Velez et al., J Clinic Experiment Pathol 2012, S5
DOI: 10.4172/2161-0681.S5-002

Clinical & Experimental Pathology

Research Article Open Access

Mutations and Tumorigenesis Pathways Driving Personalized Treatment in Non-Small Cell Lung Cancer

Michel Velez[1], Belisario Arango[1], Luis E. Raez[2] and Edgardo S. Santos[1]*

[1]Section of Thoracic Oncology, Division of Hematology/Oncology, University of Miami Miller School of Medicine, Florida, USA
[2]Memorial Cancer Institute, Memorial Health Care System, Florida International University, Miami, Florida, USA

Abstract

There has not been a more exciting time in lung pathology than now. Because of new developments in tumor biology research and molecular pathology, the entire treatment algorithm of non small cell lung cancer has completely changed. Not too long ago, we still considered "carcinoma compatible with non small cell lung cancer" as a valid histopathologic diagnosis, good enough to start a patient on chemotherapy. Advances in pathology such as immunohistochemistry, gene expression profile, and the implementation of laboratory techniques like polymerase chain reaction, fluorescent in situ hybridization, and others have moved forward this field not only to identify a more accurate classification of this disease but also to identify new tumorigenesis pathways or active mutations which could serve as biomarkers with predictive and/or prognostic power to tailor our therapeutic agents. The fact that pathologists can accurately determine tumor histology as an adenocarcinoma or squamous cell carcinoma has a tremendous impact in the treatment selection. To date, the histologic subtype of non small cell lung cancer is the first step in customization of lung cancer therapy allowing us to choose among several chemotherapeutic agents. However, tumor biology has shown to be a stronger tool to personalized medicine, and pathology plays a crucial role in developing and improving these novel techniques. In this article, we will review the well established and most promising gene abnormalities as well as upregulated pathways recently found in lung cancer patients with the goal to use them to better classify these tumors and to identify new treatments for this disease.

Keywords: B-Raf; EGFR; EML-4/ALK; HGF; Met; Mutation; Non-small cell lung cancer; PIK3CA

Introduction

There is no question that advances in laboratory techniques in addition to the clinical pathology and appropriate handle of tumor specimens have allowed the general acceptance that personalized medicine is a reality and the old concept of one-size-fits-all (non selective) approach in medicine has been left behind. The discovery of upregulated tumorigenesis pathways has helped us not only to understand lung carcinogenic processes, but also served as a target for new therapeutic developments. Delivering a targeted therapy to a well identified enriched population who harbor a specific target is perhaps the most rationale and effective use of our therapeutic armamentarium. This approach has the ability to shut down cell signalings which promote proliferation, metastasis, and progression of the malignant cells.

Lung cancer has not escaped from the development of targeted agents against critical carcinogenic pathways which have been associated with tumor progression in this disease. Among them, two genetic abnormalities have established themselves as very good target options in first-line therapy of new patients over the classic and conventional cytotoxic chemotherapy approach. The presence of gene mutation in the epidermal growth factor receptor (EGFR) and translocation of the echinoderm microtubule-associated protein-like 4/anaplastic lymphoma kinase (EML-4/ALK) are targeted now by the agents erlotinib or gefitinib (a thyrosine kinase inhibitors) and crizotinib (initially developed as a MET inhibitor, and now an ALK inhibitor), respectively [1-5]. Other pathways and mutations in lung cancer look promising for the development of novel agents. Molecular pathology has been crucial to define the best method to detect and then to validate them in clinical trials. In this review, we initially discuss the well established EGFR mutation and EML4/ALK translocation in non-small cell lung cancer (NSCLC) followed by other promising genetic abnormalities which are on active investigation in thoracic oncology and the laboratory techniques involved in their detection. Among them, hepatocyte growth factor receptor (MET), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform (PIK3CA), and serine/threonine-protein kinase B-Raf that are moving forward into clinical trials.

Epidermal Growth Factor Receptor (EGFR) Mutation (MICHEL)

EGFR, a tyrosine kinase receptor of the ErbB family is expressed in 50-80% of patients with NSCLC. Upon ligand binding and receptor dimerization and activation, downstream signaling to PI3K/AKT and Ras/Raf/MAPK occurs. There are several key mechanisms for EGFR activation that include overexpression of ligands [6], gene amplification [7,8], and activating mutations [9]. All of these led to the study of EGFR protein expression by immunohistochemistry (IHC), gene copy number (GCN) by fluorescence in situ hybridization (FISH), and *EGFR* gene mutations as potential predictive markers of response to tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib. EGFR TKIs block signal transduction pathways implicated in proliferation and survival of cancer cells as well as other processes involved in promotion of cancer cell growth.

