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REVIEW ARTICLE

# Significance of re-biopsy of histological tumor samples in advanced non-small-cell lung cancer in clinical practice

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**Abstract**
The significance of evaluating oncogenes, including EGFR mutations, ALK abnormalities, and PD-L1 expression has become broadly recognized with recent advances in molecular biology. It is now extremely important to investigate tumor oncogene status in each patient at the initial diagnosis. By contrast, the significance of conducting a re-biopsy in the salvage setting has not been systematically reviewed. This review reports that the significance of a re-biopsy varies depending on the clinical situation.

**Keywords** Lung cancer · Treatment · Re-biopsy

## Introduction

Platinum-based chemotherapy has been the mainstay of treatment for advanced non-small-cell lung cancer (NSCLC) during the past few decades [1–7]. The treatment strategy for advanced NSCLC has become diversified due to the development and clinical introduction of several effective agents. In addition, some molecular biomarkers for predicting the efficacy of each agent have also been developed simultaneously; the significance of investigating epidermal growth factor receptor (EGFR) mutations for the EGFR-tyrosine kinase inhibitor (TKI), anaplastic lymphoma kinase (ALK) abnormalities in ALK-TKI, and PD-L1 expression for immune checkpoint inhibitors (ICIs) has been successfully established [8–10]. It is now extremely important to investigate these molecular abnormalities at the initial diagnosis. The significance of conducting a re-biopsy even in the salvage setting has not been systematically reviewed.

Although liquid biopsy has been currently developed, we focus on and discuss, especially, the situations in clinical practice for which a re-biopsy of histological tumor samples is truly significant, which subpopulation should be the focus, which molecular abnormalities should be detected, and which agents should be initiated.

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### Detecting T790M mutations in EGFR-mutant tumors: is a re-biopsy worthwhile when considering initiating the third generation-EGFR-TKI?

A phase III trial (AURA 3 study) compared osimertinib monotherapy with platinum-based chemotherapy in patients with EGFR-mutant, T790M-positive tumors [11]. In this trial, progression-free survival (PFS) improved significantly in the osimertinib group [hazard ratio (HR) 0.30, 95% confidence interval (CI) 0.23–0.41]. The overall response rate was also better with osimertinib than platinum (71% vs. 31%). By contrast, the effect of osimertinib treatment in T790M-negative tumors was limited, with a response rate of 21% [12]. Therefore, conducting a re-biopsy is quite important when considering whether osimertinib therapy should be initiated.

Next, it is unknown whether it is truly unnecessary to carry out a repeat re-biopsy in patients whose tumors were once found not to harbor a T790M secondary mutation by re-biopsy. Spatiotemporal T790M heterogeneity has been reported in individual patients with EGFR-mutant tumors after acquired resistance to an EGFR-TKI [13, 14]; in 24 patients receiving repeat re-biopsies at the same lesion, T790M status of lung lesions varied in five patients after the TKI-free interval [13]. In our series [14], 25 of 55 patients (45%) with EGFR-mutated tumors who underwent re-biopsy

had T790M tumors, and osimertinib was effective in 56% of cases (10/18). Of the remaining 30 cases whose tumors did not harbor T790M, a repeat re-biopsy was conducted in 21 cases; T790M was positive in 12 cases (57%), and the osimertinib response rate was 50% (5/10). That is, the efficacy of osimertinib was quite similar, regardless of the detection of T790M in the initial re-biopsy or a repeat re-biopsy. These results and others [13–15] suggest that it may be clinically meaningful to conduct a subsequent repeat re-biopsy in patients whose tumors are found to be T790M-negative by re-biopsy, although the evidence is not firm.

By contrast, the recently reported FLAURA study greatly influenced the above-mentioned data and considerations [16]. The FLAURA study was a randomized trial that compared osimertinib with the standard EGFR-TKIs against EGFR-mutant tumors in a first-line setting. In this trial, a profound effect of osimertinib on PFS was demonstrated (HR 0.46, 95% CI 0.37–0.57). The U.S. Food and Drug Administration granted regular approval to osimertinib in April 2018 for the first-line treatment of patients with EGFR mutation-positive NSCLC. The NCCN guidelines recommend the first-line use of osimertinib monotherapy [17]. Osimertinib will be used more frequently in the earlier lines of therapy in the near future. This will also inevitably lower the significance of a re-biopsy and repeat re-biopsy.

