

ORIGINAL ARTICLE

Discordance of epidermal growth factor receptor mutations between primary tumors and corresponding mediastinal nodal metastases in patients operated on for stage N2 non-small cell lung cancer

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Abstract

The discordance of epidermal growth factor receptor (EGFR) mutations between primary lung tumors and the corresponding mediastinal nodal metastases has not yet been well elucidated. We investigate the discordance of EGFR mutations between primary tumors and the corresponding mediastinal nodal metastases, and the discordance of EGFR mutations between different mediastinal lymph node stations in patients operated on for stage N2 non-small cell lung cancer (NSCLC). Two hundred and nineteen surgically resected primary tumors and their 553 corresponding mediastinal nodal metastases were evaluated for EGFR mutations in exon 19 or 21 by TaqMan real-time polymerase chain reaction (PCR) analysis. EGFR mutation was detected in 26.0% (57/219) of the primary tumors and 14.8% (82/553) of the corresponding mediastinal nodal metastases. In 162 cases with EGFR wild-type in primary tumors, none of their 402 corresponding mediastinal nodal metastases had EGFR mutation. In 57 cases with EGFR mutations in primary tumors, EGFR mutations were detected in 82 of all 151 metastatic lymph node stations (54.3%), 34 cases had EGFR mutations in mediastinal nodal metastases, and 23 cases had lost the mutations in mediastinal nodal metastases. Among the 219 cases, 196 cases had at least two metastatic lymph node stations, 9.0% (18/196) of cases with multiple metastatic nodal stations exhibited discordance in EGFR mutations between different lymph node stations. The possibility of discordance in EGFR mutations between primary tumors and corresponding mediastinal nodal metastases, and between different mediastinal lymph node stations should be considered whenever these mutations are used for the selection of patients for EGFR-directed tyrosine kinase inhibitor therapy.

Introduction

Lung cancer is the most common cause of cancer deaths worldwide. More than 80% of these deaths are as a result of non-small-cell lung cancer (NSCLC). Approximately

55% of patients are diagnosed with local advanced or metastatic disease. The five-year survival rate is 15% for local advanced disease, and 3% for metastatic disease.¹ Although platinum-based combination chemotherapy has improved the median survival in patients with NSCLC, the prognosis

for these patients remains poor.² As some patients with NSCLC have achieved impressive responses to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), which are both dramatic and durable, there is increased interest in EGFR-directed TKI therapy for NSCLC patients.^{3,4}

EGFR gene assays are widely recommended to identify patients with NSCLC who are highly responsive to EGFR TKIs. The EGFR gene consists of 118 kbp in 28 exons, and the TK domain is encoded within exons 18–21. Although a variety of different mutations span the entire EGFR TK domain, 89% are located in exons 19 and 21. These two mutations are highly predictive of response to TKIs and survival.⁵ The discovery of somatic mutations in the TK domain of EGFR in patients with NSCLC represents a dramatic step in elucidating genomic changes in lung cancer and the role in developing treatment strategies.^{4,6,7} Subsequent retrospective and prospective trials have confirmed that the response rate to the TKIs gefitinib or erlotinib, in patients with EGFR mutations, is approximately 70%–80% with a median survival of 20–30 months.^{8–14}

Although most patients who undergo EGFR-targeted therapy are diagnosed at an advanced stage with metastases, EGFR status was analyzed using primary tumors in the majority of studies.^{4,6} However, it is still not known whether or not the EGFR mutations of primary lung tumors are concordant with the corresponding metastatic lymph nodes. Park *et al.*¹⁵ reported discordance in EGFR mutations between primary tumors and metastatic lymph nodes in surgically resected patients with stage N1 or N2 NSCLC, of 11.9% (12 of 101 cases) by direct sequencing and 16.8% (17 of 101 cases) by heteroduplex analysis. Some recent studies have shown a discordant rate of EGFR mutations between primary tumors and the corresponding lymph node metastasis in patients with NSCLC of 27% (18 of 67 cases)¹⁶, 33% (16 of 49 cases)¹⁷, and 28% (7 of 25 cases),¹⁸ respectively. However, those reports only focused on the concordance in EGFR mutations between primary tumors and one of the metastases.

