Published in final edited form as:

J Clin Oncol. 2011 May 20; 29(15): 1943–1945. doi:10.1200/JCO.2010.34.1974.

Genetic Testing for Lung Cancer: Reflex vs. Clinical Selection

Paul A. Bunn Jr., M.D. and Robert C. Doebele, M.D., Ph.D.

Division of Medical Oncology, Univ. of Colorado Cancer Center, Aurora, CO

Interest in activating “driver” mutations in tyrosine kinase genes as therapeutic targets in non-small cell lung cancer (NSCLC) has peaked recently due to the discovery that EGFR tyrosine kinase inhibitors (TKIs) produce higher response rates, longer progression free survival (PFS) times, less toxicity, improved symptom control, improved quality of life, and greater convenience compared to cytotoxic chemotherapy in the first line treatment of advanced NSCLC patients harboring *EGFR* activating mutations (1–4). A more recent study showed that advanced NSCLC patients whose tumors harbor *EML4-ALK* fusion genes, who are treated with an ALK TKI termed crizotinib, have high response rates and long progression free survival times that are similar to those of NSCLC patients harboring *EGFR* mutations treated with erlotinib (5,6).

Recent genomic studies in adenocarcinoma of the lung identified other mutations in tyrosine kinase genes including *KRAS*, *NRAS*, *BRAF*, *PI3KCA* and others in frequencies exceeding 1% (7–15). These findings raised the possibility that other mutation specific TKIs could produce similar benefits in NSCLC patients whose tumors harbor these specific “driver” mutations. *BRAF* is one of the genes identified in these early reports with studies indicating the presence of *BRAF* mutations in 0% to 17% of tumors and cell lines. In these reports, 20 of 805 NSCLC tumors harbored *BRAF* mutations (2.5%) and many of these mutations were not the typical V600E mutations observed in malignant melanoma (8–15). Lung cancer cell lines had even higher rates of *BRAF* mutations with rates of 2% to 17% (14, 15). Preclinical model studies demonstrated that activating *BRAF* mutations were sufficient for the development of lung adenocarcinomas in mice (16, 17).

V600E *BRAF* mutations in malignant melanoma were shown to be present in an even higher percentage of cases (50–70%) (13, 18). Several studies reported that melanomas harboring *BRAF* mutations are dependent on MEK/ERK signaling (17, 19, 20). PLX4032, a potent inhibitor of oncogenic *BRAF*, was developed through structure guided discovery and selectively blocked RAF/MEK/ERK signaling in *BRAF* mutant cells and caused regression of *BRAF* mutant tumor xenografts (21). In a phase 1 study, PLX4032 inhibited ERK phosphorylation and produced an 81% response rate in melanoma patients with *BRAF* mutant tumors (22). These findings led to an extension phase of the PLX4032 study in 32 melanoma patients whose tumors had V600E *BRAF* mutations. There were 24 objective partial responses and 2 complete responses and the median PFS exceeded 7 months (23). Subsequent studies showed that acquired resistance to PLX 4032 developed by mutually

exclusive PDGFR beta up-regulation and *NRAS* mutation and not through secondary mutations in *BRAF*(24).

In this issue of the *JCO*, Paik et al. report finding *BRAF* mutations in 18 of 673 (3%) adenocarcinomas of the lung (25). Although the frequency of *BRAF* mutations in NSCLC is lower than in melanoma, it is high enough to warrant evaluation BRAF specific inhibitors in this population. The 3% figure is in keeping with the 2.5% observed in other studies and the finding that half of the mutations were non-V600E is also consistent with previous reports. As the authors point out, a 3% rate could mean as many as 5600 new cases annually in the US and 35,000 annually worldwide. However, it may be imperative to distinguish between the V600E and non-V600E mutations. If there are differences in therapeutic response to current BRAF inhibitors between V600E and non-V600E, only about 1.5% of all NSCLCs may have each of these classes of *BRAF* mutation. Cell lines such as NCIH1755 that has a non-V600E, G469A *BRAF* mutation has been reported to be resistant to PLX4032 (21). Cells with non-V600E *BRAF* mutations may also be resistant to other V600E BRAF inhibitors such as GSK2118436. However, tumor cells with other *BRAF* mutations are sensitive to downstream pathways inhibitors such as MEK inhibitors (20). Thus, it is likely that it will be critical to determine the exact type of *BRAF* mutation.

