HHS Public Access

Author manuscript

Mol Aspects Med. Author manuscript; available in PMC 2016 November 01.

Published in final edited form as:

Mol Aspects Med. 2015 November ; 45: 55–66. doi:10.1016/j.mam.2015.06.009.

Biomarkers and Targeted Systemic Therapies in Advanced Non-Small Cell Lung Cancer

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Abstract

The last decade has witnessed significant growth in therapeutic options for patients diagnosed with lung cancer. This is due in major part to our improved technological ability to interrogate the genomics of cancer cells, which has enabled the development of biologically rational anticancer agents. The recognition that lung cancer is not a single disease entity dates back many decades to the histological subclassification of malignant neoplasms of the lung into subcategories of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). While SCLC continues to be regarded as a single histologic and therapeutic category, the NSCLC subset has undergone additional subcategorizations with distinct management algorithms for specific histologic and molecular subtypes. The defining characteristics of these NSCLC subtypes have evolved into important tools for prognosis and for predicting the likelihood of benefit when patients are treated with anticancer agents.

Keywords

Biomarkers; lung cancer; NSCLC; EGFR; ALK; ROS1; RET

1.0 Introduction

The last decade has witnessed significant growth in therapeutic options for patients diagnosed with lung cancer. This is due in major part to our improved technological ability to interrogate the genomics of cancer cells, which has enabled the development of biologically rational anticancer agents. The recognition that lung cancer is not a single disease entity dates back many decades to the histological subclassification of malignant neoplasms of the lung into subcategories of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). While SCLC continues to be regarded as a single histologic and therapeutic category, the NSCLC subset has undergone additional subcategorizations with distinct management algorithms for specific histologic and molecular subtypes. The defining characteristics of these NSCLC subtypes have evolved into important tools for prognosis and for predicting the likelihood of benefit when patients are treated with anticancer agents.

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A discrete and measurable factor, whether in the whole patient or within the neoplastic cancer cells, that provides information on the likelihood of treatment efficacy is termed a predictive biomarker (1, 2). In contrast, a measurable factor that provides information on the overall patient outcome irrespective of treatment intervention is classically considered a prognostic biomarker (1, 2). Various biomarkers have emerged as predictive and prognostic markers in NSCLC patients and are now employed as part of their standard management. Putative biomarkers employed in clinical trials of investigational agents in SCLC, none of which have led to a management-defining paradigm, will be outside the scope of this review. This review will therefore focus on the clinical, histologic and molecular factors that are currently employed to guide the selection of therapeutic options for NSCLC patients.

2.0 Tumor histology as a biomarker in NSCLC

The WHO/IASLC classification of NSCLC includes various subtypes characterized by distinct morphology and immunophenotype (3, 4). The squamous and adenocarcinoma categories represent the two major histologic subtypes of NSCLC. The utility of tumor histology as a biomarker for selecting therapeutic intervention is therefore relevant to this review. The impact of squamous histology as a poor prognostic factor is supported by various retrospective and prospective studies (5, 6). This strategy became an established paradigm following retrospective analysis of outcome data from prospective studies of pemetrexed in unselected NSCLC patients, where a differential efficacy was noted between patients with squamous and non-squamous tumors (7, 8). Prospective comparison of the efficacy of pemetrexed-containing and gemcitabine-containing platinum doublet chemotherapy regimens as first line treatment of advanced NSCLC confirmed the differential efficacy of a pemetrexed-containing doublet by histology (9).

Histology has also served as a surrogate biomarker for increased risk of treatment-related toxicity leading to the avoidance of specific therapeutic agents. The notable example is the increased propensity for squamous tumors, which are more likely to be cavitary and centrally located in close proximity to major blood vessels, to hemorrhage following treatment with agents targeting angiogenesis such as bevacizumab (8). Squamous histology has thus become a biomarker to exclude patients who are unsuitable for anti-angiogenesis therapies. The main drawback with the use of tumor histology as a predictive biomarker in NSCLC is the significant discordance even among expert pulmonary pathologists in establishing a pathologic diagnosis of squamous NSCLC (10). Nonetheless, an algorithm that couples cell morphology and immunophenotype in the hands of an experienced pathologist can overcome this challenge in most cases.

