

# Rebiopsy of Histological Samples in Pretreated Non-small Cell Lung Cancer: Comparison Among Rebiopsy Procedures

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**Abstract.** *Aim: The aim of the present study was to compare successful rate, failure reasons, and complications among procedures of histological rebiopsy. Patients and Methods: We retrospectively reviewed medical records of histologically rebiopsied cases with non-small cell lung cancer. Results: One hundred and eleven histological rebiopsies were performed in: 86 (77%) lung; 11 (10%) lymph node; 5 (5%) pleura; 4 (4%) liver; 2 (2%) muscle; 2 (2%) adrenal gland; and 1 (1%) rib. Successful rate by computed tomography-guided biopsy (CTGB), transbronchial biopsy (TBB), and ultrasound-guided biopsy were 86% (48/56), 90% (28/31), and 100% (24/24), respectively. Reasons for rebiopsy failure by CTGB were no/insufficient malignant cells (n=5) and pneumothorax (n=3), and those by TBB were no/insufficient malignant cells (n=2) and bleeding (n=1). Severe complications (≥grade 3): one grade 3 pneumothorax and one grade 4 air embolization were observed in two (2%, 2/111) cases receiving CTGB. Conclusion: Rebiopsy of histological samples can be highly successful and feasible by optimal procedural selection.*

Lung cancer is the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancers, and the majority are already unresectable and metastatic upon their initial diagnosis. Cytotoxic chemotherapies such as platinum-based regimens were once the primary therapeutic option for metastatic NSCLC, but their advancement has reached a plateau. Molecular-targeted therapies have been recently

developed, and they have provided a remarkable benefit to patients harboring specific genetic alterations such as epidermal growth factor receptor (EGFR) gene mutations or anaplastic lymphoma kinase (ALK) gene fusions (1-3). Efficacies of up-front EGFR- and ALK-tyrosine kinase inhibitors (TKIs) have been established for patients harboring these genetic alterations in prospective randomized phase III trials comparing platinum doublets, and the median progression-free survivals (PFSs) are approximately 12 months (4, 5). Despite an initial dramatic response, most patients receiving these TKIs finally acquire resistance.

Several acquired resistant mechanisms to EGFR-TKIs have been identified (6-9), and the "gatekeeper" EGFR mutation, a threonine-to-methionine substitution at amino acid position 790 in exon 20 (T790M), is the most common mechanism and accounts for more than half of acquired resistance to EGFR-TKIs (10). Therefore, it is clinically important to develop more effective therapies for patients with T790M. Indeed, third-generation EGFR-TKIs have been developed, and have demonstrated their remarkable efficacies for patients with T790M (11, 12). Because a third-generation EGFR-TKI is indicated only in cases where histological samples reveal T790M, histological rebiopsy to confirm T790M status becomes essential in clinical practice for patients after acquired resistance to EGFR-TKIs.

On the other hand, current advancement of immunotherapies is evolving. Among them, anti-programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) antibodies have demonstrated their notable efficacies in pretreated NSCLC. Anti-PD-1 antibodies, nivolumab, pembrolizumab and atezolizumab have shown survival benefit compared to docetaxel monotherapy in pretreated patients with NSCLC after failure of platinum doublet chemotherapies in randomized phase III trials (13-16). Based on results of these trials, anti-PD-1 antibody monotherapies have become standard treatments for pretreated NSCLC.

In cases responding to such immunotherapies, durable response is expected over 1-2 years, much longer than

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common cytotoxic agents (13-16). Unfortunately, the response rate and PFS of these immunotherapies are generally 10-20% and 2-3 months, respectively, and relatively many patients obtain no response and experience early progression. Thus, reliable predictive markers are needed for better patient selection.

Several predictive markers for anti-PD-1/PD-L1 antibodies have been developed. Among them, PD-L1 expression is the most widely investigated predictive marker for many types of cancers. Some studies for NSCLC have demonstrated correlations between PD-L1 expression and response to anti-PD-1/PD-L1 antibodies (14-17). PD-L1 expression is commonly examined by immunohistochemistry (IHC) for histological samples. Moreover, PD-L1 expression can be temporally dynamic (18), and rebiopsied histological samples may be desirable before PD-L1 IHC examinations.