However, assuming that 234,030 new lung cancer cases are projected to occur in the United States [18], approximately 20,000 patients with EGFR-mutant tumors would have initiated a standard EGFR-TKI, and some of them would not have access to a third-generation EGFR-TKI; thus, the significance of a re-biopsy and/or repeat re-biopsy should be retained for a certain period. In addition, transformation to small-cell lung cancer is a rare but well-recognized mechanism of acquired resistance to EGFR-TKI therapy in EGFR-mutated NSCLC [19]. Therefore, it would also be appropriate to perform a re-biopsy once the transformation is highly suspicious at the time of resistance to EGFR-TKI therapy, while referring to tumor behavior, such as more rapid tumor growth or a significant increase in a tumor marker value (i.e., ProGRP).

## Detecting ALK-secondary mutations against ALK-positive tumors: is it worthwhile to re-biopsy when considering initiating a second ALK inhibitor?

In the NCCN guidelines and the Japan Lung Cancer Society Clinical Practice Guidelines 2017, the first-line treatment with alectinib is strongly recommended for ALK-positive tumors, based on the ALEX and J-ALEX trial results showing a significant PFS advantage of alectinib over crizotinib (PFS-HR 0.47, 95% CI 0.34–0.65 in ALEX trial and 0.34, 99.7% CI 0.17–0.71 in the J-ALEX trial) [20, 21]. However, to date, precision medicine with the information about ALK-secondary mutations in ALK-positive tumors has not been established for the second-line or later settings.

Exploratory, small prospective phase II trials have been initiated or completed to evaluate the efficacy of second ALK inhibitors in patients harboring alectinib-resistant tumors [22, 23]. In one of these trials, ceritinib monotherapy was investigated (ASCEND-9 study); 5 of the 20 registered patients responded, with an overall response rate of 25% [22]. One of these five responders had undergone a re-biopsy before the ceritinib treatment, and L1196M was detected as an ALK-secondary mutation in that patient. In a previous translational study, a pie chart of secondary mutation patterns in tumor cells that acquired resistance to alectinib revealed G1202 R, L1196 M, 1171T/N/S, and V1180L as the representative mutations [24]. The latter three mutations were still sensitive to ceritinib, accounting for 25% of the entire pattern. Quite interestingly, (1) its frequency was close to the overall response rate of 25% obtained in the ASEND 9 trial, and (2) L1196 M, which was potentially sensitive to ceritinib in pre-clinical data, was, indeed, detectable from a tumor in one responder in the ASCEND 9 trial.

In summary, the significance of conducting a re-biopsy for detecting ALK-secondary mutations in patients with ALK-positive and alectinib-resistant tumors cancer remains unclear. We should be aware of any relevant data released in the future.

## Focusing on PD-L1 expression mainly against oncogene-driver-negative tumors: is it worthwhile to undergo a re-biopsy while considering initiation of an ICI?

According to the NCCN guidelines [17], the anti-PD-1 antibody pembrolizumab is strongly recommended as the initial treatment for advanced NSCLC if PD-L1 expression is $\geq$50% without targetable EGFR or ALK genetic aberrations [25]. The most important point is that such clinical decision-making is available only when PD-L1 status is checked at the time of diagnosis. Here, we raise the clinical question; is it clinically significant to re-evaluate PD-L1 expression level by carrying out a re-biopsy to initiate ICI therapy after relapse from platinum-based therapy?

Arguments both for and against arise from this issue. Those in favor might argue the following. First, PD-L1 expression can change before and after platinum-based therapy; PD-L1 positivity on tumor cells changed from 75 to 38% in 32 patients with stage III NSCLC [26]. Another study showed that PD-L1 expression was initially identified in 53% patients, whereas 62% with stage I–III squamous cell carcinoma of the lung were positive for PD-L1 expression after platinum-based chemotherapy [27]. These findings clearly show that PD-L1 expression

is dynamic; indeed, in some cases, PD-L1 expression changed from 0% to a quite higher level after platinum-based chemotherapy. Thus, those in favor would say that it is quite reasonable to carry out a re-biopsy after a recurrence from platinum-based chemotherapy.