It is still not well known whether or not the EGFR mutations of all the mediastinal nodal metastases are concordant with the corresponding primary lung tumors in patients operated on with complete dissection of the mediastinal lymph nodes for stage N2 NSCLC, and whether or not the EGFR mutations of metastatic lymph nodes are the same between different nodal stations. In the current study, we assessed the following two problems: (i) the discordance in EGFR mutations between the primary tumors and the corresponding mediastinal nodal metastases; and (ii) the discordance in EGFR mutations between different lymph node stations in patients operated on with complete dissection of the mediastinal lymph nodes for stage N2 NSCLC.

Patients and methods

Patient selection and tumor samples

All specimens were obtained from NSCLC patients who had undergone microscopic complete resection by either lobectomy or pneumonectomy with systemic mediastinal lymph node dissection, according to TNM classification (the International Association for the Study of Lung Cancer 2009)¹⁹ between January 2008 and March 2011 at Cancer Center of Sun Yat-sen University. The surgical procedures used for staging and treatment of the mediastinal lymphatics included complete dissection of the mediastinal lymph nodes at levels 2, 3, 4, 7, 8 and 9 during a right-sided thoracotomy and at levels 5, 6, 7, 8 and 9 during a left-sided thoracotomy. The inclusion criteria for this study were as follows: (i) pathologically-confirmed NSCLC with mediastinal nodal metastases after operation; (ii) patients who had not been exposed to TKIs before operation; (iii) tumor samples of the metastatic lymph nodes as well as primary tumors were available for analysis of EGFR mutations; and (iv) age > 18 years old.

We evaluated EGFR gene mutations in 239 patients with complete resection between January 2008 and March 2011. Twenty patients were excluded from analysis because of insufficient lymph node specimens or failure of the polymerase chain reaction (PCR) to amplify DNA. Consequently, 219 cases, who had received formalin-fixed, paraffin-embedded surgically resected lung cancer tissues, together with the corresponding metastatic lymph nodes, were included in this retrospective analysis (Fig 1).

From those 219 cases, a total of 3684 lymph nodes were resected, of which 1539 lymph nodes were metastatic. We selected the lymph node with the most tumor tissue from each mediastinal nodal station (levels 2, 3, 4, 5, 6, 7, 8 and 9), and only tissue samples with >80% tumor content were retained. Finally, 553 mediastinal nodal metastases from 553 mediastinal nodal stations were evaluated for the EGFR mutations by TaqMan real-time PCR analysis.

The histologic type of tumor was determined according to World Health Organization²⁰ criteria, and the stage of the disease corresponded to the stage of disease at the time of primary diagnosis. Smoking history was obtained during the patient's first evaluation.

All patients signed an informed consent to use their tumor samples for molecular and pathologic analyses. The Ethics and Scientific Committees of our institution approved the study.

DNA extraction and mutation analyses

Tissue sections were examined by microscopy after hematoxylin and eosin staining and only tissue samples with >80% tumor content were selected for the study. Mutational analysis of the EGFR gene was carried out using TaqMan real-time