Paik et al. also report association between clinical characteristics, *BRAF* mutation, and survival outcome. Unlike other oncogenic mutations reported to date, they found *BRAF* mutations occurred most often in former or current smokers compared to never smokers. It should be noted that previous studies have identified *BRAF* mutations in non-smokers (26, 27). Although *EGFR* activating mutations were initially identified in a subset of patients that shared clinical and histologic characteristics, a recent comprehensive study has shown that approximately 39% of all *EGFR* mutations occur in current and former smokers (28); thus basing clinical testing on unreliable clinical characteristics can deny patients the benefit of targeted therapy. The authors did not find a sex-specific association with *BRAF* mutations. One major limitation of this study is that only adenocarcinomas were evaluated for *BRAF* mutations. Previous studies have shown the presence of both exon 11 and exon 15 *BRAF* mutations in squamous cell carcinoma of the lung (26). There is poor concordance on histologic diagnosis, even among expert pathologists (29). Therefore, testing based on histology cannot be recommended. The survival outcome seems to be slightly superior compared to patients without such driver mutations and similar to the survival of patients with *EGFR* mutations but the number of patients was quite small and there was no uniformity of treatment.

One of the major implications of the association with clinical features is to determine a priority for molecular testing. Many argue that it may be most cost effective to test only the patients most likely to have a specific mutation. However, if one conducts serial analysis of each mutation individually, it may take a long time to rule out the presence of any of the mutations. Enrichment strategies may make sense when trying to identify patients for clinical trials with limited resources to screen large numbers of patients (30); however, newer technologies such as SNaPshot and Sequenom allow testing of all of these mutations at the same time and with little additional expense compared to testing single mutations. Furthermore, there are reports of NSCLC patients with more than one oncogenic driver

mutations, (e.g., *EML4-ALK* and *EGFR* or *KRAS* mutations) (31, 32). Patients with *BRAF* (or *KRAS*) mutations can also have inactivating mutations in the tumor suppressor gene, *LKB1*, and this may influence clinical benefit from *BRAF* TKIs (33). As more driver mutations are identified, we can expect the complexity of overlapping mutations to increase. This multiplexed approach is clearly practicable as the NCI-funded Lung Cancer mutation consortium is currently conducting simultaneous genetic testing for 10 oncogenic changes for which there are specific therapies. For example, there is a trial of GSK 2118436 for patients with *BRAF*V600E mutations and a trial of the MEK inhibitor GSK1120212 in patients with non-V600E *BRAF* mutations.

This study of mutation testing in NSCLC patients and others measuring other genetic changes, signal a new era of personalized medicine for patients with advanced lung cancer where it will be imperative to match the specific mutations of a patient's tumor with a specific therapy. One challenge will be obtaining adequate tumor material at the time of diagnostic biopsies to ensure successful testing. Another challenge will be developing testing platforms that can analyze for the presence of somatic mutations, gene fusions, or other genetic changes simultaneously. It is likely that routine simultaneous (reflex) testing of multiple markers will be conducted on all patients prior to initiation of therapy, irrespective of their clinical features.

Acknowledgments

Supported in part by NCI SPORE CA 5 P50 CA 058187-15 And NCI GO CA RC2 CA 148394-02

References

1. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or caboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009; 361:947–957. [PubMed: 19692680]
2. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med*. 2010; 362:2380–2388. [PubMed: 20573926]
3. Lee JS, Park K, Kim S, et al. A randomized phase III study of gefitinib (IRESSATM) versus standard chemotherapy (gemcitabine plus cisplatin) as a first-line treatment for never-smokers with advanced or metastatic adenocarcinoma of the lung. *J Thorac Oncol*. 2009; 4(9):S283–S284.
4. Zhou, C., et al. Program and abstracts of the 35th European Society of Medical Oncology Congress. Milan, Italy; 2010 Oct. Efficacy results from the randomized phase 3 OPTIMAL (CTONG 0802) study comparing first-line erlotinib versus carboplatin (CBDCA) plus gemcitabine (GEM) in Chinese advanced non-small-cell lung cancer (NSCLC) patients (pts) with EGFR activating mutations. Abstr. LBA13
5. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med*. 2010; 363:1693–1703. [PubMed: 20979469]
6. Ou SH, Bazhenova L, Camidge DR, et al. Rapid and dramatic radiographic and clinical response to an ALK inhibitor (crizotinib, PF02341066) in an ALK translocation-positive patient with non-small cell lung cancer. *J Thorac Oncol*. 2010; 5:2044–2046. [PubMed: 21102269]
7. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature*. 2008; 455:1069–1075. [PubMed: 18948947]
8. Sun Y, Ren Y, Fang Z, et al. Lung adenocarcinoma from East Asian never smokers is a disease largely defined by targetable oncogenic mutant kinases. *J Clin Oncol*. 2010; 28:4616–4620. [PubMed: 20855837]

