



EGFR mutation testing in non-small cell lung cancer (NSCLC)

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Summary Lung carcinoma is subdivided into small cell carcinoma and non-small cell carcinoma (NSCLC). NSCLC is heterogeneous group of carcinomas and accounts for 70–80% of lung cancer. NSCLC is further divided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

Activating somatic mutations of the tyrosine kinase domain of epidermal growth factor receptor (EGFR) have recently been characterized in a subset of patients with non-small cell lung cancer (NSCLC). These mutations involve exons 18, 19, 20 and 21. Patients harboring these mutations in their tumors show good response to EGFR tyrosine kinase inhibitors (EGFR-TKIs). The aim of this manuscript is to provide an overview of EGFR mutations in NSCLC as well as to briefly discuss sample requirements and testing guidelines for EGFR mutation.

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Introduction

As per current World Health Organization (WHO) [1], lung carcinoma is subdivided into small cell and non-small cell carcinoma (NSCLC). The latter comprise 70–80% of lung carcinoma. Although NSCLC consists of squamous cell carcinoma, adenocarcinoma and large cell carcinoma, it was considered as one group mainly because of lack of specific therapy for various histologic subtypes. Nowadays the

distinction between adenocarcinoma and squamous cell carcinoma is extremely important due to availability of therapy.

There is increasing interest in adenocarcinoma of lung for various reasons. One reason is adenocarcinoma incidence is increasing (now considered to be the most predominant histologic subtype). Other reason is the potential uses of targeted therapy in cases showing EGFR mutations.

Since 1980s, many studies showed EGFR over-expression in lung carcinoma particularly squamous cell carcinoma using various techniques including immunohistochemistry. However, the significance of these over-expressions as prognostic marker

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¹ On behalf of the Lung Cancer Guidelines Committee. See Appendix A.

continued to be controversial. Clinical trials revealed variability in response to tyrosine kinase inhibitor, with higher response seen in Japanese patients than European patients (27.5% vs. 10.4%). In USA, partial response was noticed in women, in non-smoker and patient with adenocarcinoma. The breakthrough took place in 2004, Lynch et al. [2] reported that activating mutations of EGFR gene kinase domain resulted in responsiveness to tyrosine kinase inhibitors (TKIs) in patients with lung adenocarcinoma. Simultaneously two independent groups reported similar results [3,4]. Up to 20% of NSCLC shows EGFR mutation and up to 80% of these patients respond to TKIs (only 10% of EGFR mutation negative cases respond to TKIs). However, most of these patients will develop resistance to treatment within one year [5]. Secondary resistance is either due to second EGFR mutation T790M, or MET amplification.

The most frequent mutations in EGFR are exon 19 deletions and exon 21 point mutation: L858R (replacement of leucine at position 858 in the protein with arginine). Mutations detection start with extracting good quality DNA followed by amplifications of exon 18–21 of EGFR tyrosine kinase domain then bidirectional sequencing.

The recommendation from International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS) and European Respiratory Society (ERS) [6] is to test for EGFR mutation in all cases of lung adenocarcinoma, possible adenocarcinoma and NSCLC—not otherwise specified. If EGFR testing is negative, Alkfusion Test should be performed. It is optional to proceed to KRAS mutation testing (codon 12 and 13). Activating mutations in KRAS gene were shown to be of negative predictive value to TKIs. Also, KRAS mutations correlate with smoking history and poor prognosis.

EGFR mutations

EGFR is a member of receptor tyrosine kinase family and a major factor in regulating cellular proliferation, invasion, metastasis, angiogenesis and inhibition of apoptosis. EGFR signals activate at least two parallel intracellular pathways [7]. One of these pathways, is the MAP kinase pathway (MAPK) that regulates G1 checkpoint in the cell cycle and control cellular proliferation [8]. Once EGFR is activated, the MAPK pathway transmits the signal to the nucleus via the active forms of RAS, RAF and MEK genes [7,9]. The RAS proto-oncogene consists of H-ras, K-ras and N-ras [10], located in the inner

plasma membrane layer and present in either the active form (GTP bound) or the inactive form (GDP bound) [11].

Mutant EGFR binds ATP less tightly and binds TKIs more tightly than wild type EGFR.