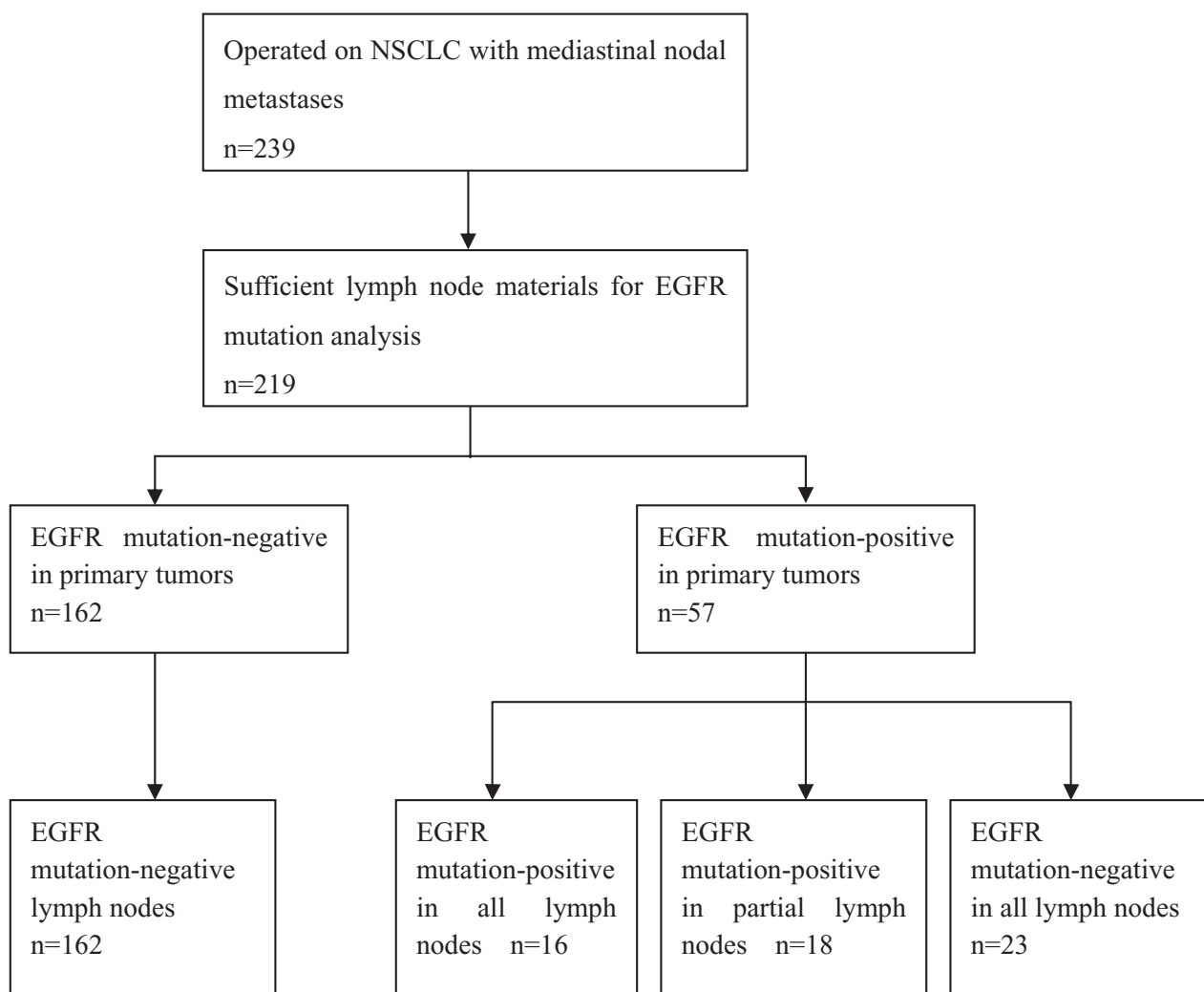


Figure 1 Two hundred and thirty-nine cases were evaluated for epidermal growth factor receptor (EGFR) gene mutations in the primary non-small-cell lung cancer (NSCLC) specimens and the corresponding mediastinal lymph nodes metastases. Twenty patients were excluded from analysis because lymph nodes specimens were insufficient or failure of polymerase chain reaction (PCR) to amplify DNA.

PCR, as previously described.²¹ Briefly, genomic DNA was extracted and purified from four microsections (10 μ m thick) of paraffin-embedded tissues obtained from the primary tumors and metastatic lymph nodes using the TIANamp Genomic DNA Kit (Tiagen Biotech, Beijing, China). A 296 bp glyceraldehyde 3-phosphate dehydrogenase (GAPDH) fragment was amplified as an internal control to ensure DNA integrity and for normalization. PCR was performed on a Peltier Thermal Cycler PCR system (Esco Technologies, Inc., St. Louis, MO, USA), as described previously,²² and the PCR products were visualized on a 1.5% agarose gel. The EGFR mutations were analyzed using a Real-time PCR Detection kit for the analysis of EGFR gene mutations (GP Medical Technologies, Beijing, China), to detect two specific in-frame deletion mutations in exon 19 (A: nucleotide, 2235-

2249del and amino acid, E746-A750del; B: nucleotide, 2240-2257del and amino acid, L747-S752del) and two point mutations in exon 21 (C: nucleotide, 2573 T > G and amino acid, L858R; D: nucleotide, 2582 T > A and amino acid, L861Q) of the EGFR gene. The TaqMan PCR and genotyping analysis were performed on Applied Biosystems 7900 Real Time PCR System (Applied Biosystems, Shanghai, China), according to the manufacturer's instructions. (The sensitivity, specificity, positive prediction or negative prediction value of the real-time PCR assay in detecting EGFR mutations was 96.3%, 97.5%, 94.0% and 98.4%, respectively.)