9. Schmid K, Oehl N, Wiba F, et al. EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. *Clin Cancer Res.* 2009; 5:4554–4560. [PubMed: 19584155]
10. Yousem SA, Nikiforova M, Nikiforov Y. The histopathology of BRAFV600E mutated lung adenocarcinoma. *Am J Surg Pathol.* 2008; 32:1317–1321. [PubMed: 18636014]
11. Sasaki H, Kawano O, Endo K, et al. Uncommon V599E mutations in Japanese patients with lung cancer. *J Surg Res.* 2006; 133:203–206. [PubMed: 16376942]
12. Naoki K, Chen TH, Richards WG, et al. Missense mutations in the BRAF gene in human lung adenocarcinoma. *Cancer Res.* 2002; 62:7001–7003. [PubMed: 12460919]
13. Brose MS, Volpe P, Feldman M, et al. BRAF and KRAS mutations in lung cancer and melanoma. *Cancer Res.* 2002; 62:6997–7000. [PubMed: 12460918]
14. Soh J, Okumura N, Lockwood WW, et al. Oncogenic mutations, copy number gains and mutant allele specific imbalance (MASI) frequently occur together in tumor cells. *PLoS One.* 2009; 4:e7464. [PubMed: 19826477]
15. Blanco R, Iwakawa R, Tang M, et al. A gene alteration profile of human lung cancer cell lines. *Hum Mutat.* 2009; 30:1199–1206. [PubMed: 19472407]
16. Ji H, Wang Z, Perera SA, et al. Mutations in BRAF and KRAS converge on activation of the mitogen activated protein kinase pathway in lung cancer mouse models. *Cancer Res.* 2007; 67:4993–4999.
17. Dankfort D, Filenova E, Collado M, et al. A new mouse model to explore the initiation, progression and therapy of BRAFV600E-induced lung tumors. *Genes Dev.* 2007; 23:379–384.
18. Wellbrock C, Hursstone A. BRAF as therapeutic target in melanoma. *Biochem Pharmacology.* 2010; 80:561–567.
19. Solit DB, Garraway LA, Pratilas CA, et al. BRAF mutations predict sensitivity to MEK inhibition. *Nature.* 2006; 49:358–362. [PubMed: 16273091]
20. Hatzivassiliou G, Song K, Yen I, et al. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature.* 2010; 464:431–435. [PubMed: 20130576]
21. Yang H, Higgs B, Kolisky K, et al. RG 7204 (PL4032), a selective BRAF V600E inhibitor, displays potent antitumor activity in preclinical melanoma models. *Cancer Res.* 2010; 70:5518–5527. [PubMed: 20551065]
22. Bollag G, Hirth P, Tsai J, et al. Clinical efficacy of a BRAF inhibitor needs broad target blockade in BRAF mutant melanoma. *Nature.* 2010; 467:596–599. [PubMed: 20823850]
23. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med.* 2010; 363:809–819. [PubMed: 20818844]
24. Nazarian R, Shi H, Wang Q, et al. Melanomas acquire resistance to BRAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature.* 2010; 468:973–977. [PubMed: 21107323]
25. Pail PK, et al. Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. *J Clin Oncol.* 2010 in press.
26. Pratilas CA, Hanrahan AJ, Halilovic E, et al. Genetic predictors of MEK dependence in non-small cell lung cancer. *Cancer Res.* 2008; 68:9375–9383. [PubMed: 19010912]
27. Yamamoto H, Shigematsu H, Nomura M, et al. PIK3CA mutations and copy number gains in human lung cancers. *Cancer Res.* 2008; 68:6913–6921. [PubMed: 18757405]
28. Pietanza M, D'Angelo SP, Johnson ML, et al. EGFR mutations in men and cigarette smokers with lung adenocarcinoma. *J Clin Oncol.* 2010; 28 abstr. 10538.
29. Field RW, Smith BJ, Platz CE, et al. Lung cancer histologic type in the surveillance, epidemiology and end results registry versus independent review. *J Natl Cancer Inst.* 2004; 96:1105–1107. [PubMed: 15265973]
30. Camidge DR, Kone SA, Flacco A, et al. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. *Clin Cancer Res.* 2010; 16:5581–5590. [PubMed: 21062932]
31. Koivunen JP, Mermel C, Zejnullahu K, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res.* 2008; 14:4275–4283. [PubMed: 18594010]

32. Martelli MP, Sozzi G, Hernandez L, et al. EML4-ALK rearrangement in non-small cell lung cancer and non-tumor lung tissues. *Am J Pathol.* 2009; 174:661–670. [PubMed: 19147828]
33. Koivunen JP, Kim J, Lee J, et al. Mutations in the LKB1 tumour suppressor are frequently detected in tumours from Caucasian but not Asian lung cancer patients. *Br J Cancer.* 2008; 99:245–252. [PubMed: 18594528]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript