18F-FDG uptake in PET/CT is a potential predictive biomarker of response to anti-PD-1 antibody therapy in non-small cell lung cancer

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To examine the association between 18F-fluorodeoxyglucose (18F-FDG) uptake in positron emission tomography/computed tomography (PET/CT) and the response to anti-programmed cell death-1 (PD-1) monoclonal antibody therapy in non-small cell lung cancer (NSCLC) patients, 89 patients with advanced or recurrent NSCLC were retrospectively analysed. Maximum standardized uptake value (SUVmax) in 18F-FDG PET/CT and the response to anti-PD-1 antibodies were recorded. A cut-off value of SUVmax was determined by receiver operating characteristic curve analysis for patient stratification. Among the 89 patients evaluated, 24 were classified as responders (all partial response), and 65 as non-responders. The average SUVmax of the responders was 15.60 (range, 6.44–51.10), which was significantly higher than that of the non-responders (11.61; range, 2.13–32.75; P = 0.0168, Student’s t-test). The cut-off SUVmax value selected for stratification was 11.16 (sensitivity and specificity, 0.792 and 0.585, respectively). The response rate of patients with SUVmax value ≥ 11.16 (41.3% [19/46]) was significantly higher than that of patients with SUVmax < 11.16 (11.6% [5/43], P = 0.0012, Chi-squared test). The SUVmax in 18F-FDG PET/CT is a potential predictive marker of response to anti-PD-1 antibody therapy in NSCLC patients. Further prospective studies of large populations are necessary to validate these results.

The interaction between programmed cell death-1 (PD-1), expressed on activated T lymphocytes, and programmed cell death-ligand 1 (PD-L1), expressed on antigen-presenting cells and tumour cells, has a major role in suppression of the anti-tumour immune response1. As such, monoclonal antibodies (mAbs) against PD-1 (e.g. nivolumab and pembrolizumab) or PD-L1 (e.g. atezolizumab) have become one of the standard treatments for patients with advanced or recurrent non-small cell lung cancer (NSCLC). Detection of PD-L1 expression in tumour samples is routinely conducted by immunohistochemistry (IHC) before initiation of treatment with anti-PD-1 or anti-PD-L1. However, expression of PD-L1 alone does not fully predict the response to anti-PD-1 mAbs, and more accurate and convenient response markers are urgently required.

18F-Fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) is an essential imaging modality for lung cancer2,3 and the majority of patients undergo 18F-FDG PET/CT before treatment initiation. Several recent studies have shown that 18F-FDG uptake is significantly associated with tumour response to PD-1 or PD-L1 inhibition. Further prospective studies of large populations are necessary to validate these results.
PD-L1 expression in NSCLC patients\(^4\). However, these studies examined patients with surgically resectable NSCLC, not those with advanced or recurrent cancer; thus, it is unknown whether the relationship between \(^{18}\)F-FDG uptake and the efficacy of anti-PD-1 mAbs is also true in more advanced disease.

In this translational study, we evaluated the relationship between \(^{18}\)F-FDG uptake on PET/CT and the response to anti-PD-1 mAbs in patients with advanced or recurrent NSCLC patients by dichotomizing the cohort according to the maximum standardized uptake value (SUV\(_\text{max}\)).

### Results

#### Patient characteristics.

Table 1 shows the characteristics of the 89 patients enrolled in this study. The median age was 67 years (range, 36–88 years); 75 (84.3%) patients were male, and 73 (82.0%) were smokers. EGFR or ALK gene mutation status was available for 75 (84.3%) patients, and PD-L1 expression data were available for 49 (55.1%) patients. The median SUV\(_\text{max}\) was 11.40 (range, 2.13–51.10).

| Characteristic                      | Value or n (%) of patients |
|-------------------------------------|-----------------------------|
| Age (years)                         | Median 67, Range 36–88      |
| Sex                                 | Female 14 (15.7%), Male 75 (84.3%) |
| ECOG PS                             | 0 18 (20.2%), 1 63 (70.8%), 2 7 (7.9%), 3 1 (1.1%) |
| Line of treatment                   | First 17 (19.1%), Second 40 (44.9%), Third or higher 32 (36.0%) |
| Smoking history                     | Never-smoker 16 (18.0%), Ex-smoker 39 (43.8%), Current smoker 34 (38.2%) |
| History of radiation therapy        | No 58 (65.2%), Yes 31 (34.8%) |
| Clinical stage                      | IIIB 14 (15.7%), IV 52 (58.4%), Recurrent 23 (25.9%) |
| Mutation status (EGFR or ALK)      | Wild type 66 (74.2%), Mutated\(^4\) 9 (10.1%), Unknown 14 (15.7%) |
| Histology                           | Adenocarcinoma 59 (66.3%), Squamous cell carcinoma 23 (25.8%), Other or unknown\(^b\) 7 (7.9%) |
| Immune checkpoint inhibitor therapy | Nivolumab 60 (67.4%), Pembrolizumab 29 (32.6%) |
| PD-L1 (22C3) TPS                    | <1% 11 (12.4%), ≥1% and <50% 16 (18.0%), ≥50% 22 (24.7%), Unknown 40 (44.9%) |
| SUV\(_\text{max}\)                   | Median 11.40, Range 2.13–51.10 |