Based on the above background, rebiopsy of histological sample is necessary to confirm T790M/PD-L1 status. However, histological rebiopsy is an invasive manner, and tissue availability and procedural feasibility make it more challenging, resulting in limited data focusing on histological rebiopsy. We thus reviewed pretreated NSCLC cases receiving rebiopsy of histological samples at our institutes.

## Patients and Methods

**Patients.** We retrospectively screened electronic medical records of patients with NSCLC who had received histological rebiopsies in our institutes (Institute of Biomedical Research and Innovation [IBRI], Japan and Kobe City Medical Center General Hospital [KCGH], Japan). The study was approved by the institutional review board, and complied with the Declaration of Helsinki.

**Rebiopsy.** Rebiopsies were performed on various sites using various procedures. Computed tomography-guided biopsy (CTGB) (using percutaneous core needle), transbronchial biopsy (TBB) with flexible bronchoscopy, or ultrasound-guided biopsy (USGB) (using percutaneous core needle) were used as rebiopsy procedures after obtaining appropriate written informed consent. Some patients underwent multiple (sites and/or times) rebiopsies.

**T790M and PD-L1 expression analyses.** We isolated tumor DNA from each specimen, and analyzed EGFR sensitive mutations and T790M mutation using highly sensitive assays: the peptide nucleic acid-locked nucleic acid PCR clamp method or the cycleave method (19, 20). In patients who were enrolled to clinical trials of a third-generation EGFR-TKI, we sent histological samples to central laboratories of each trial, and they were examined using the cobas *EGFR* Mutation Test (11).

PD-L1 expression was analyzed only in patients who were enrolled in clinical trials of anti-PD-1/PD-L1 antibodies. These clinical trials routinely demanded histological samples, but the results were not always reported to doctors in charge. Central laboratories of each trial analyzed PD-L1 expression, and we knew only whether PD-L1 expression analysis was successful or not.

**End-point.** The aim of this study was to investigate successful rate, failure reasons, and major complications of rebiopsy, according to

Table I. Patient characteristics.

Characteristics (n=81)	Number (%)
Age	
Median (range)	65 (26-84)
Gender	
Male	37 (46%)
Female	44 (54%)
Smoking status	
Never	32 (40%)
Ever	49 (60%)
Histology	
Adeno	71 (88%)
Squamous	8 (10%)
LCNEC	2 (2%)
Genotype	
EGFR-mutated	60 (74%)
ALK-fused	1 (1%)
Wild-type	20 (25%)
Frequency of rebiopsy	
1	58 (72%)
2/3/4/5	19/2/1/1 (28%)

LCNEC, Large-cell neuroendocrine carcinoma; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase.

each procedure. Successful rebiopsy was defined as obtaining sufficient malignant cells to examine T790M status or PD-L1 expression. Major complications were graded by Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

## Results

**Sample and patient characteristics.** One-hundred and eleven histological rebiopsies were performed in 81 NSCLC patients between January 2010 and October 2015 at IBRI or KCGH. Patient characteristics are shown in Table I. Median age was 65 (range=26-84) years. Histologies included: 71 (88%) adenocarcinomas; 8 (10%) squamous cell carcinomas; and 2 (2%) large cell neuroendocrine carcinomas. Patient gene profiles were: 60 (74%) *EGFR*-mutant; 1 (1%) *ALK*-fusion; and 20 (25%) *EGFR/ALK* wild-type. Twenty-three (28%) patients underwent rebiopsy more than two times.

The purposes of rebiopsies were to examine: T790M status (66/111, 59%); PD-L1 expression (25/111, 23%); or both (20/111, 18%).

**Rebiopsy site and procedure.** Rebiopsy was performed in: 86 (77%) lung; 11 (10%) lymph node (LN); 5 (5%) pleura; 4 (4%) liver; 2 (2%) muscle; 2 (2%) adrenal gland; and 1 (1%) rib (Table II). Most rebiopsies were performed in lung lesions (86/111, 77%), whereas approximately one-fourth (25/111, 23%) were extra-lung.

In 6 (5%) of 111 rebiopsies whose target lesions were difficult for us to carry out (4 liver, 1 LN, and 1 adrenal

Table II. Details of rebiopsy.