EGFR testing

The sample available is usually paraffin embedded tissue. Preferably primary tumor tissue is used, when this is not available one may consider sample from metastatic tissue. Ideally, the tissue sample should contain at least 50% of viable tumor cells. Methods with higher detection sensitivity can detect mutation with lower tumor content levels. 4–10 μ m sections of non-baked unstained slides prepared from paraffin block and one H&E reference slide to mark the area of interest. The tumor area of interest selected by the pathologist should be a minimum of 2 mm \times 2 mm.

Mutation detection

Detection of mutation can be performed using a variety of mutation platforms, direct sequencing is widely used (amplify and sequence EGFR exons 18–21). Other methods includes real-time-PCR (amplification refractory mutation system), high resolution melting analysis, and denaturing high performance liquid chromatography (DHPLS).

Testing facility

Mutation analysis testing should be performed in accredited, quality assured facility participating in external proficiency testing schemes. EGFR testing should be validated before reporting the test results. Requirements for validation for molecular testing are both analytical and clinical. There are published guidelines for validating and reporting molecular testing [12]. The College of American Pathologists developed recommendations for testing, validating and reporting molecular testing [13].

Summary

Adenocarcinoma is the most common histologic type of NSCLC. Treatment decisions of NSCLC are dependent on two important factors. The first one

is accurate histologic classification using H&E stain as well as several immunohistochemical stains particularly in poorly differentiated carcinoma. The other factor is testing the tumor tissue for the presence or absence of specific mutations for targeted therapy. Since most of the tissue specimens are biopsy specimen, the pathologists play important role in managing the tissue carefully for immunohistochemical studies, molecular testing and for possible research.

Utilizing the 2011 IASLC/ATS/ERS proposal for classification of lung adenocarcinoma is highly recommended. In this classification, histologic subtypes are correlated well with EGFR mutations [14].

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References

- [1] Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC. Pathology and genetics of tumours of the lung, pleura, thymus and heart. World Health Organization Classification (WHO) of Tumours; 2004.
- [2] Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *New England Journal of Medicine* 2004;350(21):2129–39.
- [3] Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304(5676):1497–500.
- [4] Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proceedings of the National Academy of Sciences of the United States of America* 2004;101(36):13306–11.
- [5] Suda K, Onozato R, Yatabe Y, Mitsudomi T. EGFR T790M mutation: a double role in lung cancer cell survival?
- [6] Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International association for the study of lung cancer/American thoracic society/European respiratory society international multidisciplinary classification of lung adenocarcinoma. *Journal of Thoracic Oncology* 2011;6(February (2)):244–84.
- [7] Yarden Y. The EGFR family and its ligands in human cancer. Signalling mechanisms and therapeutic opportunities. *European Journal of Cancer* 2001;37(Suppl. 4):S3–8.
- [8] Raponi M, Hans Winkler N, Dracopoli C. KRAS mutations predict response to EGFR inhibitors. *Current Opinion in Pharmacology* 2008;8(4):413–8.
- [9] Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. *New England Journal of Medicine* 2008;358(11):1160–74.
- [10] Lowy DR, Willumsen BM. Function and regulation of ras. *Annual Review of Biochemistry* 1993;62:851–91.
- [11] Castagnola P, Giaretti W. Mutant KRAS, chromosomal instability and prognosis in colorectal cancer. *Biochimica et Biophysica Acta (BBA) – Reviews on Cancer* 2005;1756(2):115–25.
- [12] Lawrence J, Van Deerlin V, Gulley ML. Recommended principles and practices for validating clinical molecular pathology tests. *Archives of Pathology and Laboratory Medicine* 2009;133:743–55.
- [13] Gulley ML, Brazier RM, Halling KC, Hsi ED, Kant JA, Niki-forova MN, et al. Clinical laboratory reports in molecular

- pathology. Archives of Pathology and Laboratory Medicine 2007;131(6):852–63.
- [14] Shim HS, Lee da H, Park EJ, Kim SH. Histopathologic characteristics of lung adenocarcinomas with epithelial growth factor receptor mutations in the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society Lung Adenocarcinoma Classification. Archives of Pathology and Laboratory Medicine 2011;135(October):132.

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