3.0 Genetic alterations as biomarker

The major advance in the treatment of NSCLC in the last decade grew from the recognition that specific genetic alterations define subsets of NSCLC (11). This paved the way for the development of an array of effective agents to specifically counteract the biological consequences of such genetic aberrations. Thus, NSCLC went from a disease defined primarily by tumor histology to an amalgam of molecular subtypes, of which, the subsets

characterized by alterations in the epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) genes are the most dominant.

3.1 Epidermal growth factor receptor (EGFR) gene mutations as a biomarker

The EGFR is a 170-kDa plasma membrane glycoprotein consisting of a large extracellular region, a single transmembrane domain, and an intracellular domain with tyrosine kinase activity and a C-terminal tail. The EGFR family consists of 4 closely related receptors, HER-1/ErbB1, HER-2/neu/ErbB2, HER-3/ErbB3 and HER-4/ErbB4 with significant homology in their kinase domains, but differences in the coding regions for the extracellular domain and the C-terminal tails (12). Dimerization of ErbB receptors upon ligand binding to the extracellular domain results in activation of their intrinsic tyrosine kinase activity. Activation of the EGFR receptor via phosphorylation relays downstream signals to the phosphatidylinositol 3-kinase (PI3K)/AKT and RAS/RAF/mitogen-activated protein kinase (MAPK) pathways. These intracellular signaling pathways that are responsible for the normal regulation of essential cellular processes such as proliferation and apoptosis are coopted by neoplastic cells harboring *EGFR* mutation (12, 13). Mutations in the *EGFR* gene occurring in NSCLC are commonly localized within the tyrosine kinase domain of the gene. Well established mutations include deletions in exon 19 (60%), missense mutation (L858R) in exon 21 (25%), point mutations in exons 18, 20 or 21, and insertion in exon 20 (14–16). These alterations result in constitutive activation of the kinase activity of EGFR and serve as the driver of neoplastic transformation and progression.

3.1.1 EGFR as a prognostic biomarker—Activating mutations in the *EGFR* gene confer a good prognosis on patients whose tumors harbor such alterations (17). In a prospective study that enrolled 647 patients for molecular profiling, 22.1% of NSCLC cases harbored a mutation in the *EGFR* gene. The patients with *EGFR* mutations had a much longer overall survival (OS, 3.51 years; 95% CI, 2.89 to 5.5 years), compared to patients without any detectable mutation (1.85 years; 95% CI, 1.61–2.13 years) (17). Patients with exon 19 deletion type EGFR mutation have also been shown to have significantly longer OS compared to patients with L858R mutation. A retrospective study of 32 patients showed an increased OS (38 *vs* 17 months) in patients with *EGFR* exon 19 deletion compared to patients with L858R mutation (18). Similar observation was made by Riely *et al* who demonstrated a significantly longer OS (34 *vs* 8 months) in patients with exon 19 deletions over those with L858R substitution (19). The prognostic impact of the less common types of *EGFR* mutations involving exon 18 or 20 has not been well studied due to the lower prevalence of these genetic alterations.

3.1.2 Predicting efficacy of EGFR inhibitors—Activating mutations in the gene encoding the EGFR protein are present in approximately 10–20% of NSCLC patients diagnosed in North America and Western Europe, and 30–50% of Asian patients (20). These mutations are commonly found within defined hotspots in the gene namely, exon 18, 19, 20 and 21 (14–16). In addition to its recognized role as a prognostic biomarker, EGFR mutation also reliably predicts the efficacy of EGFR targeted agents in prospective studies comparing cytotoxic chemotherapy to biologic agents targeting the activated kinase function of mutant EGFR. Consistent with the better prognosis associated with exon 19 deletion mutation,

EGFR inhibitor therapy is also associated with superior efficacy in patients with exon 19 deletion compared to those with exon 21 alterations. It is noteworthy that the performance of different *EGFR* mutations as a predictive biomarker of treatment efficacy varies. For instance, while exon 19 and 21 alterations are generally sensitizing, rarer alterations involving exon 18 and 20 may or may not be. Particularly, exon 20 insertion, which is a rare subtype at 4% of all *EGFR* mutations but represents the third most common *EGFR* mutation, has approximately 122 different variants most of which are not responsive to currently available kinase inhibitors (21). A retrospective study of 23 Korean patients with exon 20 insertion reported objective response in only four out of 16 (25%) patients treated with gefitinib. Median OS of the responding patients was 23 months *vs* 7 months for the non-responders. Eight of the 16 (50%) treated patients had concurrent mutations including alterations in exons 21 and 18. Of note, different exon 20 mutations and other coexisting mutations appeared to have a different role on treatment response (22). Another retrospective study identified 27 patients with exon 20 insertion and the most common variant described was (V769_D770insASV), accounting for 22%. The median OS was 16 months (20). Thus, patients with exon 20 insertion have survival rates similar to those seen in EGFR wild-type NSCLC. These patients may not be best suited for currently available EGFR targeted agents. An intriguing response to HSP 90 inhibitor was reported in preclinical studies (23) as well as in early phase clinical study, leading to an ongoing prospective evaluation of AUY922 as treatment for NSCLC patients whose tumors harbor EGFR exon 20 mutations.

