Driver mutations as predictive biomarkers in lung cancer

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Abstract Treatment decisions for patients with lung cancer have historically been based upon tumor histology. Among the numerous molecular alterations observed in cancer cells, some specific mutations have been identified as being necessary and sufficient to drive tumor formation and maintenance. These “driver” mutations occur in genes that encode signaling proteins critical for cellular proliferation and survival. With the development of agents specifically designed to target these key proteins, the paradigm is changing to “personalized medicine,” which consists of the individual genotyping of each patient tumor to identify driver mutations that are predictive biomarkers of the efficacy of these agents. This paper reviews the clinical and biologic evidence for the major driver mutations occurring in lung cancer.

Keywords Lung cancer · Chemotherapy · Targeted therapy · Anaplastic lymphoma kinase · Kinase · Driver mutation · *EGFR* · *ALK* · *HER2* · *BRAF* · *KRAS* · *PIK3CA* · *AKT1* · *MEK1* · *MET* · *DDR2* · *FGFR1*

Introduction

Lung cancer is the most frequent cause of cancer-related death worldwide, accounting for more than 1 million deaths per year [1]. Lung cancer has been historically divided into two major histologic groups: small cell lung cancer (SCLC; 15% of cases) and non-small cell lung cancer (NSCLC; 85% of cases) [2]. NSCLC is composed of three major different types: adenocarcinoma, squamous cell carcinoma (SCC), and large cell carcinoma. Adenocarcinoma now accounts for more than 50% of all cases of lung cancer. Until recently, treatment of NSCLC was confined to surgery, radiotherapy, and chemotherapy. Optimization, including histology-driven decision-making of regimen [3], adjunction of the anti-angiogenic agent bevacizumab [4], and development of maintenance strategies [5], has marginally improved median survival of patients with metastatic tumor, ranging from 12 to 15 months in recent trials. Several molecular alterations have been reported to influence the prognosis of patients receiving chemotherapy, especially in the adjuvant setting [6]; however, these prognostic features are currently not included in the standard treatment strategy algorithm, in the absence of prospective validation. Overall, long-term survival remains lower than 15% in routine clinical care. Novel treatment strategies are needed.

Over the past decade, major progresses have been achieved in the understanding of the molecular bases of lung cancer. Data from genomic [7•], expression [8•], mutational [9•, 10], and proteomic profiling studies [11] have been regarded for a long time by many clinicians as being obscure and out of touch with the overall poor reality of cancer care. Among the numerous molecular alterations observed in cancer cells, some specific mutations have been identified as being necessary and sufficient to drive tumor formation and maintenance (Fig. 1, Table 1) [12]. These

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Fig. 1 Known driver mutations in lung adenocarcinoma **a** and squamous cell carcinoma **b**. The mutations are mutually exclusive except for *PIK3CA*. (Data adapted from Ding et al. [9••], Hammerman et al. [10], and Pao et al. [12])

“driver” mutations occur in genes that encode signaling proteins critical for cellular proliferation and survival. Cancers may then rely upon the expression of these single mutant oncogenes for survival, even in the absence of other alterations, a concept referred to as “oncogene addiction” [13]. With the development of agents specifically designed to target these key proteins, the paradigm is changing from the traditional view of chemotherapy, which, even if binding specific proteins, aims at destroying cancer cells, to the potential use of targeted agents to specifically inhibit the causative oncogenic process by itself.

Over the past years, the systematic identification and targeting of driver mutations has become the leading challenge for the treatment of lung cancer, formalizing the concept of “personalized medicine,” which consists of the individual genotyping of each patient tumor to identify driver mutations that are predictive biomarkers of the efficacy of specific targeted agents.

