Effectiveness of EGFR-TKI rechallenge immediately after PD-1 blockade failure

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Abstract
Background: There is currently insufficient information available on effective therapies that can be administered to patients with non-small cell cancer (NSCLC) who develop resistance to epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs). However, sequential treatment via programmed death-1 (PD-1) blockade followed by EGFR-TKI rechallenge is suggested to improve the therapeutic efficacy in such patients.

Methods: A total of 75 patients with advanced NSCLC harboring sensitive EGFR mutations treated with afatinib, erlotinib, or gefitinib after EGFR-TKI treatment failure were retrospectively analyzed. Among them, 13 patients were treated with EGFR-TKI rechallenge immediately after the failure of PD-1 blockade therapy (experimental group) and the remaining 62 patients did not receive PD-1 inhibitor therapy before EGFR-TKI rechallenge (control group). Blood samples were collected at two time points; before the initiation of anti-PD-1 therapy and at EGFR-TKI rechallenge.

Results: The objective response rates of EGFR-TKI rechallenge in the experimental and control groups were 46.1% and 16.1%, respectively, with a significant difference (p = 0.026). In the experimental group, the median progression-free survival (PFS) and overall survival (OS) after EGFR-TKI rechallenge were 5.0 and 25.0 months, respectively, and no statistically significant difference in the percentage of lymphocytes before immune checkpoint inhibitor (ICI) therapy and EGFR-TKIs was observed in patients with partial response (PR) and without PR after EGFR-TKI rechallenge. In particular, the sequential treatment of PD-1 blockade therapy followed by EGFR-TKI rechallenge was consecutively repeated three times in two out of 13 patients in the experimental group, and EGFR-TKI rechallenge consecutively for the third time yielded a PR without increased toxicities.

Conclusions: EGFR-TKI rechallenge immediately after PD-1 blockade treatment was identified as an effective therapy for NSCLC patients with resistance to EGFR-TKIs.

KEYWORDS
EGFR-TKI, immune checkpoint inhibitor, lung cancer, PD-1, rechallenge

INTRODUCTION
Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are known to be effective in patients with advanced non-small cell lung cancer (NSCLC) harboring EGFR mutations.1 Three generations of EGFR-TKIs are available, which are gefitinib or erlotinib as first-generation agents, afatinib or dacomitinib as second-generation agents,
and osimertinib as a third-generation TKI. However, EGFR mutation is an opposite factor for the favorable prediction of the response to immune checkpoint inhibitors (ICIs), such as anti-programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) antibodies. Moreover, according to recent reports, a combination of osimertinib, durvalumab, and osimertinib immediately after nivolumab therapy is not acceptable due to the increased frequency of interstitial lung disease in patients with NSCLC harboring EGFR mutations. Currently, clinicians consider the usefulness of PD-1 blockade as a weak strategy for the prolongation of survival in patients with EGFR mutation-positive NSCLC. Notably, we have previously reported a drastic response to EGFR-TKI rechallenge immediately after PD-1 blockade in two patients with TKI-resistant EGFR mutation-positive NSCLC. It remains unknown why EGFR-TKI rechallenge immediately after the failure of PD-1 blockade could overcome its resistance. Recently, there have been several reports on the therapeutic efficacy of EGFR-TKI rechallenge in EGFR-mutant NSCLC. Yamaguchi et al. reported that afatinib rechallenge after the failure of first-generation EGFR-TKIs exhibited an objective response rate (ORR) of 17.0% and disease control rate (DCR) of 79.2% and suggested afatinib rechallenge as one of the preferred therapeutic options in such cases. In addition, first-generation EGFR-TKI rechallenge has been identified to have an ORR in patients of approximately 10%. Its rechallenge after EGFR-TKI failure gives only limited efficacy and may slightly contribute to the prolongation of survival. There are no established treatment options for patients showing resistance after EGFR-TKI failure; thus, further research is warranted to overcome its resistance aside from the T790M mutation.