Table 1. Clinicopathological characteristics of all NSCLC patients. \(^a\)Nine patients positive for mutant EGFR. \(^b\)Four patients with sarcomatoid carcinoma and three patients with NO (nototherwise specified). ALK, anaplastic lymphoma kinase; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; PD-L1, programmed cell death-ligand 1; TPS, tumour proportion score; SUV\(_\text{max}\), maximum standardized uptake value.

PD-L1 expression in NSCLC patients\(^4\). However, these studies examined patients with surgically resectable NSCLC, not those with advanced or recurrent cancer; thus, it is unknown whether the relationship between \(^{18}\)F-FDG uptake and the efficacy of anti-PD-1 mAbs is also true in more advanced disease.

In this translational study, we evaluated the relationship between \(^{18}\)F-FDG uptake on PET/CT and the response to anti-PD-1 mAbs in patients with advanced or recurrent NSCLC patients by dichotomizing the cohort according to the maximum standardized uptake value (SUV\(_\text{max}\)) on imaging.

#### Association between SUV\(_\text{max}\) and tumour response in NSCLC patients treated with anti-PD-1 mAbs.

Of the 89 patients, 24 were classified as responders (all partial response [PR]) and 65 were non-responders (progressive disease [PD], \(n = 36\); stable disease [SD], \(n = 29\)) following treatment with nivolumab or pembrolizumab. The mean SUV\(_\text{max}\) was significantly higher for the responders (15.60; range, 6.44–51.10) than the non-responders (11.61; range, 2.13–32.75; \(P = 0.0168\), Student's t-test) (Fig. 1).
Association between SUVmax and response rate in NSCLC patients treated with anti-PD-1 mAbs. To evaluate the relationship between SUVmax and response rate, we selected the optimal SUVmax cut-off value of 11.16 (area under the curve 0.6772, \( P = 0.0207 \)) by receiver operating characteristic curve analysis (Fig. 2a). The response rate of the patients with SUVmax \( \geq 11.16 \) was significantly higher than that of the patients with SUVmax \(< 11.16 \) (41.3% [19/46] vs. 11.6% [5/43]; \( P = 0.0012 \), Chi-squared test) (Fig. 2b).

We conducted univariate and multivariate analyses of the relationship between tumour response and patient characteristics, and high SUVmax (\( \geq 11.16 \)) was an independent predictor for tumour response (complete response [CR] or PR) (Table 2).
Association between SUVmax and survival of NSCLC patients treated with anti-PD-1 mAbs.

The median follow-up time of the study population was 225 days (range, 5–932). Analysis of patient survival using the Kaplan–Meier method revealed that patients with SUVmax <11.16 tended to have shorter PFS than the patients with SUVmax ≥11.16, although the difference did not reach statistical significance (P = 0.1671, log-rank test; Fig. 3a). However, no comparable trend was observed for OS (P = 0.7411, log-rank test; Fig. 3b).

Characteristics of the study population stratified by SUVmax. Table 3 shows the features of patients with SUVmax <11.16 and ≥11.16. Of the 49 patients for whom data on tumour PD-L1 expression were available, patients with SUVmax ≥11.16 tended to have higher PD-L1 expression than patients with SUVmax <11.16, although the difference was not significant (P = 0.3350, Chi-squared test).

Discussion

In this study, we evaluated the relationship between 18F-FDG uptake on PET/CT and the response to anti-PD-1 therapy in patients with advanced or recurrent NSCLC. We found that high SUVmax was significantly associated with better response to anti-PD-1 mAbs. Consistent with this, patients with high SUVmax also showed a trend towards higher tumour expression of PD-L1, although this association was not statistically significant. Nevertheless, the results of this study suggest that 18F-FDG PET/CT, which is a relatively non-invasive procedure, might be a useful tool to predict the efficacy of anti-PD-1 mAbs in patients with advanced or recurrent NSCLC.