Statistical analyses

Statistical analyses were carried out using SPSS version 16.0.

Results

Patient characteristics

Two hundred and nineteen cases with tumor specimens adequate for molecular analysis of EGFR mutations for the primary tumors and matched synchronous mediastinal nodal metastases were included in this study. No patients received EGFR-targeted therapy or chemotherapy before the tumor specimens were obtained. The median age was 57 years (range, 28–75 years) and 75.3% were male. The pathologic type of tumor was determined according to World Health Organization²⁰ criteria. The 219 tumors were distinguished as follows: 132 adenocarcinomas (60.2%); nine adenosquamous carcinomas (4.1%); and 78 squamous cell carcinomas (35.6%). Subjects were categorized according to smoking history, as “never smoker” (90/219 or 41.1%) and “ever smoker” (129/219 or 58.9%). Never-smokers were defined as those who had smoked <100 cigarettes in their lifetime.

EGFR mutations in primary NSCLC tumors and metastatic mediastinal lymph nodes

In the current study, EGFR mutations were detected in 26.0% of cases in the primary lung tumors (57/219). The types of EGFR mutations detected in primary tumors were 30 in-frame deletions (52.6%) in exon 19 and 27-point mutations (47.4%) in exon 21. The most frequent mutation identified was a simple deletion of five amino acid residues from codons 746–750 (30/57 [52.6%]). Twenty-six cases of point mutations were leucine-to-arginine mutations at codon 858 (L858R). These two types of mutation are known to be the most common mutations in lung cancer. The remaining case had a point mutation at codon 861 (L861Q).

In this study, EGFR mutations were detected in 14.8% of all the metastatic lymph nodes (82/553) and 16.0% of patients had EGFR mutations in metastatic lymph nodes (34/219). The types of EGFR mutations detected in the metastatic lymph nodes were 19 in-frame deletions (55.9%) in exon 19, and 15-point mutations (45.1%) in exon 21. Of the 34 lymph node mutation-positive cases, the most frequent mutation was a deletion from codons 746–750 (19/34 [55.9%]). Fifteen cases of the point mutations were the leucine-to-arginine mutation at codon 858 (L858R).

Comparison of EGFR mutations between primary tumors and corresponding mediastinal lymph nodes

Among the 219 patients operated on for stage N2 NSCLC, 57 patients (26.0%) were EGFR mutation-positive and 162 patients (74.0%) were mutation-negative in primary tumors. In 162 cases with EGFR wild-type in primary tumors, none of

their 402 corresponding mediastinal nodal metastases had EGFR mutation. Among the 57 cases with EGFR mutation-positive in primary tumors, 34 cases had EGFR mutations in mediastinal nodal metastases, and 23 cases had lost the mutations in mediastinal nodal metastases. EGFR mutations were detected in 82 of all 151 metastatic lymph node stations (54.3%). The mutation pattern of EGFR detected in the corresponding metastatic lymph nodes of the 34 patients was identical to that in the primary lung tumors (Table 1). Finally, 23 of 219 cases (11.0%) showed discordance in EGFR mutations between the primary tumors and the corresponding mediastinal lymph nodes metastases.

Comparison of EGFR mutations between different mediastinal lymph node stations

Among these 219 cases, 196 had at least two metastatic lymph node stations and 23 cases had just one metastatic lymph node station. For 196 patients who had multi-station metastatic lymph nodes, 11 patients (36 lymph node stations) had all lymph nodes with EGFR mutation-positive, 167 patients (431 lymph node station) had all lymph nodes that were EGFR mutation-negative, 18 (63 lymph node station) patients had some lymph nodes with EGFR mutation-positive, and the rest with EGFR mutation-negative. Eighteen of 196 cases (9.0%) showed discordance in EGFR mutations between different mediastinal lymph node stations.

Discussion

EGFR gene mutations correlate with an increased response to EGFR TKIs in patients with advanced NSCLC. The use of EGFR gene mutation assays to select patients who would most likely be hypersensitive to TKI treatment has been extensively proposed. Currently, data of the EGFR mutation status in NSCLC is mostly based on samples obtained from a single source, either primary tumors or metastases, but it is still not well known whether or not the EGFR mutation status of primary lung cancers is concordant with the corresponding metastatic tumors.