Recently, Offin et al. reported that the tumor mutational burden (TMB) on pre-EGFR-TKI was significantly lower than that after EGFR-TKI resistance, and a significantly shorter survival was associated with patients having EGFR mutation with a high TMB than those with a low TMB. An analysis using paired pretreatment and post-progression samples revealed that TMB was significantly increased at resistance. Their study suggests a close relationship between EGFR-TKI resistance and increased TMB. TMB is considered a favorable predictor of response to PD-1 blockade therapy. Although NSCLC patients with EGFR mutations are suggested to have an increased TMB after resistance to EGFR-TKIs, it remains unclear whether PD-1 blockade is likely to suppress tumor growth. A recent study showed that the estimated five-year overall survival (OS) rate was 16% for patients receiving nivolumab monotherapy. EGFR mutation was observed in fewer patients with a five-year survival rate, thus, PD-1 blockade provides the possibility to contribute to the long-term survival of patients with EGFR mutation-positive NSCLC.

Based on this background, we investigated the effectiveness of EGFR-TKI rechallenge after EGFR-TKI failure and elucidated the possibility of PD-1 blockade therapy followed by EGFR-TKI rechallenge. Moreover, we evaluated the correlation between the transition of plasma lymphocytes and the treatment course.

**METHODS**

**Patients**

From May 2014 to December 2019, 83 patients with advanced NSCLC harboring sensitive EGFR mutations were treated with afatinib, erlotinib, or gefitinib after EGFR-TKI treatment failure at the Saitama Medical University International Medical Center. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institution and/or the National Research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study was approved by the Institutional Ethics Committee of the Saitama Medical University International Medical Center. In our retrospective study, patients who had received osimertinib immediately after the failure of PD-1 blockade therapy were excluded, and the therapeutic analysis of osimertinib as the rechallenge or in EGFR T790M-positive NSCLC was not performed. Among 83 patients, eight without evaluable lesions were excluded and the remaining 75 were eligible to be included in the analysis. In the experimental group, 13 of 75 patients who received EGFR-TKI rechallenge immediately after the failure of PD-1 blockade therapy were chosen, and the remaining 62 patients who did not receive PD-1 inhibitor before EGFR-TKI rechallenge were selected as the control group. This control group overlapped with the previously reported cases. Figure 1(a) shows the CONSORT flow diagram for patient selection.

**Treatment and evaluation**

Afatinib, erlotinib, or gefitinib were orally administered daily, and the starting dose of these EGFR-TKIs was chosen by the chief physician. In addition, erlotinib plus bevacizumab was selected based on the judgment of the chief physician, and bevacizumab was intravenously administered at a dose of 15 mg/kg every three weeks. As PD-1 blockade monotherapy, nivolumab and pembrolizumab were intravenously administered at a dose of 240 mg/day (or 3 mg/kg) and 200 mg/day, respectively. The treatment schedule of the experimental and control groups is shown in Figure 1(b). Complete blood cell count, differential count, routine biochemistry measurements, physical examination, and toxicity levels were evaluated. Acute toxicity was graded according to the Common Terminology Criteria for Adverse Events v4.0. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors v1.1 (RECIST).

**Blood sample analysis**

The patients in the experimental group (n = 13) participated in the observational study on peripheral blood mononuclear
cell (PBMC) analysis as a predictor of PD-1 blockade. Written informed consent was obtained from all 13 patients included for the analysis of PBMC. Blood samples were collected at two time points before the initiation of PD-1 blockade therapy and EGFR-TKI rechallenge (Figure 2(b)). PBMC analysis was performed according to our previous study.13 Cells were stained with monoclonal antibodies (mAbs) using the BD Accuri C6 plus flow cytometer (Becton Dickinson and Company) and the BD LSR Fortessa (Becton, Dickinson and Company). The following mAbs were used in the study: fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (HIT3a) and anti-CD4 (RPA-T4), phycoerythrin (PE)-conjugated anti-CD8 (RPAT8) and anti-CD25 (M-A251), PE-Cy7-conjugated anti-CD25 (MA251), PE-Cy5-conjugated anti-CD62L (Dreg 56; all from BD Pharmingen), and FITC-conjugated anti-CD62L (Dreg 56; eBioscience).

Statistical analysis
In this study, Fisher’s exact test was used to determine the association between two categorical variables. The Kaplan–Meier method was used to estimate survival as a function of time and survival differences were analyzed using the log-rank test. Progression-free survival (PFS) was defined as the time from the initial administration of the EGFR-TKI rechallenge to tumor recurrence or death from any cause, while OS was defined as the time from the initial administration of the EGFR-TKI rechallenge to death from any cause. Statistical analyses were performed using the GraphPad Prism 8 software and JMP 14.0 software (SAS Institute Inc.). p < 0.05 was considered statistically significant.