***Corresponding author:** Edgardo S. Santos, Associate Professor of Clinical Medicine, Associate Director, Hematology/Oncology Fellowship Program, Member, Thoracic Oncology Program, Division of Hematology/Oncology, Sylvester Comprehensive Cancer Center, University of Miami Leonard M. Miller School of Medicine, Florida, USA, Tel: 305-243-6554; Fax: 305-243-3289; E-mail: esantos2@med.miami.edu

Received December 06, 2011; **Accepted** February 21, 2012; **Published** February 23, 2012

Citation: Velez M, Arango B, Raez LE, Santos ES (2012) Mutations and Tumorigenesis Pathways Driving Personalized Treatment in Non-Small Cell Lung Cancer. J Clinic Experiment Pathol S5:002. doi:10.4172/2161-0681.S5-002

Copyright: © 2012 Velez M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Activating somatic mutations of the tyrosine kinase domain of EGFR have been described in a subset of patients with advanced NSCLC. Specific mutations were identified in 2004 which confer sensitivity to TKIs targeting *EGFR* mutated tumors [10-12]. Mutations in the *EGFR* gene that comprise about 90% of activating mutations include exon 19 microdeletion and exon 21 L858R point mutation [13,14]. Other less common sensitizing *EGFR* mutations include exon 18 G719 and exon 21 L861; in the other hand, mutations in exon 20 (like T790M mutation) are generally associated with resistance to TKIs [15].

Cappuzzo et al. [16] evaluated tumors from 102 NSCLC patients treated daily with 250 mg of gefitinib for EGFR status by FISH, DNA sequencing, and IHC. Amplification of the *EGFR* gene was seen in 33 of 102 patients and high protein expression was evident in 58 of 98 patients. These two EGFR tests were significantly associated with better response rate: 36% vs 3% ($P < 0.001$); disease control rate: 67% vs 26% ($P < 0.001$); time to progression (TTP): 9.0 vs 2.5 months ($P < 0.001$); and survival: 18.7 vs 7.0 months ($P = 0.03$). The presences of EGFR mutations seen in 15 of 89 patients were also statistically significant in terms of response and TTP. In a multivariate analysis, only high *EGFR* GCN remained statistically significantly associated with better survival.

Erlotinib demonstrated a survival benefit when compared to placebo in the landmark BR. 21 study [1]. In this phase III trial, patients with NSCLC who had progressed after standard chemotherapy were randomized to either placebo or erlotinib. Tsao et al. [17] evaluated if responsiveness and impact on survival to erlotinib were associated with EGFR expression, gene amplification and mutation status. A total of 325 tumors were analyzed by IHC of those 57% where found to be EGFR positive, of which 50% were adenocarcinomas. FISH was successfully performed in 125 samples (57%), of which 45% had high polysomy or amplification and 48% were adenocarcinomas. Mutational analysis for exon 19 and 21 were successful in 107 of 197 available samples, of these 24(22%) samples had one or more mutations. Of the remaining 90 samples, 87 contained small amount of tissue which yielded DNA available for analysis for exon 19 and 21 mutations, mutations were found in 28% of the adenocarcinomas.

Polysomy or amplification of EGFR was associated with response ($P = 0.03$), mutational status had no association, with responses in 7% of patients with EGFR wild type compared to 16% with EGFR mutated tumors ($P = 0.37$). Logistic regression analyses revealed that patients that never smoker, adenocarcinoma histology, and expression of EGFR by IHC were associated with response. Survival was not associated with EGFR expression, number of copies or mutational status in patients in both the erlotinib and placebo groups. Survival in patients with EGFR expression was longer in those patients receiving erlotinib ($P = 0.02$) compared to placebo; this was also seen in those with polysomy or amplification ($P = 0.008$).