This study compared EGFR mutations in pairs of primary tumors and the corresponding mediastinal nodal metastases in patients operated on for stage N2-NSCLC. To our knowledge, it is the first report of potential changes in EGFR mutations between different mediastinal lymph node stations in patients operated on for stage N2 NSCLC. In our study, we determined 219 pairs of primary lung tumors and multiple synchronous mediastinal nodal metastases to assess the differential mutations of EGFR gene between the primary tumors and corresponding mediastinal nodal metastases and the concordance of EGFR mutations between different mediastinal lymph node stations. Interestingly, the data shows that in postoperative stage N2 NSCLC, the metastatic lymph

Table 1 Epidermal growth factor receptor (EGFR) mutations in primary tumors and corresponding mediastinal nodal metastases

	Mutation in primary tumor	No. of dissected LN	No. of metastatic LN	No. of Metastatic LN stations	No. of mutation in LN stations	Mutation in Metastatic LN
1	exon 19	32	6	1	1	exon 19
2	exon 21	19	9	3	3	exon 21
3	exon 19	25	8	5	5	exon 19
4	exon 21	25	10	4	4	exon 21
5	exon 21	13	7	3	3	exon 21
6	exon 19	18	15	1	1	exon 19
7	exon 21	17	17	3	3	exon 21
8	exon 21	16	9	3	3	exon 21
9	exon 19	36	9	1	1	exon 19
10	exon 19	10	5	3	3	exon 19
11	exon 19	9	7	3	3	exon 19
12	exon 19	9	6	3	3	exon 19
13	exon 19	27	4	3	3	exon 19
14	exon 21	17	5	3	3	exon 21
15	exon 19	19	16	3	2	exon 19
16	exon 21	9	3	3	2	exon 21
17	exon 21	11	6	4	3	exon 21
18	exon 19	7	2	3	2	exon 19
19	exon 21	14	6	3	2	exon 21
20	exon 21	22	11	4	2	exon 21
21	exon 21	17	5	3	2	exon 21
22	exon 19	15	7	4	3	exon 19
23	exon 21	28	7	4	3	exon 21
24	exon 21	19	5	3	2	exon 21
25	exon 21	14	8	3	2	exon 21
26	exon 19	22	9	4	3	exon 19
27	exon 19	15	8	3	2	exon 19
28	exon 21	17	10	1	1	exon 21
29	exon 19	30	11	4	3	exon 19
30	exon 21	18	13	4	2	exon 21
31	exon 19	22	7	4	2	exon 19
32	exon 21	9	5	1	1	exon 21
33	exon 21	15	8	3	2	exon 21
34	exon 19	18	6	4	2	exon 19
35	exon 21	17	8	1	0	wild
36	exon 21	10	5	2	0	wild
37	exon 19	31	14	1	0	wild
38	exon 19	11	8	3	0	wild
39	exon 19	9	4	1	0	wild
40	exon 19	9	3	1	0	wild
41	exon 19	24	11	3	0	wild
42	exon 21	16	9	2	0	wild
43	exon 19	18	4	2	0	wild
44	exon 21	9	3	1	0	wild
45	exon 21	15	11	3	0	wild
46	exon 19	8	3	1	0	wild
47	exon 21	15	10	3	0	wild
48	exon 21	19	7	2	0	wild
49	exon 21	15	9	3	0	wild
50	exon 19	22	9	2	0	wild
51	exon 21	27	11	2	0	wild
52	exon 21	16	5	2	0	wild
53	exon 21	18	8	2	0	wild
54	exon 19	23	13	4	0	wild
55	exon 19	17	9	2	0	wild
56	exon 21	14	6	2	0	wild
57	exon 19	13	6	2	0	wild
Sum		990	406	151	82	

EGFR, epidermal growth factor receptor; No., number, LN, lymph node.

nodes do not always carry EGFR mutations as the primary tumors, and there is discordance in EGFR mutations between different mediastinal lymph node stations.