RESULTS

Patient demographics
Table 1 shows the patient characteristics (n = 13; nmales = 5, nmales = 8; median age = 65 years; age range = 39–79 years) of the experimental group compared with the control group. Six patients had a history of smoking, and a performance status (PS) of 0–1 in 11 patients and 2–3 in two patients. All patients were histologically confirmed to have adenocarcinoma. EGFR mutation analysis revealed seven patients with exon 19 deletion, four with L858R (exon 21), and two with other mutations. As the first-line TKI before EGFR-TKI rechallenge, eight patients were treated with gefitinib or erlotinib, four with afatinib, and one with osimertinib. In the EGFR-TKI rechallenge, eight patients were treated with gefitinib or erlotinib, four with afatinib, and one with osimertinib. In the EGFR-TKI rechallenge, eight patients were treated with gefitinib, erlotinib, or erlotinib plus bevacizumab and five with afatinib. Table 2 shows the demographics of 13 patients who received EGFR-TKIs after ICI failure. In particular, the sequential treatment with ICIs followed by EGFR-TKIs was consecutively repeated three times in Cases 11 and 13 (Figure 2).

Next, 62 patients in the control group were compared with the experimental group (Table 1). The frequency of recurrence after surgical resection was significantly higher in the control group than in the experimental group. The frequency of the T790M mutation, the number of therapeutic regimens before the EGFR-TKI rechallenge and usefulness of cytotoxic agents between first-line and EGFR-TKI rechallenge were significantly higher in the experimental group than in the control group. The administration of afatinib as an EGFR-TKI rechallenge was more frequent in the control group, and the ORR of the EGFR-TKI rechallenge was higher in the experimental group.

There were no serious side effects to EGFR-TKIs with more than grade 3 adverse events in the patients who did and did not receive ICIs.

Efficacy and survival benefit with EGFR-TKI rechallenge (experimental group)
Figure 3(a) shows the objective responses to the EGFR-TKI rechallenge after PD-1 blockade treatment. The ORR and DCR were 46.1% and 76.1%, respectively. Six of 13 patients yielded a partial response (PR), four had stable disease (SD), and three had progressive disease (PD). The ORRs of EGFR-TKI rechallenge in the experimental and control
groups were 46.1% and 16.1%, respectively, with a significant difference ($p = 0.026$). Of all patients who received PD-1 blockade monotherapy, four patients had SD and nine had PD, indicating an ORR of 0% and a DCR of 30.7%. In the experimental group, the ORR and DCR of the first-line EGFR-TKIs were 84.6% and 92.3%, respectively.

Figure 3(b) shows the total therapeutic sequence and the treatment period from the initiation of PD-1 blockade therapy is shown in Figure 3(c). PD-1 blockade monotherapy, consisting of pembrolizumab and nivolumab, was administered to four and nine patients, respectively.

Next, we analyzed the survival data of the experimental group. The median PFS of patients who received PD-1 blockade therapy was two months (range, 1.0–9.5 months), and the median PFS and OS of patients treated with the EGFR-TKI rechallenge were five and 25 months, respectively (Figure A1, online only).
**Analysis of PBMCs in the experimental group**

The analysis of peripheral blood mononuclear cells (PBMCs) was successfully performed in 11 of 13 patients at two time points, that is before the ICI and EGFR-TKI treatments; however, PBMCs were collected only before ICI therapy without EGFR-TKIs in the remaining two patients. In particular, blood samples at two time points, that is before the ICI and EGFR-TKI treatments, were collected three times in Cases 11 and 13. Figure 3 shows the transition of CD4, CD8, Foxp3, and CD62L<sup>low</sup> before the ICI and EGFR-TKI treatments. No statistically significant differences in the percentage of these lymphocytes were observed before the ICI and EGFR-TKI treatments in patients with PR (Figure 4(a)) and without PR (Figure 4(b)) after the EGFR-TKI rechallenge.

**Dramatic response by EGFR-TKI rechallenge in two cases**

As described above, Case 11 and Case 13 repeatedly received the sequential ICI regimens followed by EGFR-TKIs (Figure 2), as previously reported.  