To date, many clinical trials have been conducted using EGFR as a predictive biomarker. Overexpression of EGFR protein whether assessed by *in situ* hybridization or immunohistochemistry failed to reliably identify patients likely to benefit from biologic agents targeted against the kinase activity of EGFR receptor. Activating mutation in the kinase domain of the *EGFR* gene is the most reliable predictive biomarker for this class of agents and has been successfully employed in paradigm-defining clinical trials of EGFR inhibitors in lung cancer. Please refer to the comprehensive review of EGFR targeted therapies by Conor *et al* in this issue of the journal.

3.1.3 Predicting resistance to EGFR tyrosine kinase inhibitors—Loss of treatment efficacy is nearly universal in patients treated with EGFR targeted agents (24). Various mechanisms that mediate resistance have been uncovered and can be classified as pharmacologically or biologically mediated resistance (Table 1). Pharmacological resistance is an acquired resistance that occurs through inadequate drug delivery to the target due to various factors such as limited delivery to sanctuary sites like the central nervous system, poor adherence to dosing schedule, decreased gastrointestinal absorption, and altered hepatic metabolism. The fluctuations and low levels of drug exposure consequently facilitate the development of resistance by cancer cells exposed to suboptimal drug concentration.

Biological mechanisms of resistance can develop through a positive selection pressure that favors the outgrowth of specific subpopulations of cancer cells that are able to adapt and proliferate in the presence of the EGFR inhibitor. Adaptations in the resistant clones may occur either through an alteration in the target or by activation of alternative or bypass signaling pathways (24). Alterations in the drug target preserve the oncogenic drive despite

adequate drug exposure. The T790 mutation acquired following extended period of therapy with an EGFR inhibitor is a classic example of this type of resistance mechanism. This mutation is the most frequently described and may be found in more than 50% of patients with acquired resistance to an EGFR tyrosine kinase inhibitor (25). Similar but rarer mutations associated with resistance include D761Y and L747S mutations (26, 27). Activation of bypass signaling pathways is the other common mechanism of biological resistance observed in EGFR-mutant patients. In this scenario, an alternative receptor tyrosine kinase is coopted to reactivate a critical signaling cascade downstream of the EGFR kinase blockade. Escape from the consequence of target inhibition enables the cell to continue to proliferate and survive in the face of an effective drug level and preserved drug target. *MET* gene amplification is the most commonly observed bypass signaling pathway that mediates failure of EGFR inhibitors (28, 29), but mutations in the *PIK3CA* and *BRAF* genes as well as *HER2* gene amplification can have the same consequence (30–32).

3.2 Anaplastic lymphoma kinase (ALK)

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase whose biological function is yet to be defined but is normally expressed in the small intestine, testes and the nervous system of adult human tissues (33). Morris and colleagues first reported oncogenic activity of ALK in anaplastic large cell lymphoma more than 2 decades ago (33). In 2007, Soda and colleagues in Japan used retrovirus-mediated expression screening to identify a novel rearrangement of the ALK gene in NSCLC. The genes encoding echinoderm microtubule associated protein like 4 (*EML4*) and *ALK* are both located on the short arm of chromosome 2 (2p21 and 2p23) (34). An inversion mutation within the short arm of chromosome 2 (p21p23) results in the formation of a fusion gene comprising *EML4* and *ALK*, whereby exon 1–13 of *EML4* is joined to exon 20–29 of *ALK*. The EML4-ALK fusion kinase is potently oncogenic and is the driver in a subgroup of NSCLC patients (34). The coiled coil domain of EML4-ALK is involved in cellular proliferation and apoptosis inhibition through downstream signaling relays involving PI3K/AKT, MAPK/extracellular-related kinase (ERK1/2) and JAK/STAT pathways (35, 36). Since the initial discovery of the ALK-EML4 fusion protein, other fusion partners of ALK have been identified (35). To date, 11 different ALK fusion variants have been reported based on demonstrable oncogenic activity in NIH-3T3 cells or in Ba/F3 cells (35, 37). These include fusions of ALK with tropomyosin-related kinase (TRK), kinesin family member 5B (KIF5B), kinesin light chain 1 (KLC1), protein tyrosine phosphatase, nonreceptor type 3, huntingtin interacting protein 1 (HIP1) and TRP (38–42).