Gefitinib was evaluated in the Iressa Survival Evaluation in Lung Cancer (ISEL) trial [18]. This was a double-blind, placebo-controlled, parallel-group, multicenter, phase III survival study which randomly assigned 1,692 patients with previously treated, locally advanced or metastatic NSCLC patients in a 2:1 ratio to receive either gefitinib or placebo plus best supportive care. In ISEL, the treatment with gefitinib was associated with a trend towards improvement in the overall survival ($P = 0.087$) and the presence of adenocarcinoma histology ($P = 0.089$) [18]. Crino et al. [19] evaluated the relationships between biomarkers and outcomes in the study population. A total of 114 patients (30.8%) had a high *EGFR* GCN by FISH and achieved statistically significant better survival with gefitinib compared with placebo than patients with a low *EGFR* GCN ($P = 0.045$). Median survival in patients with a high *EGFR* GCN was 8.3 and 4.5 months for gefitinib and placebo groups, respectively. No difference in survival between gefitinib and placebo was observed in patients with a low *EGFR* GCN ($P = 0.417$). Moreover, patients who had a high *EGFR* GCN achieved better objective response rate (16.4%) and time to treatment failure (4.5 months) than those with a low *EGFR* GCN (3.2% and 2.4 months, respectively). High EGFR protein expression was present in 264 patients (69.6%); EGFR protein expression positive patients achieved a significant better survival with gefitinib than those who had EGFR protein expression negative tumors ($P = 0.049$). There was a trend for survival benefit for patients with EGFR protein expression positive tumors treated with gefitinib ($P = 0.126$). EGFR protein expression positive patients also seemed to achieve better response rates (8.2% vs 1.5%) than EGFR protein expression negative patients. Regarding mutational status, 215 patients were assessable for mutational analysis. Twenty-six (12.1%) patients were positive for *EGFR* mutation. Sequencing detected 16 *EGFR* mutations and ARMS detected 17 *EGFR* mutations involving exon 19 and 21 with ARMS being more sensitive than sequencing. Female patients who were never smokers and of Asian origin, or had adenocarcinoma histology were more likely to have *EGFR* mutations. Objective response rates with gefitinib were higher among patients with tumors positive for *EGFR* mutation than those patients with non mutated tumors (37.5% vs 2.6%). Survival outcomes and TTF were limited because there were only 10 deaths and 15 treatment failures among the 26 patients with tumors positive for *EGFR* mutation. Five different *K-ras* mutations were detected in 12 of 152 (7.9%) patients, whereas no *B-Raf* mutations were detected in 118 patients. No *K-ras* mutations were found in samples positive for an *EGFR* mutation. Almost all *K-ras* mutations were in samples from smokers and no patients treated with gefitinib who had a *K-ras*-positive mutation had a tumor response.

More recently, the results of the phase III Iressa Pan-ASia Study (IPASS) which compared gefitinib versus the combination of carboplatin and paclitaxel in untreated patients with NSCLC adenocarcinoma histology added to the cumulative data in regards to these biomarkers [2]. *EGFR* mutation data was available only for 437 patients (35.9%). Of these, 261 (59.7%) patients had tumor with a mutation: 140 (53.6%) had exon 19 deletion, 111 (42.5%) had a mutation at exon 21 (L858R), 11 (4.2%) had a mutation at exon 20 (T790M), and 10 (3.8%) had other mutations. Progression-free survival (PFS) was significantly longer among patients receiving gefitinib than among those receiving carboplatin/paclitaxel in the mutation-positive subgroup ($P < 0.001$) and significantly shorter among patients receiving gefitinib than among those receiving carboplatin/paclitaxel in the mutation-negative tumors ($P < 0.001$). The overall response rate (ORR) was 71.2% with gefitinib versus 47.3% with carboplatin/paclitaxel combination in the mutation-positive subgroup ($P < 0.001$) whereas in the mutation-negative subgroup the ORR was 1.1% versus 23.5% ($P = 0.001$) for those who received gefitinib and conventional chemotherapy, respectively [2].

Other randomized studies such as INVITE trial (gefitinib as single agent versus vinorelbine in chemonaive elderly patients) [19],

INTEREST trial (gefitinib versus docetaxel in previously treated patients) [20], and the TRIBUTE trial (erlotinib with carboplatin/paclitaxel versus carboplatin/paclitaxel alone) [21] failed to reveal a survival advantage in patients with EGFR FISH positive tumors when treated with TKIs. TRIBUTE demonstrated an improvement in TTP in patients who received erlotinib despite no OS benefit [21]. These results are difficult to interpret due to the lack of uniform sampling and testing and interpretation among these studies. Thus, a consensus guideline has been developed for analysis and interpretation of the EGFR FISH assay including sample preparation and storage, specimen selection, tissue sectioning and assay interpretation by properly trained personnel [22]. Moreover, a consensus from a European workshop was published regarding *EGFR* mutation testing [23]. It highlighted the importance of close collaboration between all parties involved in the management of lung cancer, who should be tested based on high frequency of mutations, biopsy and tissue sample preparation and fixation. No consensus was reached in regards to what is the best DNA extraction method, but may be tailored based on specimen availability.

Echinoderm Microtubule Associated Protein Like-4/Anaplastic Lymphoma Kinase (EML-4/ALK) Translocation

The *EML-4* and *ALK* (already known in anaplastic non-Hodgkin's lymphoma) genes were identified in NSCLC by Soda et al. [4]. This alteration occurs from chromosome 2p inversion and is found in 3-13% of NSCLC patients [24]. The inversion of chromosome 2 leads to the fusion gene and subsequently, a fusion protein that induces a constitutive activation of the intracellular domain of ALK; therefore, a downstream cascade of events that lead to carcinogenesis [4]. The majority of patients with EML4-ALK rearranged tumors are younger than other patients with lung cancer and is never smokers or light smokers (less than 10 pack/yr) [4,5,25]. Tumors are mostly adenocarcinomas and they do not have *EGFR* and *K-ras* mutations [24]. It does not seem to be a difference in the incidence among female and male patients, compare with *EGFR* mutations.