Epidermal growth factor receptor mutations

Epidermal growth factor receptor activating mutations

Epidermal growth factor (EGF) receptor (EGFR) mutations are the best illustration of the therapeutic relevance of identifying molecular clusters of NSCLC based on driver genetic

alterations. Epidermal growth factor receptor is a transmembrane receptor harboring an intracytoplasmic tyrosine kinase domain that undergoes autophosphorylation after extracellular binding of a ligand, such as EGF, leading to homo-dimerization or hetero-dimerization with other HER family receptors [14••]. Following EGFR phosphorylation, key kinase-binding domains of downstream signaling proteins are activated, that control multiple cellular processes, including proliferation and survival [14••].

EGFR inhibitors have been developed along the clinical lines. The leading agents are orally available reversible, ATP-competitive inhibitors of the EGFR kinase: gefitinib (Iressa; AstraZeneca, Macclefields, UK) and erlotinib (Tarveva; Roche, Basel, Switzerland). Following promising phase 1 and 2 studies, gefitinib was compared to placebo in 1692 patients with refractory NSCLC [15]. A statistically significant difference in survival was not observed (5.6 vs 5.1 months, $P=0.11$).

At the same time clinical trials were ongoing, several groups took different approaches to identify predictors of response to EGFR tyrosine kinase inhibitors (TKIs). One approach hypothesized that patients who experienced striking responses to these agents had somatic mutations in *EGFR* that would indicate the essential role of the EGFR signaling pathway in the tumor [16]. Another approach involved high-throughput re-sequencing of exons encoding the kinase domains of several receptor tyrosine kinases, to

Table 1 Genes with driver mutations in non-small cell lung cancer: differences by histology

Mutation	Adenocarcinoma	Squamous cell carcinoma
<i>KRAS</i>	15%–25%	<5%
<i>EGFR</i> *	5%–15%	5%–10%
<i>ALK</i>	5%–10%	<5%
<i>HER2</i>	<5%	0%
<i>BRAF</i>	<5%	0%
<i>PIK3CA</i>	<5%	<5%
<i>MEK1</i>	<5%	0%
<i>MET</i>	<5%	<5%
<i>AKT1</i>	0%	<5%
<i>DDR2</i>	0%	4%
<i>PDGFR</i>	0%	5%–10%
<i>FGFR2</i>	<5%	5%–10%
<i>SOX2</i> **	0%	15%–25%
<i>FGFR1</i> **	0%	15%–25%

*EGFR kinase domain mutations are found predominantly in adenocarcinoma, while EGFR “vIII” mutations (that result from deletion of exons 2–7) are found predominantly in squamous cell carcinoma. Amplification may be identified in both histologies.

**Amplification.

determine whether mutations in specific kinases played a causal role in NSCLC [17]. Investigators focused their analyses on patients with clinical characteristics predictive of response to EGFR inhibitors—a so-called “clinical enrichment” strategy [18, 19•]: Asians, never-smokers, women, adenocarcinoma tumors with bronchioloalveolar features. These studies demonstrated that somatic mutations in the *EGFR* kinase domain were present in a subset of NSCLCs, and were predictive of sensitivity to EGFR TKIs (Table 1, Fig. 1) [16, 17, 20•]. Most frequent *EGFR* mutations arise within the first 4 exons (exons 18 to 21) encoding the tyrosine kinase domain, and include in-frame deletions within exon 19 (codons 746–750; 44% of cases), and L858R point mutations within exon 21 (41% of cases).

These mutations confer ligand-independent activation and prolonged receptor kinase activity after ligand stimulation, and are sufficient for in vitro oncogenic transformation of fibroblasts and lung epithelial cells [20•, 21]. Additionally, tetracycline-regulatable mouse model systems indicate that expression of either *EGFR* exon 19 deletions or L858R alleles in mouse lung epithelia leads to formation of tumors [22•]. These experiments demonstrate the driver nature of *EGFR* mutations.

Back to the clinical side, four prospective randomized phase 3 trials are now available that all demonstrated that *EGFR*-activating mutations are the best predictor of efficacy of EGFR TKIs. These studies were conducted in patients with previously untreated metastatic *EGFR*-mutant NSCLC, and compared gefitinib or erlotinib to standard chemotherapy [23–26]. In these studies, contrary to trials conducted in unselected populations, response rates with EGFR TKIs ranged from 55% to 83%. Median progression-free survival was significantly higher in the experimental arms (9.2–13.0 months) than in the chemotherapy arms (4.6–6.3 months). Median overall survival reached unprecedented values, higher than 27.0 to 30.5 months.