Institute	Procedure	Location	Successful rate
IBRI (n=105)	CTGB (n=55)	Lung (n=50) Pleura (n=5)	85% (47/55)
	TBB (n=31) USGB (n=19)	Lung (n=31) LN (n=10); Cervical (n=4); Supraclavicular (n=3); Mediastinal (n=1); Axillary (n=1); and Inguinal (n=1) Lung (n=5) Muscle (n=2) Rib (n=1) Adrenal gland (n=1)	90% (28/31) 100% (19/19)
KCGH (n=6)	USGB (n=5)	Liver (n=4) Axillary LN (n=1)	100% (5/5)
	CTGB (n=1)	Adrenal gland (n=1)	100% (1/1)

IBRI, Institute of Biomedical Research and Innovation; CTGB, computed tomography-guided biopsy; TBB, transbronchial biopsy; USGB ultrasound-guided biopsy; LN, lymph node; and KCGH; Kobe City Medical Center General Hospital.

gland), we asked another cooperating institute (KCGH) to perform rebiopsies (Table II).

**Successful rate.** Overall successful rate of rebiopsy was 90% (100/111). Successful rate by CTGB, TBB, and USGB were 86% (48/56), 90% (28/31), and 100% (24/24), respectively.

**Reasons for rebiopsy failure.** Table III shows reasons for rebiopsy failure by each procedure. Reasons for rebiopsy failure by CTGB (n=8) were no or insufficient malignant cells (n=5) and pneumothorax during procedure before biopsy (n=3), and those by TBB (n=3) were no or insufficient malignant cells (n=2) and bleeding during procedure before biopsy (n=1). All USGBs were successful.

**Major complications.** Lower Table III shows major complications by each procedure. In cases receiving CTGB, 3 (3/56, 5%) grade 1 and 7 (7/56, 13%) grade 2 bleeding, 14 (14/56, 25%) grade 1, 1 (1/56, 2%) grade 2, and 1 (1/56, 2%) grade 3 pneumothorax, and 1 (1/56, 2%) grade 4 air embolization were confirmed in a total of 25 (25/56, 45%) cases. In cases receiving TBB, 12 (12/31, 39%) grade 1 and 9 (9/31, 29%) grade 2 bleeding were observed in a total of 21 (21/31, 68%) cases. There were no major complications in USGB cases. Severe complications ( $\geq$ grade 3): one grade 3 pneumothorax and one grade 4 air embolization were observed in 2 (2%, 2/111) cases receiving CTGB.

Table III. Reasons for failure and major complications.

	CTGB (n=56)	TBB (n=31)	USGB (n=24)
<b>Reasons for failure</b>			
No or insufficient malignant cells	5 (9%)	2 (6%)	0 (0%)
Pneumothorax	3 (5%)	0 (0%)	0 (0%)
Bleeding	0 (0%)	1 (3%)	0 (0%)
Total	8 (14%)	3 (10%)	0 (0%)
<b>Major complications</b>			
<b>Bleeding</b>			
Grade 1	3 (5%)	12 (39%)	0 (0%)
Grade 2	7 (13%)	9 (29%)	0 (0%)
<b>Pneumothorax</b>			
Grade 1	14 (25%)	0 (0%)	0 (0%)
Grade 2	1 (2%)	0 (0%)	0 (0%)
Grade 3	1 (2%)	0 (0%)	0 (0%)
<b>Air embolization</b>			
Grade 4	1 (2%)	0 (0%)	0 (0%)
Total	25 (45%)*	21 (68%)	0 (0%)

CTGB, Computed tomography-guided biopsy; TBB, transbronchial biopsy; USGB ultrasound-guided biopsy. \*Both bleeding and pneumothorax occurred in two cases.

## Discussion

In order to confirm T790M status and/or PD-L1 expression, rebiopsy of histological samples will become more common in our clinical practice. Approval of third-generation EGFR-TKIs and anti-PD-1/PD-L1 antibodies will make rebiopsy more essential. In our institute, the number of rebiopsies has been increasing in patients with *EGFR*-sensitive mutations, to enroll clinical trials of third-generation EGFR-TKIs. Moreover, rebiopsy has been performed even in patients without driver oncogene alterations to examine PD-L1 expression before clinical trials. Indeed, twenty (25%) of 81 patients were *EGFR/ALK* wild-type and underwent rebiopsy to confirm PD-L1 expression. Until development and spread of liquid biopsy, rebiopsy is necessary to confirm gene alterations and protein expressions.