Initially there have been significant variability and conflicting reports regarding these tumors histologic and morphologic findings. Based on the study by Yoshida et al. [26], we can now say that almost uniformly all ALK-positive tumors have an adenocarcinoma component with few exceptions that we found this translocation in squamous cell carcinomas. ALK-positive tumors have distinct histologic findings compared to ALK-negative tumors. Mucinous cribiform pattern was present in 56% of ALK-positive tumors, as much as 43% of them had a solid signet-ring pattern [26]. There has been observed a lack of significant nuclear pleomorphism, and most of the architecture is composed of solid or acinar growth pattern, cribiform structure, presence of mucous cells with extracellular mucous, and paucity of lipidic growth [26]. However, no a single histologic parameter was still completely sensitive or specific to predict ALK rearrangement.

Lung adenocarcinomas which have these histologic characteristics and are negative for *EGFR* and *K-Ras* should be tested for EML-4/ALK translocation. IHC has proven to be a reliable way for screening specimens in a daily routine practice because is less time consuming and cheaper than performing FISH analysis on the specimens [27]. However, FISH still remains the standard and only FDA approved tool for testing for EML-4/ALK rearrangement.

Shaw et al. [5] proposed the use of *EML4-ALK* translocation as a predictive biomarker of resistance to EGFR TKIs. Of the 19 patients with *ALK* translocation, no clinical response (as defined by CR + PR) to the oral EGFR TKI erlotinib was observed in comparison with 70% response rate in patients with *EGFR* mutations ($P < 0.001$). Recently, the United States Food and Drug Administration (US FDA) approved the use of crizotinib (formerly known as PF-01241066), an inhibitor of ALK and c-MET receptor TK, which has activity on *ALK* mutant tumors. In the clinical trial conducted by Kwak et al.[28] the ORR was 64% and an impressive disease control rate (CR + PR + SD) of 90% in 50 patients with metastatic *ALK* mutant lung adenocarcinomas of the lung in patients treated with crizotininib [28]. Herein, there were 5 patients who were treated with this novel agent as first line, and response rate was 80%. This novel ALK inhibitor was approved without a phase III clinical trial due to the overwhelmed data of the impact of genetic abnormality presence as a target for novel agents not only in lung cancer but also in many other tumor types.

Hepatocyte Growth Factor

The hepatocyte growth factor (HGF) is a transcription factor which modulates expression of cytoskeletal proteins, cell cycle regulators and anti-apoptotic effectors. HGF is deregulated in cancer tumors and associated with tumor growth and metastatic invasion [29-31]. The receptor for HGF is known as Met, a tyrosine kinase (TK). Met is amplified, mutated, or overexpressed, as part of the pathway activation in many tumors including NSCLC [32,33] and its expression has been associated with a worse prognosis in NSCLC. As we discussed already EGFR pathway, now we can add the fact that activation of Met has been described as one of the mechanisms of resistance for EGFR TKIs. This is important now that EGFR TKIs have become standard of care as front line therapy for patients whose tumors harbor *EGFR* mutation. EGFR TKIs have increased PFS with better quality of life in NSCLC patients in many randomized clinical trials [2-3,34-35]. Unfortunately, sooner or later some of these *EGFR*-driven tumors acquire resistance to these TKIs. Thus, there is a strong rationale in looking for a dual inhibition of Met/EGFR as a potential approach in oncology. Novel therapies are needed to rescue these patients after progression to EGFR TKI either alone or in combination with other agents and research efforts are ongoing nowadays in this arena. One of these examples is MetMAb, a monovalent monoclonal antibody (MoAb), that specifically binds the Met receptor. In a recent randomized multicenter phase II study, this novel agent was combined with erlotinib in patients with advanced and previously treated NSCLC patients [36]. This combination improved PFS and OS in those patients whose tumors overexpressed Met by IHC ("MET high"). Because of these encouraging results, a phase III trial will be launched soon using this combination. As any other agent, MetMab is not exempted from resistance by tumor cells. Thus far, two mechanisms of resistance have been described including mutation in the Met activation loop and increased expression of transforming growth factor alpha (TGF-α) [37].