Our study showed a trend, albeit not significant, towards longer PFS for patients with high SUVmax, which is also consistent with the positive correlation between SUVmax and response rate. However, no such difference was observed for OS. Previous work has shown that FDG uptake by lung cancer cells is regulated by hypoxia, angiogenesis, glucose metabolism, and mammalian target of rapamycin (mTOR) signalling. Thus, tumours with
sample size. However, this is the first report to show a relationship between 18F-FDG uptake in PET/CT and require further study in the future.

from the analyses because the data were available for only 49 (55.1%) patients. We should conduct the same anal-
was an independent predictor for tumour response (CR or PR). However, we excluded PD-L1 expression data
multivariate analyses of the relationship between tumour response and patient characteristics, and high SUVmax.
Moreover, we conducted univariate and PD-L1 expression data were available for only 49 (55.1%) patients, which may have been insufficient to obtain
the efficacy of anti-PD-1 mAbs in NSCLC patients. Second, a definitive cut-off value for SUVmax has yet to be
analyses with PD-L1 expression data in a sufficient sample size in future studies.

The current study has several limitations. First, this was a single-institution retrospective study with a small
sample size. However, this is the first report to show a relationship between 18F-FDG uptake in PET/CT and the
efficacy of anti-PD-1 mAbs in NSCLC patients. Second, a definitive cut-off value for SUVmax has yet to be
established and our results should be validated in further prospective studies of larger patient populations. Third,
PDL1 expression data were available for only 49 (55.1%) patients, which may have been insufficient to obtain
robust data on the association between PD-L1 expression and SUVmax. Moreover, we conducted univariate and
multivariate analyses of the relationship between tumour response and patient characteristics, and high SUVmax
was an independent predictor for tumour response (CR or PR). However, we excluded PD-L1 expression data
from the analyses because the data were available for only 49 (55.1%) patients. We should conduct the same anal-
yses with PD-L1 expression data in a sufficient sample size in future studies.

In conclusion, the SUVmax in 18F-FDG PET/CT obtained at the time of treatment initiation may be important
for predicting the efficacy of anti-PD-1 mAbs in NSCLC patients. Consideration of 18F-FDG SUVmax and
tumour expression of PD-L1 in combination could be a more effective marker of the response to this targeted
therapy than the current use of PD-L1 expression alone.

| Characteristic       | n (%)          | SUVmax, n (%)               | P value |
|----------------------|----------------|----------------------------|---------|
|                      | <11.16         | ≥11.16                     |         |
| Age (years)          |                |                            |         |
| <67                  | 43 (48.3%)     | 20 (46.5%)                 | 0.7421  |
| ≥67                  | 46 (51.7%)     | 23 (53.5%)                 |         |
| Sex                  |                |                            |         |
| Female               | 14 (15.7%)     | 9 (20.9%)                  | 0.1907  |
| Male                 | 75 (84.3%)     | 34 (79.1%)                 |         |
| ECOG PS              |                |                            |         |
| 0 or 1               | 81 (91.0%)     | 38 (88.4%)                 | 0.3984  |
| 2 or 3               | 8 (9.0%)       | 5 (11.6%)                  |         |
| Line of treatment    |                |                            |         |
| First or second      | 57 (64.0%)     | 27 (62.8%)                 | 0.8116  |
| Third or higher      | 32 (36.0%)     | 16 (37.2%)                 |         |
| Smoking history      |                |                            |         |
| Never-smoker         | 16 (18.0%)     | 10 (23.3%)                 | 0.2085  |
| Smoker               | 73 (82.0%)     | 33 (76.7%)                 |         |
| History of radiation |                |                            |         |
| No                   | 58 (65.2%)     | 28 (65.1%)                 | 0.9920  |
| Yes                  | 31 (34.8%)     | 15 (34.9%)                 |         |
| Clinical stage       |                |                            |         |
| IIIb or IV           | 66 (74.2%)     | 30 (69.8%)                 | 0.3601  |
| Recurrent            | 23 (25.8%)     | 13 (30.2%)                 |         |
| Mutation status      |                |                            |         |
| Wild type            | 66 (74.2%)     | 32 (74.4%)                 | 0.9566  |
| ECOG or ALK          | 23 (25.8%)     | 11 (25.6%)                 |         |
| Others               | 66 (74.2%)     | 29 (67.4%)                 | 0.1608  |
| Histology            |                |                            |         |
| Non-SCC              | 23 (25.8%)     | 14 (32.6%)                 | 0.196   |
| SCC                  | 66 (74.2%)     | 29 (67.4%)                 |         |
| PD-L1 (22C3) TPS     |                |                            |         |
| <50%                 | 27 (55.1%)     | 16 (61.5%)                 | 0.3350  |
| ≥50%                 | 22 (44.9%)     | 10 (38.5%)                 |         |