The *ALK-EML4* fusion oncogene is reported in 2–7% of advanced NSCLC tumors (43). Initial reports from Soda *et al* showed five (6.7%) out of 75 Japanese NSCLC patients were positive for EML4–ALK (34). Other studies showed variable frequency of ALK fusion in lung cancer. Taheuchi *et al* reported a frequency of 4.4% (11 of 253) in Japanese patients (44). A lower frequency of 1.4% was reported in 136 samples obtained from Caucasian patients while a rate of 3.6% was reported in Korean patients (45). Similarly, a rate of 4.9% was described in 266 Chinese patients tested for EML4-ALK (46), while a prevalence of 7.5% was reported in a population of Italian and Spanish patients (47). Using clinical characteristic such as female gender, Asian ethnicity, never or light smoker and

adenocarcinoma histology, Shaw *et al* from the Massachusetts General Hospital screened 141 tumor samples of which 19 (13%) cases were found to harbor *EML4-ALK* translocation (48). Overall, *EML4-ALK* gene translocation more frequently presents in patients who are relatively young, male, with light or no smoking history, and predominantly with adenocarcinoma histology (34, 35, 37, 48). Nonetheless, these clinical characteristics are relatively insensitive and imprecise as a predictive biomarker to select patients for ALK-targeted therapies. Indeed, the first *EML4-ALK* fusion gene was identified in a smoker with lung cancer.

3.2.1 ALK translocation as a prognostic biomarker—Patients whose tumors harbor alterations in the *ALK* gene appear to have a worse prognosis than those without ALK alteration. In an observational study in non-smoking patients with advanced adenocarcinoma, patients with ALK positive disease had a higher risk of disease progression in comparison to ALK negative patients (49). ALK translocation has also been associated with an increased risk of brain involvement (40% *vs* 21%), liver metastases and a greater number of metastatic sites (49, 50). Kim *et al* reported a shorter median OS of 14.3 *vs* 33.3 months in patients with ALK(+) *vs* ALK(−) disease who were well matched with respect to age, gender, stage and smoking status (51). Similar observations were made by other investigators, although there have been occasional reports that failed to show ALK translocation as a negative prognostic biomarker. While the majority of patients with known diagnosis of ALK(+) NSCLC presents at advanced stages (52), the prognostic role of ALK translocation has also been studied in early stage disease. Paik *et al* analyzed 735 cases of stage I–III NSCLC and found 3.8% of cases positive for ALK translocation. There was no significant difference in the mean OS (97.7 *vs* 78.9 months; p=0.10) and disease free survival (76.4 *vs* 71.3 months; p = 0.66) between ALK(+) and ALK(−) patients (53). However, ALK rearranged tumors had a lower stage of the primary site but more frequent regional lymph node involvement (53–56).

3.2.2 ALK translocation as a predictive biomarker—ALK gene translocation assessed by FISH assay has been used successfully as a predictive biomarker of the efficacy of different agents targeting the ALK kinase activity including crizotinib, ceritinib and alectinib. This has resulted in the approval of two biologic agents, crizotinib and ceritinib, by the US FDA as standard therapy for the ALK(+) subset of NSCLC. Crizotinib is a first in class, orally available small molecule with potent inhibitory effects on cell proliferation through the induction of apoptosis and arrest in G1-S phase cell-cycle (57). The ALK kinase inhibition induced by crizotinib results in potent suppression of downstream survival signaling and induction of apoptosis (58). Initial phase 1 clinical trial experience in previously treated NSCLC patients harboring *ALK* translocation showed an objective response rate (ORR) of 60.8% and median progression free survival (PFS) of 9.7 months, leading to FDA approval of crizotinib in this subset of NSCLC (43, 59). Subsequently, randomized comparison of crizotinib to standard second line chemotherapy (docetaxel or pemetrexed) in 347 patients previously treated with platinum-based chemotherapy confirmed the superiority of crizotinib with a median PFS of 7.7 months *vs* 3 months and response rates of 65% *vs* 20% (60). More recently, the benefit of crizotinib over frontline platinum-based doublet chemotherapy (pemetrexed plus cisplatin or carboplatin) in newly

diagnosed patients with advanced *ALK*-positive non-squamous NSCLC was demonstrated in a randomized phase III study showing a PFS of 10.9 *vs* 7 months and response rate of 74% *vs* 45% with crizotinib and chemotherapy, respectively (61).