The landmark study conducted by Spigel et al. [36,38] was a global, randomized, double-blind phase II study comparing MetMAb plus erlotinib (MetMAb/E) to placebo plus erlotinib (P/E) in the setting of second- and third line treatment for advanced NSCLC. Tissue collection was mandatory to assess c-Met IHC expression levels (Met Diagnostic; Met Dx). A Met Dx positive was defined as majority (> 50%) tumor cells with moderate or strong staining intensity. A total of

128 NSCLC patients were randomized to receive MetMAb/E or P/E. Ninety-three percent of patients had adequate tissue for evaluation of Met by IHC; and 52% patients with evaluable tissue were Met Dx positive [36,38]. In the Met Dx positive group, MetMAb/E resulted in a statistically and clinically significant improvement in both PFS (2.9 vs. 1.5 months; $P = 0.04$; HR: 0.53) and OS (12.6 vs. 3.8 months; $P = 0.002$; HR: 0.37) [10,16]. Interestingly, from the pathology point of view, is the analysis of several potential biomarkers including *EGFR* and *K-ras* mutations as well as *Met* but measured through FISH analysis. An OS benefit from MetMAb/E was observed in *Met* FISH positive NSCLC as well as in FISH negative/IHC positive. When patients with *EGFR* mutations in their tumors were removed from the analysis, it did not influence the results. Selective benefit of MetMAb/E was not observed in other subgroups. When the patients who received P/E were analyzed based on Met expression, those who had Met Dx positive tumors had a worse outcome, confirming prior observations that Met expression is a negative prognostic factor. Importantly, the benefit was not only exclusive to *EGFR* mutation or *Met* FISH positive, but also to FISH negative/IHC positive patients. This may suggests that IHC is a more sensitive predictor of benefit from MetMAb treatment [36,38].

PIK3CA Mutation

Phosphatidylinositol 3-kinases (the PI3K protein family) are lipid kinases that regenerate phosphatidylinositol-3-phosphate, which is a key mediator between growth factor receptors and intracellular downstream signaling pathways [39]. PIK3CA gene encodes the main catalytic subunit of PI3K proteins, p110a isoform [40]. *PIK3CA* mutations have been identified in around 2% of NSCLC tumors [40], are as frequent in squamous cell histology as in adenocarcinoma, and may occur in *EGFR* mutated NSCLC patients [41]. When *PIK3CA* gene is mutated, it activates the protein B signaling pathway in the absence of growth factors and induces oncogenic cellular transformation [42,43]. Gene amplification has also been reported in NSCLC, especially in men, smokers, and squamous cell carcinoma [44-46].

BEZ235, a small molecule inhibitor which targets PI3K and mTOR proteins, has shown antitumour activity in mice [47]. To date, multiple PI3K inhibitors are in early clinical development, but response rate are still low [48].

B-Raf Mutation

B-Raf is a serine/threonine kinase which links Ras GTPases to downstream proteins of the MAPK family involved in control cell proliferation [49]. B-Raf is one of three members of the Raf kinase family: A-Raf, B-Raf, and Raf-1 [50]. In NSCLC, *B-Raf* mutations are found in 1–3% of tumors, and most of them are adenocarcinoma [50-53]. In NSCLC, *B-Raf* mutation is found as non-Val600Glu mutations including *Leu596Val* mutation in the kinase domain and the *Gly468Ala* mutation in the G loop of the activation domain [50,52,53]. This differs from *B-Raf* mutations found in melanoma which are Val600Glu. Although it was thought that *B-Raf* mutations are mutually exclusive to *EGFR* and *K-ras* mutations, in a recent publication from Marchetti et al. *EGFR* and *B-Raf* mutations were described concomitantly in the tumors of two patients [54].

In a retrospective series of 1,046 NSCLCs (739 adenocarcinomas and 307 squamous cell carcinomas tumors) were analyzed for *B-Raf* mutations [54]. High-resolution melting analysis followed by sequencing and strip hybridization assay were used. A total of 37 cases were found to carry on the mutation. *B-raf* mutation was found more commonly in adenocarcinoma patients and V600E mutation was the most common type. This particular mutation was also more prevalent in females (16 of 187 patients; 8.6%) than in males (5 of 552 patients; 0.9%). V600E-mutated tumors showed an aggressive histotype characterized by micropapillary features in 80% of patients and were significantly associated with shorter disease-free and OS. All non-V600E mutations (16 out of 37; 43.2%) were found in smokers (P = 0.015) and were associated with neither clinicopathologic parameters nor prognosis. This study provides clinicopathological insights of this novel mutation in NSCLC [54]. V600E seems to be more common in females and being a poor prognostic factor. Interestingly, the clinicopathologic features described may help us to select patients who are carrying *B-Raf* mutations.

There are multiple B-Raf inhibitors under development. One of the first drug approved for clinical use which is a multi-TKI of Raf-1, B-Raf, vascular endothelial growth factor receptors (VEGFRs)-1, 2, 3, platelet-derived growth factor receptor (PDGFR), and c-Kit was sorafenib [55]. Sorafenib is approved for the treatment of hepatocellular carcinoma and kidney cancer [56,57]. Regarding advanced NSCLC, a phase III trial of first-line chemotherapy alone or in combination with sorafenib (Evaluation of Sorafenib, Carboplatin, And Paclitaxel Efficacy; ESCAPE study) failed to demonstrate OS benefit in the sorafenib arms [58]. Another promising B-Raf inhibitors are in the near horizon such as PLX4032, a small molecule inhibitor selective for B-Raf. Thus far, it has shown an impressive clinical activity in melanoma *B-Raf* mutated Val600Glu with an ORR of 80% and PFS of 7 months [59].

Conclusion

The discovery of multiple and clinical significant gene mutations in NSCLC has completely remodeled our non selective therapeutic concept that "one-size-fits-all". In thoracic oncology, the introduction of molecular pathology was not as fast as in other malignancies. When some of the targeted agents were introduced in lung cancer few years ago, we still relied on clinicopathological features only such as gender, smoking history, and histopathologic subtype. Even today, just the correct histopathologic diagnosis has a crucial impact on treatment selection. Histology, a "rudimentary" biomarker, is the main cornerstone to decide which patient will receive antiangiogenic therapy with bevacizumab (or other new small molecule with antiangiogenic properties) or antifolate therapy with pemetrexed. Thus, it is extremely important that the pathologist and the medical oncologist obtain the most accurate histological subtype. Nonetheless, histology continues to be a major challenge in thoracic oncology and pathology due to the lack of sufficient tumor sampling for histopathological, and now, molecular analysis. Today, when there is doubt in the diagnosis of lung cancer (including "compatible with non-small cell lung cancer), we strongly recommend a re-biopsy to obtain more tissue for analysis and Classification in a specific histologic subtype and genetic testing matter now.

This manuscript has highlighted the two genetic abnormalities which have already become standard of care in the management of NSCLC as three therapeutic targeted agents are approved for these genetic abnormalities; erlotinib and gefitinib for those tumors which harbor *EGFR* mutations and crizotinib for those who carry the EML-4/ALK translocation. We have also discussed other tumorigenesis

pathways which look very promising to be considered into this molecular identification process in the near future. PIK3CA, B-Raf and Met are potential targets which are now in the clinical arena due to the efficacy of novel agents being developed specifically for these overexpressed or amplified or altered pathways. There is no question that all these recent genetic discoveries have demonstrated the complex network of lung carcinogenesis. As we recently changed the lung cancer staging, we also ask ourselves if a molecular classification should also be incorporated into the equation for not only diagnosis but also for staging and treatment algorithms.

To date, there are multicenter, organizations, and international efforts to study the human genome and specifically the phenotype of lung cancer through the implementation of mass scale mutation analyses. Moreover, new laboratory techniques are allowing us to perform more precise and sensitive tests including genetic profile, mass assisted laser desorption ionization (MALDI), and others. To succeed in this task, we need to obtain, handle, and store (for future studies) an adequate amount of tumor tissue in each patient. Therefore, it is important to have a multidisciplinary team of pulmonologists, pathologists, and oncologists, and to create algorithm and guidelines that help us not only to serve our patients with the best clinicopathological data but also to interface and collaborate with national and international efforts.