**Case 11** A 64-year old woman with adenocarcinoma repeatedly received sequential treatments, including EGFR-TKI and cytotoxic agents (Figure 2(a)). As there was a resistance to the EGFR-TKI and systemic chemotherapy, nivolumab was initiated. One month after its administration, PD was observed; therefore, the patient was treated with afatinib rechallenge. Two weeks after treatment, a drastic response of tumor shrinkage in multiple pulmonary metastases was identified. Ten months after the rechallenge, pulmonary metastases exhibited marked regrowth, and nivolumab was restarted. Because of immediate tumor progression, afatinib rechallenge was repeated for a second time. The tumor regression continued for seven months, and the patient also had tumor regrowth of pulmonary metastases. Nivolumab as a rechallenge was administered for the third time; however, progression of pulmonary metastases also occurred. Considering the possibility of nivolumab followed by afatinib therapy, afatinib as a rechallenge was administered for the third time and the patient had tumor regression and continued to receive treatment.
| Case | Age | Gender | PS | Smoking | TNM    | EGFR mutation | Therapeutic sequences before ICIs | ICI Drug | OR | Gr3 or 4 AE / ILD |
|------|-----|--------|----|---------|--------|---------------|----------------------------------|-----------|----|------------------|
| 1    | 62  | Male   | 0  | Yes     | T2aN3M1c | L858R | Afa → Osi → CDDP/DTX | Pem       | SD | Afa PD None / none |
| 2    | 66  | Female | 0  | No      | T4N3M1a  | L858R | Erl → CDDP/PEM/Bev → Erl → Osi | Pem       | SD | Afa PD None / none |
| 3    | 46  | Female | 3  | Yes     | T4N3M1c  | Del 19 | Gef → Erl → Afa → Osi → Erl/Bev → PEM/Bev | Nivo      | PD | Afa PD None / none |
| 4    | 71  | Male   | 2  | Yes     | T2aN3M1b | Del 19 | Erl → Osi → CBDCA/nabPTX → PEM | Nivo      | SD | Erl/Bev SD None / none |
| 5    | 65  | Female | 1  | No      | T1cN3M1c | Del 19 | Afa → CBDCA/PEM/Osi | Nivo      | PD | Erl/Bev SD None / none |
| 6    | 79  | Male   | 1  | Yes     | Ope rec.| Del 19 | Gef → CDDP/PEM → Gef → PEM → Gef → Afa → DTX/S-1 | Nivo      | SD | Erl SD None / none |
| 7    | 77  | Female | 1  | Yes     | T1bN0M1b | Del 19 | Gef → CBDCA/PEM → Afa → DTX → Osi | Nivo      | SD | Erl/Bev SD None / none |
| 8    | 72  | Male   | 1  | No      | T2aN3M1b | L858R | Erl → CBDCA/PEM/Bev → Erl → Osi | Nivo      | PD | Erl/Bev PR None / none |
| 9    | 67  | Female | 1  | Yes     | T2bN3M1b | Del 19 | Afa → CDDP/VP-16 | Nivo      | PD | Afa PR None / none |
| 10   | 65  | Female | 1  | No      | T3N0M1c  | L858R | Osi → CBDCA/PEM → Erl/Bev | Nivo      | PD | Erl/Bev PR None / none |
| 11   | 64  | Female | 0  | No      | T4N3M1a  | L851Q | Gef → CBDCA/PEM/Bev → Erl → DTX → Afa → CBDCA/PEM → Erl/Bev | Nivo      | PD | Afa PR None / none |
| 12   | 40  | Male   | 0  | Yes     | T2aN3M1b | Del 19 | Erl → CBDCA/PEM/Bev | Pem       | SD | Erl/Bev PR None / none |
| 13   | 39  | Female | 1  | No      | T2bN3M1c | Del 19 | Afa → Osi → CDDP/PEM/Bev → Osi | Nivo      | PD | Erl/Bev PR None / none |

Abbreviations: AE, adverse events; Afa, afatinib; Bev, bevacizumab; CBDCA, carboplatin; CDDP, cisplatin; Del 19, deletion 19; DTX, docetaxel; EGFR mutation, epidermal growth factor receptor mutation status; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; Erl, erlotinib; Gef, gefitinib; ICI, immune checkpoint inhibitor; ILD, interstitial lung disease; nabPTX, nab-paclitaxel; Nivo, nivolumab; Ope rec., recurrence after operation; OR, objective response; Osi, osimertinib; PD, progressive disease; Pem, pembrolizumab; PEM, pemetrexed; PR, partial response; PS, performance status; RT, radiation; SD, stable disease; VP-16, etoposide.
Case 13 A 39-year old woman with adenocarcinoma was repeatedly treated with second- or third-generation EGFR-TKI or platinum-based regimens; however, the patient experienced therapeutic resistance (Figure 2 (b)). Thus, nivolumab was initiated, but there was evidence of marked expansion of multiple brain metastases one month after its administration. Erlotinib plus bevacizumab was sequentially administered, and the brain metastases almost disappeared after one month. Nine months after combination
therapy, the patient experienced obvious recurrence of brain metastasis, and was treated with nivolumab rechallenge. However, exacerbation of brain metastases was identified one month after treatment. Erlotinib plus bevacizumab was started as a rechallenge for the second time, and tumor regression was observed. Five months after its rechallenge, there was slight regrowth of brain metastases. Thus, docetaxel with ramucirumab followed by osimertinib was initiated. However, the patient also experienced exacerbation of brain metastases, and nivolumab followed by afatinib was considered an effective sequential therapy. Tumor regression of brain metastases was then observed.

In Cases 11 and 13, a significant transition of lymphocytes, such as CD4, CD8, Foxp3, and CD62L<sub>low</sub>, before the ICI and EGFR-TKI treatments was not identified (Figures A2 and A3, online only).

**DISCUSSION**

This is a complementary investigation to improve the efficacy of EGFR-TKI rechallenge in patients with advanced NSCLC with EGFR-TKI resistance. Although the poor response to PD-1 blockade therapy in NSCLC patients with **EGFR** mutations is consistent with a previous study, the
administration of PD-1 blockade therapy just before the administration of EGFR-TKIs may be helpful for the recovery of drug resistance related to the EGFR signaling pathway. Some patients benefited clinically from EGFR-TKI rechallenge after exacerbation of PD-1 blockade; however, the tumors of other patients failed to evade drug resistance. We observed that EGFR-TKI rechallenge immediately after PD-1 blockade could improve its therapeutic response compared to that without the previous administration of PD-1 blockade therapy. Moreover, the administration of PD-1 blockade therapy in patients with EGFR-TKI resistance did not affect the transition of lymphocytes such as CD4, CD8, and Foxp3 in the peripheral blood. However, it remains unclear why PD-1 blockade immediately before EGFR-TKI administration could change the sensitivity of tumors to EGFR-TKIs. Further studies are warranted to elucidate the detailed mechanism of the synergistic relationship between the immune microenvironment and EGFR driver mutations.

Little is known about the precise mechanism by which PD-1 blockade is potentially effective in patients with resistance to EGFR-TKIs. In our study, approximately half of the enrolled patients showed a good response to EGFR-TKI rechallenge, whereas there was no response to EGFR-TKI rechallenge in about 25% of all patients. In particular, Case 11 and Case 13 had long-term exposure to EGFR-TKI treatment, and their sensitivity to EGFR-TKI rechallenge repeatedly recovered after exposure to PD-1 blockade. Moreover, these two cases acquired complete resistance to PD-1 blockade, and a significant transition of lymphocytes was not identified before and after PD-1 blockade initiation. We suspect therefore that some changes in immune cells and PD-L1 expression within tumor cells may occur in the tumor immune microenvironment after PD-1 blockade administration. As it is impossible to obtain tumor specimens by biopsy, this is a matter for speculation. Recently, Sugiyama et al. reported that erlotinib as an EGFR inhibitor decreased CD positive effector regulatory T cell infiltration in the tumor microenvironment, but the combination of an EGFR inhibitor with PD-1 blockade yielded favorable anti-tumor effects than either treatment alone based on their experimental model. Their study suggests that EGFR-TKIs when administered in conjunction with PD-1 blockade potentially improve the efficacy of immunotherapy. As the mechanism of potential effect, a synergistic effect of EGFR-TKI with PD-1 blockade may recover the antitumor effect in the situation of long-term exposure to EGFR-TKI treatment.