In our study, 23 of 219 cases (11.0%) showed discordance in EGFR mutations between the primary tumors and the mediastinal nodal metastases. The lack of any correlation in the mutation status between primary tumors and metastasis is most likely not as a result of technical problems. First, all tumor specimens analyzed were required to contain >80% tumor cells. Second, the mutation rate in the primary tumors in our study is the same as that of previously published data. Finally, our results are in accordance with those from other reports.^{15–18} Park *et al.*¹⁵ reported that the discordance in EGFR mutations between primary tumors and metastatic lymph nodes was 11.9% (12 of 101 cases) by direct sequencing and 16.8% (17 of 101 cases) by heteroduplex analysis. Our data was nearly the same as that reported by Park *et al.*,¹⁵ but our results were from 219 primary tumors and all their 553 corresponding mediastinal nodal metastases. Similar figures were presented by other recent studies; specifically, the discordant rate of EGFR mutations between primary tumors and corresponding metastases in patients with NSCLC was 27% (18 of 67 cases),¹⁶ 33% (16 of 49 cases),¹⁷ and 28% (7 of 25 cases),¹⁸ respectively.

However, some other studies have contradictory findings. Matsumoto *et al.*²³ reported that in six cases of brain metastases that were EGFR mutation-positive, the corresponding primary lung tumor had identical EGFR mutations. Another study involving lung cancer metastases reported that the mutational status of EGFR and p53 of the primary tumors was preserved.²⁴

By examining the EGFR gene in synchronous mediastinal nodal metastases corresponding to primary lung tumors, we found that EGFR mutations have a trend to differ not only between primary tumors and the mediastinal nodal metastases (11.0% discordant rate), but also between different mediastinal lymph node stations (9.0% discordant rate). This biological phenomenon of discordant EGFR mutations could partially account for the fact that some patients with well-known EGFR TKI-sensitive mutations in primary tumors fail to respond to EGFR-directed TKI therapy in mediastinal nodal metastases.

As EGFR TKIs have been remarkably effective against advanced NSCLC with mutant EGFR, there is great potential for their use as a therapeutic option in patients with local advanced NSCLC, especially for neo-adjuvant treatment of N2 diseases. EGFR mutation status in primary or metastatic tumor has been proposed to guide patient selection in advanced NSCLC. The possibility of discordance in EGFR mutations between primary tumors and corresponding mediastinal nodal metastases, and between different mediastinal lymph node stations, should be considered whenever these mutations are used for the selection of patients with local advanced NSCLC for EGFR-directed tyrosine kinase inhibitor

therapy. As for mediastinal lymph nodes, the tissue is usually taken by endobronchial ultrasound (EBUS) or cervical mediastinoscopy. If the mutation status is detected only at one mediastinal lymph node, patients with EGFR mutation in the primary tumor are not eligible for TKI therapy. In addition, the response to TKI treatment of mediastinal lymph nodes may not be evaluated accurately. A more aggressive pursuit of tissue specimens from primary tumor and corresponding mediastinal nodal metastases may be indicated to accurately determine EGFR mutations to select patients for neo-adjuvant therapy, and to accurately evaluate the N-down staging.

The main limitation of our study is its retrospective nature. Additional studies to confirm the potential implications of our results are suggested. Large-scale studies with prospective clinical trials, including EGFR mutation analysis and the EGFR TKI response on mediastinal nodal metastases, are needed.

Conclusion

In conclusion, we observed an 11.0% discordance of EGFR mutations between primary tumors and corresponding mediastinal nodal metastases, and 9.0% discordance between different lymph node stations in patients operated on for stage N2 NSCLC. These discordant results of EGFR mutations between primary tumors and corresponding mediastinal nodal metastases may have therapeutic implications for EGFR-targeted therapy strategies. Given that EGFR mutation is considered as a positive predictive factor of TKI therapy, the results of EGFR mutations according to the site of the biopsy specimens (primary vs. mediastinal lymph nodes) should be cautiously interpreted. Analysis of EGFR mutations in the primary lung tumor and mediastinal lymph nodes might be considered for the selection of patients with local advanced NSCLC for neo-adjuvant EGFR-directed TKI therapy, and to accurately evaluate the N-down staging. In patients of relapsed NSCLC after surgical resection, determination of the EGFR status using metastatic lesion might be considered in making a decision for TKI therapy. Further study will be required to ascertain the potential importance of discordance in EGFR mutations between primary tumors and mediastinal nodal metastases in determining clinical response to TKIs.

Disclosure

No authors report any conflict of interest.

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