References

1. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, et al. (2005) Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 353: 123-132.
2. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, et al. (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361: 947-957.
3. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, et al. (2009) Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 361: 958-967.
4. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, et al. (2007) Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 448: 561-566.
5. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, et al. (2009) Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 27: 4247-4253.
6. Rusch V, Baselga J, Cordon-Cardo C, Orazem J, Zaman M, et al. (1993) Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. Cancer Res 53: 2379-2385.
7. Brabender J, Danenberg KD, Metzger R, Schneider PM, Park J, et al. (2001) Epidermal growth factor receptor and HER2-neu mRNA expression in non-small cell lung cancer Is correlated with survival. Clin Cancer Res 7: 1850-1855.
8. Hirsch FR, Varella-Garcia M, Bunn PA Jr, Di Maria MV, Veve R, et al. (2003) Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 21: 3798-3807.
9. Sordella R, Bell DW, Haber DA, Settleman J (2004) Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 305: 1163-1167.
10. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, 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.
11. Pao W, Miller V, Zakowski M, Doherty J, Politi K, et al. (2004) EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A 101: 13306-13311.
12. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, et al. (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304: 1497-1500.
13. Marchetti A, Martella C, Felicioni L, Barassi F, Salvatore S, et al. (2005) EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. J Clin Oncol 23: 857-865.
14. Sharma SV, Bell DW, Settleman J, Haber DA (2007) Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer 7: 169-181.
15. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, et al. (2005) Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2: e73.
16. Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, et al. (2005) Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 97: 643-655.
17. Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, et al. (2005) Erlotinib in lung cancer - molecular and clinical predictors of outcome. N Engl J Med 353: 133-144.
18. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, et al. (2005) Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: Results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 36: 1527-1537.
19. Crinò L, Cappuzzo F, Zatloukal P, Reck M, Pesek M, et al. (2008) Gefitinib versus vinorelbine in chemotherapy-naive elderly patients with advanced non-small-cell lung cancer (INVITE): a randomized, phase II study. J Clin Oncol 26: 4253-4260.
20. Jean-Yves o, Edward K, Vera H, Tony M, Mark S (2007) Gefitinib (Iressa) versus docetaxel in patients with locally advanced or metastatic non-small cell lung cancer pre-treated with platinum based chemotherapy: a randomized, open-label phase III study (INTEREST). J Thorac Oncol 2:S305-306.
21. Hirsch FR, Varella-Garcia M, Dziadziuszko R, Xiao Y, Gajapathy S, et al. (2008) Fluorescence in situ hybridization subgroup analysis of TRIBUTE, a phase III trial of erlotinib plus carboplatin and paclitaxel in non-small cell lung cancer. Clin Cancer Res 14: 6317-6323.
22. Varella-Garcia M, Diebold J, Eberhard DA, Geenen K, Hirschmann A, et al. (2009) EGFR fluorescence in situ hybridisation assay: guidelines for application to non-small-cell lung cancer. J Clin Pathol 62: 970-977.
23. Pirker R, Herth FJ, Kerr KM, Filipits M, Taron M, et al. (2010) Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. J Thorac Oncol 5: 1706-1713.
24. Horn L, Pao W (2009) EML4-ALK: honing in on a new target in non-small-cell lung cancer. J Clin Oncol 27: 4232-4235.
25. Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, et al. (2008) EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 14: 4275-4283.
26. Yoshida A, Tsuta K, Nakamura H, Kohno T, Takahashi F, et al. (2011) Comprehensive histologic analysis of ALK-rearranged lung carcinomas. Am J Surg Pathol 35: 1226-1234.
27. McLeer-Florin A, Moro-Sibilot D, Melis A, Salameire D, Lefebvre C, et al. (2012) Dual IHC and FISH Testing for ALK Gene Rearrangement in Lung Adenocarcinomas in a Routine Practice: A French Study. J Thorac Oncol 7: 348-354.
28. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, et al. (2010) Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 363: 1693-1703.
29. Trusolino L, Bertotti A, Comoglio PM (2010) MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol 11: 834-848.
30. Mahtouk K, Tjin EP, Spaargaren M, Pals ST (2010) The HGF/MET pathway as

target for the treatment of multiple myeloma and B-cell lymphomas. Biochim Biophys Acta 1806: 208-219.

31. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF (2003) Met, metastasis, motility and more. Nat Rev Mol Cell Biol 4: 915-925.

32. Danilkovitch-Miagkova A, Zbar B (2002) Dysregulation of Met receptor tyrosine kinase activity in invasive tumors. J Clin Invest 109: 863-867.

33. Mitra AK, Sawada K, Tiwari P, Mui K, Gwin K, et al. (2011) Ligand-independent activation of c-Met by fibronectin and (5)β(1)-integrin regulates ovarian cancer invasion and metastasis. Oncogene 30: 1566-1576.

34. Zhou C, Wu Y-L, Chen G, et al (2010) Efficacy results from the randomized phase III OPTIMAL (CTONG 0802) study comparing first-line erlotinib versus carboplatin (CBDCA) plus gemcitabine (GEM) in Chinese advanced non-small cell lung cancer (NSCLC) patients with EGFR activating mutations. Presented at: 35th European Society of Medical Oncology Congress. Milan, Italy (abstr LBA13).

35. Rosell R, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. (2011) Erlotinib versus chemotherapy (CT) in advanced non-small cell lung cancer (NSCLC) patients (p) with epidermal growth factor receptor (EGFR) mutations: Interim results of the European Erlotinib Versus Chemotherapy (EURTAC) phase III randomized trial. Presented at: 2011 American Society of Clinical Oncology Meeting. Chicago, IL, USA (abstr 7503).

36. Spigel DR, Ervin TJ, Ramlau R, Daniel DB, Goldschmidt JH, et al (2011) Final efficacy results from OAM4558g, a randomized phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. Presented at: 2011 American Society of Clinical Oncology Meeting. Chicago, IL, USA (abstr 7505).

37. Qi J, McTigue MA, Rogers A, Lifshits E, Christensen JG, et al. (2011) Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors. Cancer Res 71: 1081-1091.