Meanwhile, TMB might have increased compared to prior EGFR-TKIs, although TMB testing was not performed in all cases. A previous report suggested that TMB was increased in response to EGFR-TKIs; therefore, a majority of our patients may have exhibited a high TMB before the initiation of the PD-1 blockade therapy. Of note, five (83.3%) of six patients with a PR to EGFR-TKIs immediately after PD-1 blockade showed a PD after PD-1 blockade therapy, whereas five (71.4%) of seven patients who showed an SD or a PD with the EGFR-TKI rechallenge yielded an SD after PD-1 blockade treatment (Table 2). The introduction of PD-1 blockade therapy may restore EGFR-TKI sensitivity in patients with drug resistant tumors. In particular, the noneffective microenvironment associated with PD-1 blockade therapy seems to encourage the therapeutic efficacy of the EGFR-TKI rechallenge. Although it is difficult to elucidate the detailed mechanism regarding the recovery of sensitivity to the EGFR-TKI rechallenge immediately after PD-1 antibody therapy, further investigations on such promising subsequent therapies should be considered.

Recently, Isomoto et al. demonstrated the immune microenvironment in tumor rebiopsy samples after progression during the EGFR-TKI treatment. In their investigation, the expression level of PD-L1 increased, TMB tended to be higher, and CD8 and Foxp3 tumor-infiltrating lymphocytes were significantly lower after EGFR-TKI treatment than before treatment. Therefore, the efficacy of the PD-1 blockade therapy is suggested to be dependent on the expression level of PD-L1 after EGFR-TKI treatment.

In the present study, we explored the transition of lymphocytes in the systemic peripheral blood at two time points before PD-1 blockade therapy and EGFR-TKI rechallenge using PBMC analysis. The results of our study indicated that the initiation of PD-1 blockade therapy did not have an impact on the movement of peripheral lymphocytes. As we could not determine the dynamic transition of the immune microenvironment apart from the peripheral blood, it remains unknown how immune cells change within tumor specimens. However, the case presentations with regard to Cases 11 and 13 revealed interesting findings. The sequential treatment of PD-1 blockade followed by EGFR-TKI rechallenge was repeated in the same patient; however, this sequence was consistently effective, suggesting the recovery of sensitivity to EGFR-TKI. To our knowledge, this is the first study to show an improved tolerance. If dynamic monitoring of the immune microenvironment in tumor specimens is possible, we may be able to discover new evidence on therapeutic responses. In fact, dynamic monitoring of tumor tissue is actually impossible; thus, it may be difficult to elucidate the detailed mechanisms underlying the present findings. Considering the paradoxical phenomenon in our cases, we hypothesize that the effect of cancer immunoediting by PD-1 blockade therapy before EGFR-TKI administration encourages driving out the resistant tumor cells with additional gene mutations, then EGFR signal-dependent cancer cells are dominantly close to the original one without additional mutations. Therefore, the therapeutic response of EGFR-TKIs immediately after PD-1 blockade therapy may be similar to that of first-line EGFR-TKIs. However, little is known about the detailed mechanism that can be used to elucidate the phenomenon involved in our study.

There are several limitations to our study. First, the present study involved a small sample size assessed using a retrospective approach; thus, it may have contributed to biased results. Further confirmation of the therapeutic significance of PD-1 blockade therapy followed by EGFR-TKIs is warranted in a prospective large-scale study. Second, a
comprehensive analysis of genomic alterations after resistance to EGFR-TKIs was not performed. Although the analysis of genomic alterations before and after the initiation of the PD-1 blockade therapy is an interesting issue, we could not obtain the tumor specimens for these analyses. Recently, liquid biopsy has been described to be available for the detection of genomic alterations using next-generation sequencing (NGS) analysis, but this procedure appears to yield several limitations. Finally, the EGFR-TKIs administered in our study were not uniform; thus, it remains unclear which generation of EGFR-TKIs, such as first- or second-generation, are most effective as a sequential therapy after PD-1 blockade therapy. A prospective study is therefore required to resolve this limitation.

In conclusion, EGFR-TKI rechallenge immediately after PD-1 blockade treatment was identified as an effective therapy for NSCLC patients with resistance to EGFR-TKIs. Although the reason for this finding is unclear, EGFR-TKI rechallenge may be effective for patients with PD after PD-1 blockade as a prior treatment. Physicians should therefore be alerted to the choice of PD-1 blockade therapy followed by EGFR-TKI rechallenge as a clinical practice.

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CONFLICT OF INTEREST
All authors completed the ICMJE uniform disclosure form. A. Mouri, K. Kaira, O. Yamaguchi, and H. Kagamu received their salary from Ono Pharmaceutical Company and Bristol-Myers Company. All remaining authors declare no conflict of interest.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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