Overall successful rate of rebiopsy was 90%, and those by CTGB, TBB, and USGB were 86%, 90%, and 100%, respectively. We selected the most suitable procedure to successfully obtain histological samples. Since USGB was performed only in easy cases whose targetable lesions were large enough to detect by US, it was natural that the successful rate was extremely high. Successful rate of CTGB was similar to TBB, but this result was highly biased. TBB could be done only in cases whose targetable lesions were confirmed by X-ray fluoroscopy. CTGB was generally adopted to peripheral small nodules which were too difficult for TBB to hit. On the other hand, CTGB is not indicated to

central located lesions where TBB is the best procedure. It is important to select the most suitable procedure, by taking tumor size, location, and organ into consideration.

Most rebiopsies were performed in lung lesions (86/111, 77%), whereas approximately one-fourth (25/111, 23%) were in extra-lung. This result suggests that clinical physicians should prepare rebiopsy for extra-lung lesions. Some of our physicians can perform rebiopsy using CTGB, TBB, and USGB by ourselves. However, in 5 (5%) of 111 rebiopsies whose target lesions were difficult for us to carry out, we asked another cooperating institute (KCGH) to perform rebiopsy. Some of these 5 patients were enrolled into a clinical trial of third-generation EGFR-TKI, and were able to benefit from the agent in its trial. In Japan, patients with lung cancer are commonly treated by thoracic oncologists/respiratory physicians. TBB is relatively easily and competently carried out by them, but other procedures are not so familiar. Thus, it is important to establish a cooperative system to ask other departments/institutes for optimal procedures which are difficult to be done in original departments/institutes.

We examined major complications with rebiopsy according to each procedure. There were no major complications in USGB cases, because USGB was performed only in easy cases whose targetable tumors were superficial and large enough to be detect by US. TBB recorded 68% of bleedings, but all were less than grade 2 and manageable, suggesting a safe procedure. CTGB revealed several complications (45%), including 2 severes: grade 3 pneumothorax and grade 4 air embolization. Particularly, air embolization is rare, but a possible fatal complication with CTGB. A pooled case study reported that air embolization was confirmed in 6 (0.06%) of 9,783 cases, and 1 of these 6 was fatal (21). CTGB could have a high successful rate and obtain a sufficient tissue, whereas a rare fatal complication such as air embolization should be noted.

Rebiopsy of histological samples includes several disadvantages regarding invasiveness, convenience, and feasibility. As we described above, rare but fatal complications could occur in histological rebiopsy. The more difficult targeted lesions, the more time and effort are taken, resulting in lower feasibility. Notably, liquid biopsy has been currently developed, and this technique can perform rebiopsy, non-invasively. Revolutionally advancement of this non-invasive procedure would raise the convenience and feasibility of rebiopsy. Indeed, liquid biopsy using digital PCR has demonstrated its effectiveness to detect T790M mutation after acquired resistance to EGFR-TKIs (22). Liquid biopsy would compensate for the disadvantages of histological rebiopsy in the near future.

Our study includes several limitations. First, it is retrospective in its design and includes a small sample size. However, to the best of our knowledge, our study is the largest to focus on histological rebiopsy. Although potential biases are inevitable, its retrospective nature might well reflect our actual

clinical practice. Second, the situation of our Institute may be unique. In Japan, rebiopsy is mostly done by TBB. On the other hand, in western countries, radiologists seem to mostly perform rebiopsy by CTGB. Some of our physicians can carry out TBB, CTGB and USGB by ourselves, and we can easily select the most suitable procedure. Therefore, our data may be unique compared with rebiopsy data from many other institutes.

## Conclusion

Rebiopsy of histological samples can be highly successful and feasible by optimal procedural selection. To select the most suitable procedure, it is important to establish a cooperative system to carry-out optimal procedures. After approval of third-generation EGFR-TKIs, histological rebiopsy to confirm T790M status will become essential in clinical practice for patients after acquired resistance to EGFR-TKIs. Moreover, development of novel therapies such as immunotherapies will encourage histological rebiopsy, even in patients without driver gene alterations. Further studies are warranted to investigate optimizing a histological rebiopsy for better clinical practice.

## Conflicts of Interest

The Authors declare they have no conflicts of interest.

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