On the other hand, the argument against would be as follows. Even if PD-L1 expression is negative in the archival specimen, sub-analysis data from the OAK trial showed [28] a significantly prolonged overall survival (OS) after taking the anti-PD-L1 antibody, atezolizumab, compared with docetaxel in patients refractory to platinum-based chemotherapy (HR 0.61, 95% CI 0.45–0.84 for PD-L1-negative tumors) [29], which was similar to the whole cohort (HR: 0.73, 95% CI 0.62–0.87). Therefore, there is a possibility for the early ICI use even for such subgroups. In addition, the magnitude of the survival-prolonging effect of pembrolizumab over docetaxel in patients with PD-L1-positive tumors detected in the archival specimens was almost identical to that in new specimens in the KEYNOTE-010 study (HR 0.70, 95% CI 0.54–0.89 and HR 0.64, 95% CI 0.50–0.83 for archival and new specimens, respectively) [30]. That is, the survival advantage of pembrolizumab monotherapy in the salvage setting seems secure regardless of the timing of investigating PD-L1 expression. Thus, at the moment, it would hardly be worthwhile to conduct a re-biopsy to recheck PD-L1 status in each patient when considering initiating an ICI in the salvage setting.

By contrast, very recently, the KEYNOTE-189 phase III trial was reported with great fanfare [31]. That trial evaluated adding pembrolizumab to platinum and pemetrexed in patients with advanced non-squamous NSCLC in the first-line setting, regardless of PD-L1 status without targetable EGFR or ALK genetic aberrations. A large benefit to OS was clearly demonstrated with an HR of 0.49 and 95% CI of 0.38–0.64. Remarkably, this significant survival advantage was detected regardless of PD-L1 expression level (HR 0.42, 95% CI 0.26–0.68 for PD-L1 expression $\geq 50\%$; HR 0.55, 95% CI 0.34–0.90 for 1–49%; HR 0.59, 95% CI 0.38–0.92 for $< 1\%$).

These results indicate that it would be appropriate to use the combination regimen of platinum and pembrolizumab without examining PD-L1 expression before initiating treatment. However, whether this combination therapy is truly superior to pembrolizumab alone remains unknown in patients possessing tumors with high ($\geq 50\%$) PD-L1 expression, so checking the PD-L1 expression level at the time of diagnosis remains important. Nevertheless, once the trial results were published, the significance or necessity for "re-biopsy" would decrease.

## Focusing on PD-L1 expression mainly against EGFR-mutant tumors, is it worthwhile to undergo a re-biopsy while considering initiating an ICI?

Most of the reported evidence regarding ICIs for advanced NSCLC is targeted at oncogene-driver-negative tumors, meaning that the efficacy of ICI in the oncogene-driver-positive tumors has not been fully evaluated. EGFR-TKIs are key agents to treat EGFR-mutant tumors [8, 10, 32–34], so ICIs are often used in later lines of therapy in daily practice. This approach can simply lead to a longer interval between the initial biopsy at the time of diagnosis and initiating an ICI for an EGFR-mutant tumor than a wild-typed tumor. Under such situations, it is unknown whether it is significant to investigate the latest PD-L1 status in each patient using new specimens by re-biopsy to initiate ICI therapy.

There are three important points that should be raised when discussing this issue. First, PD-L1 expression can change actively in EGFR-mutant tumors before and after EGFR-TKI treatment, similar to platinum-based chemotherapy [35, 36]. Among patients who had EGFR-mutant tumors with paired, pre-, and post-TKI-resistant biopsies, the positivity of PD-L1 expression accounted for 16% (10/62) and 29% (18/63) before and after EGFR-TKI therapy, respectively [35]. Second, a three-arm, randomized trial for non-squamous NSCLC called the IMpower 150 investigated the significance of adding atezolizumab to carboplatin–paclitaxel–bevacizumab therapy [37]. The results have been partially reported and show a PFS advantage in the platinum plus atezolizumab group (HR 0.62, 95% CI 0.52–0.74). Notably, 80 patients with EGFR-mutant tumors were enrolled among the entire cohort in that study, and their HR was 0.60 according to a sub-analysis, suggesting that efficacy can be obtained even in EGFR-mutated tumors after adding atezolizumab to platinum plus bevacizumab, despite the small-scaled sub-analysis with a limited number of patients. Third, a retrospective study reported a more profound effect of ICIs in EGFR-mutant tumors at higher PD-L1 expression levels [38]. Despite a quite small-scaled study, this observation might indicate that the effect of the ICI could be affected by PD-L1 expression in the tumors. Here, re-biopsy specimens after TKI-failure, and not archival specimens, were used for the PD-L1 expression analysis.