Given the major implications of these results for routine clinical practice, *EGFR* genotyping is now recommended as part of standard care to customize treatment with EGFR TKIs [27]. The Spanish group reported the feasibility of large-scale real-time screening of 2105 patients with lung cancer, 350 of whom had *EGFR* mutant tumors and 113 received treatment with erlotinib [28]. Median progression-free and overall survival rates were strikingly similar to that reported in clinical trials, 14.0 and 27.0 months respectively.

Acquired resistance to EGFR TKIs

Besides *EGFR* non-activating mutations, such as exon 20 insertions and duplications, that are associated with limited response to EGFR TKIs [29], and molecular alterations, that may modulate the efficacy of EGFR TKIs, including PTEN loss, IGF-1R overexpression, and *PIK3CA* mutations (see

below) [14•], acquired resistance to EGFR TKIs represents a major challenge in the management of *EGFR*-mutant lung cancer [30]. Analyses of tumor tissue from patients exhibiting tumor progression after initial response to these agents have revealed the presence of additional second-site *EGFR* mutations, the most frequent being the *EGFR* T790M point mutation in exon 20 [31, 32], [33•]. The T790M mutation, while blocking the binding of erlotinib or gefitinib to the kinase ATP binding pocket, also causes drug resistance by increasing the affinity of the kinase domain for ATP [34]. The emergence of T790M mutation may involve a pre-existing T790M clone, that becomes prominent after apoptosis of EGFR TKIs sensitive cells [35•]. Paradoxically, the T790M mutation may occur in vitro after repeated exposure of *EGFR*-mutant cells to EGFR TKIs [36].

Understanding the basis for acquired resistance has led to the identification of agents that may overcome acquired resistance. The presence of second-site mutations was thought to predict the efficacy of such “second-generation” EGFR inhibitors. Afatinib (BIBW2992; Boehringer-Ingelheim, Ingelheim am Rhein, Germany) is the “irreversible” EGFR TKI whose clinical development is the most advanced. Afatinib also targets HER2, and has demonstrated its potency to overcome T790M-mediated acquired resistance to erlotinib and gefitinib in vitro and in vivo [37]. However, the LUX-LUNG1 trial comparing afatinib to placebo in 585 patients with refractory NSCLC, without integrating *EGFR* genotyping in the inclusion criteria, did not reported statistically convincing results, even in the subgroup of tumors most likely to harbor *EGFR*-activating mutations [38]. Following promising in vivo results in mice, combination of afatinib with cetuximab was recently evaluated in a phase 2 trial, including 47 patients with *EGFR*-mutant tumor, presenting with acquired resistance, 27 of which associated with T790M second-site mutations [39•, 40]. Disease control was observed in 92% of these patients, a promising figure for future phase 3 trials with this combination. Again, these data show the importance of using predictive driver mutations to select patients for specific targeted therapies.

Another mechanism of acquired resistance may involve a “kinase switch,” the most studied example being *MET* amplification. *MET* is a receptor tyrosine kinase also known as the hepatocyte growth factor (HGF) receptor (HGFR) [41, 42]. The *MET* gene is located on chromosome 7q21–q31. Collectively, *MET* amplification has been found in about 20% of samples from patients with acquired resistance, with or without *EGFR* T790M mutations. As described below, *MET* activation may predict efficacy of *MET* inhibitors.

Fibroblast growth factor receptor-1 mutations

Similar to EGFR, fibroblast growth factor receptor-1 (FGFR1) is a transmembrane tyrosine kinase receptor,

stimulating cell proliferation and survival. The *FGFR1* gene is located in 8p12 region. *FGFR1* was recently demonstrated to harbor focal amplification in 15 (9.7%) of 155 SCC tumors included in a large-scale single nucleotide polymorphism array genomic analysis [43•]. Using fluorescent in situ hybridization (FISH) with a more sensitive cutoff to define amplification, focal *FGFR1* amplification was observed in 22% of cases in an independent cohort of 153 SCC tumors (Table 1, Fig. 1). Contrary to *EGFR* amplification, *FGFR1* amplification is exceptionally associated with activating *FGFR1* mutations. *FGFR1* amplification is exclusively observed in smokers and SCC tumors

Biologically, *FGFR1* amplification leads to FGFR1 activation. In vitro studies demonstrated the driver nature of *FGFR1* amplification, which predicted the activity of PD173074 (TGen, Phoenix, AZ), a FGFR-specific TKI. Similar to *EGFR* T790M mutations, the ectopic expression of the *FGFR1* V561M mutation, designed to block the FGFR1 ATP binding pocket, led to resistance to this drug. Taken together, these data suggest that *FGFR1* amplification represents an exploitable target for SCC tumors, that predicts the efficacy of FGFR inhibitors. Such agents, including BIBF1120 (Boehringer-Ingelheim), are currently tested in phase 1/2 trials.

Anaplastic lymphoma kinase fusions

The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase, normally not expressed in the lung [44•]. Fusions of ALK with another upstream partner, the echinoderm microtubule-associated protein-like 4 (*EML4*), were recently found in NSCLC (Table 1, Fig. 1) [45•]. *EML4-ALK* fusions result from diverse small inversions within the short arm of chromosome 2 [45•, 46, 47•]. At least nine different variants have been identified [47•]. Biologically, *EML4-ALK* fusions result in protein oligomerization and constitutive activation of the kinase. In vitro, the fusions demonstrate gain-of-function properties [45•, 46]. Mice expressing an *EML4-ALK* variant under the control of a lung epithelial cell promoter develop multiple lung adenocarcinoma tumors [48].

The frequency of *EML4-ALK* translocation ranges from 3% to 7% in unselected NSCLC cohorts [45•, 46, 47•]. It has been associated with acinar, signet-ring variant, adenocarcinoma histology. The reference detection method is FISH, as reverse-transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry may lack sensitivity and specificity. Similar to *EGFR* mutations, *EML4-ALK* fusions are more frequently found in adenocarcinomas, in younger patients, and in never and light smokers [46, 47•]. *EML4-ALK* translocations are usually found in tumors wild-type for *EGFR* and *KRAS* [9•], and ALK-positive lung tumors are resistant to treatment with EGFR TKIs.

EML4-ALK fusions are strongly predictive of response to pharmacologic ALK inhibition. Crizotinib (Xalkori; Pfizer, New York, NY) is a small-molecule TKI with sub-micromolar activity against ALK and MET. Contrary to EGFR TKIs, the drug was directly evaluated in tumors harboring *EML4-ALK* fusion. After screening 1500 patients for the fusion, 82 patients were enrolled in the landmark phase 1 trial with crizotinib [49]. Overall, objective response and stable disease rates were 57% and 33%, respectively. The estimated probability of 6-month progression-free survival was 72%. These results were recently confirmed in a phase 2 trial [50], and phase 3 trials are ongoing to compare crizotinib to standard chemotherapy in *EML4-ALK*-rearranged tumors. Crizotinib is already approved in the US for *EML4-ALK*-rearranged tumors.

EML4-ALK translocations are also predictors of the efficacy of heat shock protein 90 (HSP90) inhibitors [51]. *EML4-ALK* associates in complex with multiple cellular chaperones including HSP90. Inhibitors of HSP90 disrupt this complex, lead to degradation of *EML4-ALK* fusion protein, and produce tumor regression in vitro and in vivo. As for *EGFR*, acquired resistance to crizotinib has been associated with *ALK* secondary mutations, involving both the “gatekeeper” residue (L1196M mutation) of the ATP binding pocket, and residues away from the kinase domain [44•, 51]. Cell lines harboring these mutations remain equally sensitive to HSP90 inhibitors compared to ones without the secondary mutations.

Discoidin domain receptor-2 mutations

Discoidin domain receptor-2 is another transmembrane tyrosine kinase receptor, which binds collagen as its endogenous ligand, and is implicated in cell proliferation and migration. The *DDR2* gene is located in region 1q23 and was found to harbor activating mutations in a subset (3.2%) of SCC lung tumors (Table 1, Fig. 1) [52•]. These mutations are not identified in other histologies, and are widely distributed on the gene, occurring both in the kinase domain and in other regions of the protein sequence. In vitro and xenograft studies demonstrated the oncogenic potency of the most frequent mutations (L239R and I638F), that were associated with gain-of-function of the receptor kinase activity and were transforming when introduced in fibroblast and epithelial cell lines.

DDR2 mutations were found to be a strong predictor of efficacy for dasatinib (Sprycel; Bristol-Myers Squibb, New York, NY), a multikinase inhibitor with potent activity on SRC-family kinases, nonreceptor tyrosine kinases, and receptor tyrosine kinases (including Ephrin receptors, *DDR1*, and *EGFR*), both in vitro and in vivo in xenograft studies.

Clinically, one observation of a patient with SCC lung tumor harboring a *DDR2* S768R mutation was reported;

partial response was observed after treatment with dasatinib, what further supports the use of these mutations as a predictive biomarker in this setting. A phase 2 trial with dasatinib is ongoing.

MET amplification and mutations

MET amplification, besides being found in tumors showing acquired resistance to EGFR TKIs, may be identified in EGFR TKI-naïve NSCLC tumors, at a frequency ranging from 1.4% to 21% (Table 1, Fig. 1) [53–55]. Such variability may be related to the method and cutoff used to define *MET* amplification. *MET* amplification occurs both in SCC and adenocarcinoma [54]. In adenocarcinoma, *MET* amplification, *KRAS* mutations, and *EGFR* amplification are independent events [53–55]. In vitro studies showed that *MET* amplification is associated with increased levels of phosphorylated MET. The ability of activated MET to initiate tumorigenesis has been shown in transgenic mouse models with ectopic expression of the activated receptor [56]. These data suggest that *MET* amplification is necessary and sufficient to drive the proliferation of cancer cells.

Mutations in the kinase domain of *MET* are rare in NSCLC. In a cohort of 188 lung adenocarcinomas, only three somatic *MET* mutations were identified, two in exon 13 encoding the juxtamembrane domain (R988del and Y1021N) and one in exon 18 encoding the kinase domain (G1260C) [9•]. The biological significance of these mutations is currently unknown. In addition, an intronic splice variant leading to exon 14 deletions has also been reported in about 2%–3% of NSCLC tumors in Japanese cohorts [53, 57]. Preliminary data indicate that this mutation leads to delayed receptor down-regulation and enhanced ligand-mediated proliferation [57].

Specific small-molecule MET inhibitors are being developed [58]. METMAb, a monovalent monoclonal antibody that specifically binds the MET receptor, has been evaluated in combination with erlotinib versus erlotinib alone as second-line and third-line treatment in unselected patients with NSCLC [59•]. In this study, MET expression level at immunohistochemistry was a significant predictor of survival benefit.

HER2 mutations

Like EGFR, HER2 (ERBB2/human EGFR related (HER)-2) protein is a member of the HER family of receptor tyrosine kinases. HER2 has no identified ligand and forms homodimers or hetero-dimers with other members of the HER family [60]. The protein is overexpressed in about 20% of NSCLCs, but gene amplification occurs in only 2% of cases [61]. Early clinical trials with trastuzumab, a humanized anti-HER2 monoclonal antibody that is active in HER2-

amplified breast cancer, have reported modest or disappointing effects in unselected NSCLC [62]. Interestingly, *HER2* mutations are present in 2% of NSCLCs (Table 1, Fig. 1) [61]. The mutations involve in-frame insertions in exon 20, mostly in the amino acid sequence YVMA at codon 776. Similar to *EGFR* mutations, *HER2* mutations are more frequent in never smokers, females, Asian patients, and those with adenocarcinoma. *HER2* mutations are not present in tumors harboring *EGFR* or *KRAS* mutations.