Table 3: Characteristics of NSCLC patients stratified by SUVmax. *For the 49 cases with available data. ALK, anaplastic lymphoma kinase; ECOG PS, Eastern Cooperative Oncology Group performance status; EGF, epidermal growth factor receptor; PD-L1, programmed cell death-ligand 1; TPS, tumour proportion score; SCC, squamous cell carcinoma; SUVmax, maximum standardized uptake value.
Methods

Patients. We retrospectively identified 89 patients with advanced (stage IIIB to IV) or recurrent NSCLC who were treated with anti-PD-1 mAbs (nivolumab or pembrolizumab) between January 2016 and August 2018 at Kyushu University Hospital in Japan. All patients underwent 18F-FDG PET/CT before treatment initiation. Anti-PD-1 therapy was administered intravenously at a dose of 3 mg/kg every 2 weeks (nivolumab) or at a fixed dose of 200 mg every 3 weeks (pembrolizumab).

Clinical information and follow-up data were obtained from medical records. The clinicopathological features examined were: age at the time of treatment initiation, sex, Eastern Cooperative Oncology Group performance status, treatment, smoking history, radiation therapy history, clinical stage (7th edition)15, driver oncogene status (epidermal growth factor receptor [EGFR] and anaplastic lymphoma kinase [ALK]), histology, PD-L1 expression status, and SUVmax in 18F-FDG PET/CT. EGFR status in tumour tissue was determined using the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method (Mitsubishi Chemical Medience, Tokyo, Japan)16. ALK status was assessed by fluorescence in situ hybridisation of tumour tissue sections using Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular, Des Plaines, IL, USA)17. PD-L1 IHC was performed using clone 22C3 pharmDx antibody (Agilent/Dako, Carpinteria, CA, USA) according to the manufacturer's recommended methods18. In patients with multiple lesions, the highest recorded SUVmax was used for the analysis. Tumour response was assessed by CT every 6 to 8 weeks according to the Response Evaluation Criteria in Solid Tumours (RECIST), version 1.119. According to RECIST criteria, we defined patients with CR or PR as ‘responders’ and patients with SD or PD as ‘non-responders’ in this study. The end of the follow-up period was 30 September 2018. This study was approved by the institutional review board of Kyushu University and was conducted in accordance with the Declaration of Helsinki. This research was defined as a study with human samples by the Japanese guidelines presented by the Ministry of Health, Labour, and Welfare. All methods were performed in accordance with the relevant guidelines. All patients provided written informed consent.

18F-FDG PET/CT. After fasting for at least 4 h, each patient was intravenously administered 4 MBq/kg 18F-FDG. One hour later, scans were conducted from the middle of the thigh to the top of the skull. Images were obtained using an integrated PET/CT scanner (Discovery STE; GE Medical Systems, Milwaukee, WI, USA) or Biograph mCT (Siemens Medical Solutions, Erlangen, Germany). All emission scans were performed in three-dimensional mode, and the acquisition time per bed position was 3 min for Discovery STE and 2 min for Biograph mCT. PET images were reconstructed using the ordered-subset expectation–maximization method (VUE Plus) with two full iterations of 28 subsets for the Discovery STE, and iterative TrueX algorithm and TOF (Ultra HD-PET) with two full iterations of 21 subsets for the Biograph mCT. The TrueX algorithm incorporates an additional specific correction for the point-spread function. The full width at half-maximum values of the Discovery STE and Biograph mCT were 5.2 and 4.4 mm, respectively. A low-dose 16-slice CT (tube voltage 120 kV; effective tube current 30–250 mA, Discovery STE) and a low-dose 32-slice CT (tube voltage 120 kV; use of angular and longitudinal dose modulation, CAREDose4D®, Biograph mCT) from the vertex to the proximal thigh were performed for attenuation correction and for determining the precise anatomical location of lesions before acquisition of PET images. CT scans were reconstructed by filtered back projection into 512 × 512 pixel images with a slice thickness of 5 mm to match the PET scan. 18F-FDG uptake in lesions was evaluated using SUVmax, which was calculated by the dedicated workstation for each scanner.

Statistical analysis. Patient demographics and baseline characteristics were summarised using descriptive statistics or contingency tables. Progression-free survival (PFS) was defined as the time from treatment initiation to clinical or radiographic progression or death, and overall survival (OS) was defined as the time from treatment initiation to the date of the last follow-up or death. Survival curves were constructed using the Kaplan–Meier method and analysed with the log-rank test. SUVmax values between non-responders and responders were compared using Student’s t-test. The cut-off value for SUVmax was determined by receiver operating characteristic curve analysis. Associations between SUVmax and response rate or patient characteristics were evaluated using a Chi-squared test. A P value of <0.05 was considered statistically significant. All statistical analyses were performed using JMP 13.0 (SAS Institute, Cary, NC).

Data Availability

All data generated or analysed in this study are included in this published article.

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Additional Information
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