Newer ALK inhibitors such as ceritinib, alectinib and brigatinib (AP26113) with greater potency over crizotinib have been evaluated in the clinical setting. Ceritinib, which is 5 to 20 times more potent than crizotinib, has a distinct chemical structure, is orally available, and has activity against some of the known mechanisms mediating resistance to crizotinib. It, however, lacks activity against the G1202R and F1174C mutations in preclinical models (62–65). Ceritinib achieved a response rate of 58% and a median PFS of 7 months when tested in 130 patients with ALK positive NSCLC. This efficacy was independent of the type of ALK gene resistance mutations consistent with the *in vitro* modeling experiments (66). This study led to the approval of ceritinib as a standard treatment for patients with ALK translocated NSCLC who have failed crizotinib (67). Other new generation ALK kinase inhibitors such as alectinib with potent *in vitro* activity have also shown interesting clinical activity at extracranial and intracranial sites (68, 69).

3.2.3 ALK-fusion partners and EML4 variants as predictors of efficacy—

Following the original description of the ALK and EML4 fusion, other fusion partners have been identified including TRK, KIF5B, KLC1, HIP1 and TRP (38–42). The currently approved diagnostic testing for ALK translocation positive lung cancer is the Vysis break-apart FISH assay, which does not differentiate between various fusion partners of ALK. It is therefore currently unknown whether or not the partner protein with ALK influences response of patients to targeted therapies. It is however, noteworthy that similar to the experience with EML4-ALK translocation, crizotinib has been shown to achieve comparable efficacy in patients harboring novel fusion partner translocations (41).

Similarly, the breakpoint region in the *EML4* gene can vary, resulting in significant differences in the physicochemical characteristics of the EML4-ALK fusion protein variants. To date, up to 11 different EML4-ALK variants have been described and studies of the biological function of the protein variants have been conducted with intriguing results (70). Wu *et al* compared EML4-ALK fusion variants in 39 patients including 24 with variant 1 and 15 with non-variant 1 fusion genes (two v2, six v3a, five v3b, and two other variant types). There was no difference in age, sex or OS (14.1 *vs* 16.8 months; $p=0.869$) for variant 1 and non-variant 1 cases but the variant 1 cases were more likely to be heavy smokers (54). In preclinical work using Ba/F3 cell lines to test the efficacy of ALK kinase and HSP90 inhibitors against different fusion variants of EML4-ALK, v2, which is the least stable protein variant, showed the greatest sensitivity to ALK kinase inhibition, v1 and v3b showed intermediate sensitivity, while the v3a variant was the least sensitive. The findings from these preclinical experiments suggest that ALK fusion variants with different physicochemical properties may also differ in terms of drug sensitivity and responsiveness to ALK kinase inhibitors (71). The overall rarity of ALK translocated NSCLC and the even rarer frequency of subsets defined by the different fusion protein variants make clinical confirmation of this observation very challenging. Notably, correlative analysis using banked tissue samples collected as part of prospective studies of crizotinib did not show any significant association between mutant variants and response to therapy (43). It is

anticipated that larger numbers of banked tissue samples from ongoing and future prospective clinical trials could be harnessed to elucidate the impact of fusion partner proteins and fusion protein variants as predictive biomarkers of treatment efficacy.

