 4

Glut1: glucose transporter 1

Declarations

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest

The authors declare that they have no conflict of interest.
**Funding**

None.

**Consent to participate**

For this type of study, formal patient consent was not required.

**Consent for publication**

Not applicable.

**Availability of data and material**

Datasets are available upon reasonable request.

**Code availability**

Not applicable.

**Author contributions**

YK and SS contributed to the study conception and design. YK, SS, AE, NM, and TK collected clinical data. YK and KA evaluated the pathological findings. YK and SS analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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**Figures**

**Figure 1**

The representative imaging of TILs (a) and the immunohistochemical staining of CD8 (b), FOXP3 (c), PD-
1 (d), and PD-L1 (e). FOXP3, forkhead box P3; PD-1, programmed cell death-1; PD-L1, programmed death-
ligand 1; TILs, tumor-infiltrating lymphocytes
Figure 2

Correlation with SUVmax on FDG PET/CT and tumor factors and TILs subset. FDG PET/CT, 18F-fluorodeoxyglucose positron emission tomography/computed tomography; FOXP3, forkhead box P3; PD-1, programmed cell death-1; PD-L1, programmed death-ligand 1; SUVmax, maximum standardised uptake value; TILs, tumor-infiltrating lymphocytes

Figure 3
Representative images of FDG PET/CT, hematoxylin and eosin stain and immunohistochemical findings in cases with for similar tumor biological features. (a) Infiltrating duct carcinoma not otherwise specified with tumor size of 26 mm, nuclear grade 3, Ki-67 labeling index 100%, SUVmax 11.2, TILs 50% and CD8/FOXP3 ratio 25.1. pCR was obtained. (b) Infiltrating duct carcinoma not otherwise specified with tumor size of 26 mm, nuclear grade 3, Ki-67 labeling index 87%, SUVmax 4.4, TILs 50%, and CD8/FOXP3 ratio 2.6. pCR was not obtained. Arrows point to the primary breast tumors. FDG PET/CT, 18F-fluorodeoxyglucose positron emission tomography/computed tomography; FOXP3, forkhead box P3; pCR, pathological complete response; SUVmax, maximum standardised uptake value; TILs, tumor-infiltrating lymphocytes

**Figure 4**

The relationship between TILs subsets and pCR rate. FOXP3, forkhead box P3; OR, odds ratio; pCR, pathological complete response; PD-1, programmed cell death-1; PD-L1, programmed death-ligand 1; TILs, tumor-infiltrating lymphocytes