Conclusively, based on the above-mentioned three discussion points, in EGFR-mutant tumors, (1) PD-L1 expression changes over time, (2) an ICI might be effective, and (3) its effect could be affected by the PD-L1 expression level confirmed in the re-biopsy specimens. These results suggest that it might be meaningful to conduct a re-biopsy to reassess PD-L1 expression in EGFR-mutant tumors, while considering introducing an ICI.

## Conclusions

Targeted therapy, especially with regard to EGFR mutations, ALK gene aberrations, and PD-L1 expression, has been successful for treating advanced NSCLC with substantial prolongation of survival [9, 25]. Thus, it is extremely important to investigate tumor oncogene status in each patient. We have also shown that the significance of a re-biopsy varies depending on the clinical situation. Any timely pivotal results from future relevant studies should be cautiously interpreted in daily clinical practice.

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### Compliance with ethical standards

**Conflict of interest** No other authors declare any conflicts of interest regarding this study.

## References

1. Hotta K, Matsuo K, Ueoka H et al (2004) Meta-analysis of randomized clinical trials comparing cisplatin to carboplatin in patients with advanced non-small-cell lung cancer. J Clin Oncol 22:3852–3859
2. Hotta K, Matsuo K, Ueoka H et al (2004) Addition of platinum compounds to a new agent in patients with advanced non-small-cell lung cancer: a literature based meta-analysis of randomised trials. Ann Oncol 15:1782–1789
3. Hotta K, Matsuo K (2007) Long-standing debate on cisplatin- versus carboplatin-based chemotherapy in the treatment of advanced non-small-cell lung cancer. J Thorac Oncol 2:96
4. Hotta K, Fujiwara Y, Matsuo K et al (2007) Recent improvement in the survival of patients with advanced non small cell lung cancer enrolled in phase III trials of first-line, systemic chemotherapy. Cancer 109:939–948
5. Hotta K, Takigawa N, Hisamoto-Sato A et al (2013) Reappraisal of short-term low-volume hydration in cisplatin-based chemotherapy: results of a prospective feasibility study in advanced lung cancer in the Okayama Lung Cancer Study Group Trial 1002. Jpn J Clin Oncol 43:1115–1123
6. Ninomiya K, Hotta K, Hisamoto-Sato A et al (2016) Short-term low-volume hydration in cisplatin-based chemotherapy for patients with lung cancer: the second prospective feasibility study in the Okayama Lung Cancer Study Group Trial 1201. Int J Clin Oncol 21:81–87
7. Hotta K, Ninomiya K, Takigawa N et al (2015) Reappraisal of short-term low-volume hydration in cisplatin-based chemotherapy; hoping for it as a public domain. Jpn J Clin Oncol 45:603–604
8. Lynch TJ, Bell DW, Sordella R et al (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129–2139
9. Kris MG, Johnson BE, Berry LD et al (2014) Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA 311:1998–2006
10. Hotta K, Kiura K, Toyooka S et al (2007) Clinical significance of epidermal growth factor receptor gene mutations on treatment outcome after first-line cytotoxic chemotherapy in Japanese patients with non-small-cell lung cancer. J Thorac Oncol 2:632–637
11. Mok TS, Wu Y-L, Ahn M-J et al (2017) AURA3 Investigators. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med 376:629–640
12. Jänne PA, Yang JC, Kim DW et al (2015) AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. N Engl J Med 372:1689–1699
13. Hata A, Katakami N, Yoshioka H et al (2015) Spatiotemporal T790M Heterogeneity in individual patients with EGFR-mutant non-small-cell lung cancer after acquired resistance to EGFR-TKI. J Thorac Oncol 10:1553–1559
14. Ichihara E, Hotta K, Kubo T et al (2018) Clinical significance of repeat rebiopsy in detecting the EGFR T790M secondary mutation in patients with non-small cell lung cancer. Oncotarget 9(50):29525–29531
15. Hata A, Masago K, Katakami N et al (2014) Spatiotemporal T790M heterogeneity in a patient with EGFR-mutant non-small-cell lung cancer. J Thorac Oncol 9:e64–e65
16. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T et al (2018) FLAURA Investigators. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. N Engl J Med 378:113–125
17. National Comprehensive Cancer Network homepage. https://www.nccn.org/. Accessed 5 May 2018
18. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68:7–30
19. Tokaca N, Wotherspoon A, Nicholson AG et al (2017) Lack of response to nivolumab in a patient with EGFR-mutant non-small cell lung cancer adenocarcinoma sub-type transformed to small cell lung cancer. Lung Cancer 111:65–68
20. Peters S, Camidge DR, Shaw AT et al (2017) ALEX Trial Investigators. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. N Engl J Med 377:829–838
21. Hida T, Nokihara H, Kondo M et al (2017) Alectinib versus crizotinib in patients with ALK-positive non-small-cell lung cancer (J-ALEX): an open-label, randomised phase 3 trial. Lancet 390:29–39
22. Horinouchi H, Maemondo M, Hida T et al (2017) Phase 2 study of ceritinib in patients with ALK + NSCLC with prior alectinib treatment in Japan: ASCEND-9. J Thorac Oncol 12(Supple 2):S1952–S1953
23. Isozaki H, Hotta K, Ichihara E et al (2016) Protocol design for the bench to bed trial in alectinib-refractory non-small-cell lung cancer patients harboring the EML4-ALK fusion gene (ALRIGHT/OLCSG1405). Clin Lung Cancer 17:602–605
24. Gainor JF, Dardaei L, Yoda S et al (2016) Molecular mechanisms of resistance to first- and second-Generation ALK inhibitors in ALK-rearranged lung cancer. Cancer Discov 6:1118–1133
25. Reck M, Rodríguez-Abreu D, Robinson AG et al (2016) KEYNOTE-024 Investigators. Pembrolizumab versus chemotherapy