3.2.4 Alterations in ALK and other genes predicting resistance to ALK inhibitors—

Despite the impressive efficacy of targeted agents such as crizotinib and ceritinib in ALK-translocated lung cancer, the vast majority of patients ultimately progress within a median period of 9–13 months. The mechanisms responsible for treatment failure have been elucidated using preclinical models as well as through detailed analysis of tissue samples obtained from patients at the time of progression (72–74). Resistance to ALK targeted therapy is now known to occur either in an ALK-dependent manner, whereby the cancer cells remain dependent on ALK signaling, or through an ALK-independent process (Figure 1). Acquired mutations in the ALK kinase domain as well as copy number gains of the fusion gene can impair the ability of the targeted therapy to inhibit the fusion protein. Such acquired mutations include L1196M, S1206Y, C1156Y, G1202R, 1151Tins and L1152R occurring around the ATP binding site of the kinase enzyme, which lead to a restoration of ALK signaling in the presence of crizotinib (72, 74–76). ALK-independent mechanisms of resistance include the cooption by the cancer cells of alternative oncogenic drivers, such as KRAS and EGFR, or through ligand-driven activation of the HER family, IGF-1R and KIT (74, 76–80). It has yet to be shown whether these bypass mechanisms occur *de novo* in previously untreated patients. Moreover, these genetic alterations appear to be agent-specific since new generation inhibitors of ALK fusion protein, such as alectinib and ceritinib, demonstrated efficacy in the salvage setting across patient subgroups with different mechanisms of resistance to crizotinib (66). Nonetheless, novel mutations, such as the V1180L gatekeeper mutation and the I1171T mutations occurring post alectinib, remain sensitive to ceritinib (81–83). It is conceivable that these resistance mutations will guide the choice of salvage therapy in patients who have failed a prior ALK-targeted agent.

3.3 KRAS mutations

KRAS is the most commonly detected mutation in NSCLC, present in up to a third of all cases (85). It is more common in tumors with adenocarcinoma histology as opposed to squamous type NSCLC (86). Although there is currently no targeted therapy with established efficacy in NSCLC harboring mutant *KRAS*, this genetic mutation was previously considered a negative predictive biomarker for efficacy of EGFR targeted inhibitors. However, this presumed negative association has not been conclusively borne out by larger studies (87, 88). A meta-analysis of 17 studies reporting on a total of 165 patients with tumors harboring *KRAS* mutation suggested that concurrent presence of *KRAS* and *EGFR* mutations showed a significant association with lack of clinical benefit from EGFR inhibitors (89). Mao *et al* also conducted a separate meta-analysis of 22 studies and 231 patients with *KRAS* mutation. They reported an ORR of 3% *vs* 26% in patients whose tumors harbor mutant EGFR and mutant or wild type *KRAS* gene, respectively. The overall pooled relative rate of response in the presence of *KRAS* mutation was reported as 0.29 (95% CI: 0.18–0.47; P<0.01). There was, however, no significant difference in OS (90). The presence of *KRAS* mutation was previously considered a marker of poor prognosis based on small data series reported from single institution studies (91–93). Recently, Riely *et al*

conducted a comprehensive assessment in 677 patients with advanced recurrent NSCLC looking at the frequency and impact of *KRAS* mutation as well as the impact of specific codon mutation, and failed to show any association with outcome (94). The study showed that *KRAS* mutation, whether codon 12 or 13, had no significant impact on survival. Also, comparison of *KRAS* transition type mutation with transversion type mutations also showed no significant difference in outcome ($p = 0.99$). While patients whose tumors harbor *KRAS* codon 13 mutation had shorter OS compared with patients with codon 12 mutation (1.1 *vs* 1.3 years; $p = 0.009$), this finding could not be confirmed in an independent validation set consisting of samples from 682 patients with KRAS mutant lung cancers (1.0 *vs* 1.1 years, respectively; $p=0.41$) (94). Nonetheless, *KRAS* mutation remains the most frequently detected genetic alteration in NSCLC. While it does not at present offer any clinical value either as a prognostic indicator or as a therapeutic guide, targeted therapies against activating *KRAS* mutation are undergoing active testing as a therapeutic strategy in lung cancer. Table 2 provides a summary of ongoing clinical trials in *KRAS* mutant NSCLC. The success of some of these clinical trials may establish *KRAS* as a *bona fide* predictive biomarker in NSCLC.

3.4 Rare genetic alterations as biomarkers

In addition to EGFR and ALK gene alterations which can be present in up to 40% of NSCLC patients, other genetic alterations involving ROS1, MET, RET, BRAF, and HER2, among others, have been described in smaller subsets of NSCLC. These alterations are currently employed as predictive biomarkers for therapeutic agents likely to be effective in patient subsets defined by the presence of these mutations, based on supporting clinical experience in other tumor types and/or from preclinical models.