38. Spigel D, Ervin T, Ramlau R, et al (2011) Final Efficacy Results from a Randomized Phase II Study (OAM4558g) Evaluating MetMAb or Placebo in Combination with Erlotinib in Advanced NSCLC. Presented at: 14th World Conference on Lung Cancer. Amsterdam, Netherlands (abstr O19.03).

39. Jimenez C, Jones DR, Rodríguez-Viciana P, Gonzalez-García A, Leonardo E, et al. (1998) Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3-kinase. EMBO J 17: 743-753.

40. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, et al. (2004) High frequency of mutations of the PIK3CA gene in human cancers. Science 304: 554.

41. Kawano O, Sasaki H, Endo K, Suzuki E, Haneda H, et al. (2006) PIK3CA mutation status in Japanese lung cancer patients. Lung Cancer 54: 209-215.

42. Kang S, Bader AG, Vogt PK (2005) Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. Proc Natl Acad Sci U S A 102: 802-807.

43. Ikenoue T, Kanai F, Hikiba Y, Obata T, Tanaka Y, et al. (2005) Functional analysis of PIK3CA gene mutations in human colorectal cancer. Cancer Res 65: 4562-4567.

44. Okudela K, Suzuki M, Kageyama S, Bunai T, Nagura K, et al. (2007) PIK3CA mutation and amplification in human lung cancer. Pathol Int 57: 664-671.

45. Angulo B, Suarez-Gauthier A, Lopez-Rios F, Medina PP, Conde E, et al. (2008) Expression signatures in lung cancer reveal a profile for EGFR-mutant tumours and identify selective PIK3CA overexpression by gene amplification. J Pathol 214: 347-356.

46. Kawano O, Sasaki H, Okuda K, Yukiue H, Yokoyama T, et al. (2007) PIK3CA gene amplification in Japanese non-small cell lung cancer. Lung Cancer 58: 159-160.

47. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, et al. (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med 14: 1351-1356.

48. Shapiro G, Kwak E, Baselga J, J. Rodon, C. Scheffold, et al. (2009) Phase I dose-escalation study of XL147, a PI3K inhibitor administered orally to patients with solid tumors. J Clin Oncol 27:15s (abstr 3500).

49. Leicht DT, Balan V, Kaplun A, Singh-Gupta V, Kaplun L, et al. (2007) Raf kinases: function, regulation and role in human cancer. Biochim Biophys Acta 1773: 1196-1212.

50. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, et al. (2002) Mutations of the BRAF gene in human cancer. Nature 417: 949-954.

51. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, et al. (2008) Somatic mutations affect key pathways in lung adenocarcinoma. Nature 455: 1069-1075.

52. Naoki K, Chen TH, Richards WG, Sugarbaker DJ, Meyerson M (2002) Missense mutations of the BRAF gene in human lung adenocarcinoma. Cancer Res 62: 7001-7003.

53. Sasaki H, Kawano O, Endo K, Suzuki E, Haneda H, et al. (2006) Uncommon V599E BRAF mutations in Japanese patients with lung cancer. J Surg Res 133: 203-206.

54. Marchetti A, Felicioni L, Malatesta S, Grazia Sciarrotta M, Guetti L, et al. (2011) Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. J Clin Oncol 29: 3574-3579.

55. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, et al. (2004) BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 64: 7099-7109.

56. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, et al. (2009) Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. J Clin Oncol 27: 3312-3318.

57. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. (2008) Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 359: 378-390.

58. Scagliotti G, Novello S, von Pawel J, Reck M, Pereira JR, et al. (2010) Phase III study of carboplatin and paclitaxel alone or with sorafenib in advanced non-small-cell lung cancer. J Clin Oncol 28: 1835-1842.

59. Tsai J, Lee JT, Wang W, Zhang J, Cho H, et al. (2008) Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci U S A 105: 3041-3046.

This article was originally published in a special issue, **The Biology and Molecular Pathology of Lung Cancer: Implications for Treatment** handled by Editor(s). Dr. Katerina Politi, Yale University, USA; Dr. Guoping Cai, Yale University, USA

Submit your next manuscript and get advantages of OMICS Group submissions

Unique features:

User friendly/feasible website-translation of your paper to 50 world's leading languages
Audio Version of published paper
Digital articles to share and explore

Special features:

200 Open Access Journals
15,000 editorial team
21 days rapid review process
Quality and quick editorial, review and publication processing
Indexing at PubMed (partial), Scopus, DOAJ, EBSCO, Index Copernicus and Google Scholar etc
Sharing Option: Social Networking Enabled
Authors, Reviewers and Editors rewarded with online Scientific Credits
Better discount for your subsequent articles

Submit your manuscript at: http://www.omicsonline.org/submission