for PD-L1-positive non-small-cell lung cancer. N Engl J Med 375:1823–1833
26. Sheng J, Fang W, Yu J et al (2016) Expression of programmed death ligand-1 on tumor cells varies pre and post chemotherapy in non-small cell lung cancer. Sci Rep 6:20090
27. Song Z, Yu X, Zhang Y (2016) Altered expression of programmed death-ligand 1 after neo-adjuvant chemotherapy in patients with lung squamous cell carcinoma. Lung Cancer 99:166–171
28. Rittmeyer A, Barlesi F, Waterkamp D et al (2017) Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 389:255–265
29. Gadgeel S, Kowanetz F, Zou W et al Clinical efficacy of atezolizumab (Atezo) in PD-L1 subgroups defined by SP142 and 22C3 IHC assays in 2L + NSCLC: Results from the randomized OAK study. ESMO 2017 (Abs 1296O)
30. Herbst RS, Baas P, Kim DW et al (2016) Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 387:1540–1550
31. Gandhi L, Rodriguez-Abreu D, Gadgeel S et al (2018) Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl J Med 378(22):2078–2092
32. Kato Y, Hotta K, Takigawa N et al (2014) Factor associated with failure to administer subsequent treatment after progression in the first-line chemotherapy in EGFR-mutant non-small cell lung cancer: Okayama Lung Cancer Study Group experience. Cancer Chemother Pharmacol 73:943–950
33. Hotta K, Kiura K, Tabata M et al (2014) A Survey of Japanese thoracic oncologists' perception of diagnostic and treatment strategies for EGFR-mutant or EML4-ALK-fusion non-small cell lung cancer. Chest 146:e222–e225
34. Hotta K, Tabata M, Kiura K et al (2007) Gefitinib induces premature senescence in non-small-cell lung cancer cells with or without EGFR gene mutation. Oncol Rep 17:313–317
35. Gainor JF, Shaw AT, Sequist LV et al (2016) EGFR Mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: a retrospective analysis. Clin Cancer Res 22:4585–4593
36. Kim TJ, Hong SA, Kim O et al (2017) Changes in PD-L1 expression according to tumor infiltrating lymphocytes of acquired EGFR-TKI resistant EGFR-mutant non-small-cell lung cancer. Oncotarget 8:107630–107639
37. Kowanetz M, Socinski MA, Zou W et al (2018) IMpower150: Efficacy of atezolizumab plus bevacizumab and chemotherapy in 1L metastatic nonsquamous NSCLC across key subgroups. Presented at: 2018 AACR Annual Meeting, 2018; Chicago, Illinois. Abstract CT076
38. Haratani K, Hayashi H, Tanaka T et al (2017) Tumor immune microenvironment and nivolumab efficacy in EGFR mutation-positive non-small-cell lung cancer based on T790M status after disease progression during EGFR-TKI treatment. Ann Oncol 28:1532–1539