Biologically, *HER2* insertions lead to constitutive activation of the receptor. Transgenic mice expressing the *HER2* YVMA mutant develop lung adenosquamous carcinomas; in these models, significant tumor shrinkage was observed when combining afatinib with rapamycin, an inhibitor of the downstream effector mammalian target of rapamycin (mTOR) [63]. Consistent with this, a trial using afatinib has shown promising activity in patients with *HER2* mutant lung adenocarcinoma [64].

Non-receptor kinase mutations

KRAS mutations

KRAS is a GTPase normally tethered at the cell membrane that is activated by growth factor transmembrane receptors, such as EGFR, HER2, ALK, MET, and FGFR. Point mutations involving amino acids 12, 13, and 61 lead to impaired GTPase activity, resulting in constitutive activation of downstream signaling cascades [65]. *KRAS* mutations are found in 20%–30% of lung adenocarcinoma tumors in Caucasian patients but are rarer in Asian populations (Table 1, Fig. 1) [65, 66•, 67]. These mutations are mutually exclusive with other driver mutations, and are more frequent in smokers. Mice expressing an inducible mutant *KRAS* in lung epithelial cells develop lung adenocarcinoma tumors that regress after silencing of the transgene [68•].

Unfortunately, no effective targeted agents have yet been developed against mutant *KRAS*. An obstacle to the development of specific RAS inhibitors is that mutant *RAS*, which has gained constitutive activity, has lost its normal enzymatic function. Sorafenib (Nexavar; Bayer, Leverkusen, Germany) is a BRAF inhibitor, with multikinase inhibition potential against Raf1, BRAF, vascular endothelial growth factor (VEGF) receptor (VEGFR)-1, -2, and -3, platelet-derived growth factor receptor (PDGFR), CD117/KIT, and receptor tyrosine kinase (RET). Given the direct interaction of RAS with RAF, sorafenib has been evaluated, after failure of platinum-based chemotherapy, in 57 patients with *KRAS*-mutant NSCLC [69]. Overall, disease control was observed in 53% of patients after 6 weeks of treatment; progression-free survival was however limited (2.3 months). A more promising approach may involve combined PI3K and MEK inhibition, which both are downstream partners of RAS [69].

PIK3CA mutations

Phosphatidylinositol 3-kinases (PI3K) are lipid kinases that regenerate phosphatidylinositol-3-phosphate, a key mediator between growth factor receptors and intracellular downstream signaling pathways [71]. The main catalytic subunit of PI3K is the p110 α isoform which is encoded by the *PIK3 catalytic subunit alpha* (PIK3CA) gene. Mutations in *PIK3CA* occur in about 2% of cases (Table 1, Fig. 1) [72], affecting residues E542 and E545 in exon 9 encoding the catalytic domain [9•, 72, 73]. These mutations are as frequent in SCC as in adenocarcinoma, and, contrary to other driver mutations that are mutually exclusive, may occur in *EGFR*-mutant tumors [73]. Mutant PIK3CAs display gain of enzymatic function in vitro and may induce oncogenic cellular transformation [74]. GDC-0941 (Genentech, South San Francisco, CA) is a specific PIK3CA inhibitor that is being tested in a phase 2 trial following a favorable safety profile in phase 1 trials [75].

AKT mutations

Protein kinase B (AKT1) is a serine-threonine kinase that is activated by PIK3CA and mediates PI3K signaling [76]. A major recurrent *AKT1* mutation (E17K) has been identified in several solid tumors, including breast, colon, and ovarian cancer [77]. Collectively, the incidence of *AKT1* mutations in NSCLC is about 1% (Table 1, Fig. 1). These mutations have only been identified in SCC tumors and mostly in cohorts of Western patients [77].

The E17K change occurs in the AKT pleckstrin homology domain. This mutation alters the phosphoinositide-binding pocket, is transforming in vitro, and leads to PI3K-independent AKT1 activation. The E17K mutation does not alter sensitivity to ATP-competitive AKT inhibitors. MK-2206 (Merck, Whitehouse Station, NJ), an allosteric AKT inhibitor, is being tested in phase 1 trials.

BRAF mutations

BRAF (V-raf murine sarcoma viral oncogene homolog B1) is a serine/threonine kinase linking RAS GTPases to downstream proteins of the MAPK family that control cell proliferation [78]. In NSCLC, *BRAF* mutations are found in 1%–3% of tumors, mostly of adenocarcinoma subtype (Table 1, Fig. 1) [9•, 79, 80]. Contrary to melanomas, NSCLC mostly harbor non-V600 mutations (88% of cases), including the L596V mutation in the kinase domain and the G468A in the G loop of the activation domain [79, 80]. *BRAF* mutations are mutually exclusive with *EGFR* and *KRAS* mutations. Biologically, *BRAF* mutations display elevated kinase activity and lead to constitutive activation of extracellular signal-regulated kinase (ERK) 1/2.

Sorafenib has been evaluated in NSCLC mostly as an anti-angiogenic agent in combination with chemotherapy [81]. Other inhibitors are currently evaluated specifically in *BRAF*-mutant NSCLC, such as GSK2118436 (GlaxoSmithKline, London, UK).

MEK mutations

MEK1 is a serine-threonine kinase downstream of BRAF, that activates ERK1/2 [82]. In cancers, at least three mutations have been found in *MEK1*, including Q56P, K57N, and D67N [83]. All occur in the non-kinase portion of the kinase. Somatic mutations in *MEK1* have been identified in 1% of NSCLCs, predominantly adenocarcinomas (Table 1, Fig. 1) [83]. *MEK1* mutations are mutually exclusive with *EGFR*, *KRAS*, *HER2*, *PIK3CA*, and *BRAF* mutations. The K57N and Q56P mutants display gain-of-function properties in vitro [83], and cells harboring these mutations can display sensitivity to the small-molecule non-ATP competitive MEK inhibitor, AZD6244 (AstraZeneca). AZD6244 has been used to treat NSCLC, in a trial comparing the drug to pemetrexed as second-line treatment.

Conclusions

The identification of multiple clinically relevant “driver mutations” in NSCLC has led to an improved understanding of lung cancer pathogenesis. A major hope now is that knowledge of these lesions can be used to improve patient care, as these mutations are the best predictors of efficacy of specific targeted therapies. To this end, multiple academic centers have begun to develop multiplex mutational profiling assays in clinical molecular diagnostic facilities, in order to genotype prospectively patients’ tumors [28•, 84•]. Such effort requires development of multiplex assays [85•], and high-level quality control to ensure consistency in testing methods [86].

Besides a static molecular picture of NSCLC cases at time of diagnosis, systematic re-biopsy of tumors harboring driver mutations and presenting with acquired resistance to specific inhibitors is emerging as a crucial step to identify targetable secondary mutations and possible phenotypic change. In a series of 37 patients with *EGFR*-mutant adenocarcinoma, presenting with acquired resistance to EGFR TKIs, five (14%) tumors transformed into SCLC, and three (8%) tumors into spindle-cell sarcomatoid tumors [33]. Repeated phenotypic and molecular assessment is then mandatory to guide treatment decisions throughout the course of the disease.

To conclude, driver mutations are predictive biomarkers of response to specific targeted therapies. While the identification of *EGFR* mutations was based on empiric clinical

observation of response in a small number of patients in whom mutations were discovered, the current development of targeted agent is directly linked with the early identification of predictive biomarkers leading to select the accurate population to which the drugs is specifically delivered. From bench to bedside, personalized medicine represents a major revolution in the treatment of lung cancer.

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