3.4.1 ROS1—ROS1 was initially discovered as homolog of chicken c-ros, encodes a receptor tyrosine kinase and has significant homology with ALK kinase (95). It is arranged as an intracellular C-terminal tyrosine kinase domain and a large extracellular N-terminal domain. The normal biologic function of ROS1 has not yet been defined but it is highly expressed in the kidney but not in the lung (95, 96). The initial identification of ROS1 fusion gene as a driver of NSCLC arose from preclinical and correlative work conducted in the HCC78 cell line and in a patient tumor sample where SLC34A2 and CD74 were observed to be fused to the transmembrane region of ROS, resulting in a constitutively active truncated fusion protein with two transmembrane domains (39). Subsequently, Rimkunas and group reported 9 (1.6%) tumors expressing ROS1 in Chinese NSCLC patients with CD74-ROS1, SLC34A2-ROS1, and FIG-ROS1 fusions determined by reverse transcriptase PCR (97). Multiple ROS1 fusion proteins have been described and include SDC4, EZR, SLC34A2, TPM3, LIMA1 (LIM domain and actin binding 1), LRIG3 and MSN (98–101). Analysis of archival tumor samples revealed a prevalence of 1.7 to 2.4% with most patients being relatively young non-smokers with tumors of adenocarcinoma histology (102, 103).

The presence of ROS1 translocation has not been associated with prognostic difference in early stage lung cancer but a negative impact was noted in patients with advanced stage tumors harboring ROS1 relative to EGFR mutant patients (103). There is partial homology between ROS1 and ALK and preclinical studies have shown activity of crizotinib against a

ROS1(+) cell line (102). Clinical evaluation of crizotinib in 50 patients with advanced NSCLC harboring ROS1 translocation showed an ORR of 72%, including three patients with complete response with median duration of response of 17.6 months and median PFS of 19.2 months (98). The most common ROS1 fusion partner is CD74 (44%) but there was no significant difference in the efficacy of crizotinib against the CD74 fusion partner as compared to other fusion partners (98). As expected, resistance to crizotinib has been observed in patients with ROS1(+) NSCLC. Awad *et al* reported the experience with a patient whose tumor harbored the *CD74–ROS1* rearrangement treated with crizotinib. Initial response followed by resistance led to the identification of an acquired mutation with a glycine to arginine substitution at codon 2032 (G2032R) in the ROS1 kinase domain (104). Newer agents such as foretinib (GSK1363089), which is more potent than crizotinib and also has activity against the acquired G2032R mutation that mediates crizotinib resistance, have already been identified and are now in clinical development (100).

3.4.2 HER2—HER2, human epithelial receptor 2 (HER2/erbB2) is a member of the HER family that is activated by homo-or heterodimerization with another member of the erbB family (erbB1-4) leading to downstream activation of the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway (105, 106). Amplification of the gene encoding HER2 and/or protein overexpression is well established as a biomarker for HER2 targeted therapies in breast, ovarian, gastric and uterine cancers (107–109). Mutation involving the tyrosine kinase domain of the *erbB2* gene has also been reported in NSCLC at a prevalence of 1.6% and with a predilection for never smokers and adenocarcinoma histology but without regard to sex, race, or tumor stage (105, 109–111). A meta-analysis of published data showed HER2 protein expression but not HER2 gene amplification to be a marker of poor prognosis in lung cancer (112). Trastuzumab, a humanized monoclonal antibody against HER2, showed antitumor activity in preclinical models of NSCLC both alone and in combination with cytotoxic agents (113). However, HER2 protein expression failed to predict patients likely to benefit from trastuzumab or lapatinib, a tyrosine kinase inhibitor of HER1 and HER2, in clinical trials of patients with HER2 deregulated NSCLC (114, 115). There are multiple clinical trials currently underway examining newer generation kinase inhibitors such as dacomitinib, afatinib, and neratinib for HER2(+) lung cancer.

3.4.3 RET—Rearranged in transfection (RET) is a receptor tyrosine kinase that has been established as a driver mutation in various cancers including medullary thyroid cancer and subset of papillary thyroid cancer (11, 116, 117). RET oncogene aberration in lung cancer was first reported in a young, male, never smoker with metastatic NSCLC. Using parallel whole-genome and transcriptome sequencing, a fusion gene between KIF5B and the RET proto-oncogene caused by a pericentric inversion of 10p11.22–q11.21 was identified (118). Subsequent screening of larger sets of NSCLC tumor samples found RET fusions in approximately 1–2% of NSCLC, almost exclusively in adenocarcinoma (101, 117, 119–122). Although *KIF5B* is the most common fusion partner with RET, being present in 90% of reported cases, other fusion partners have been described including CCDC6, NCOA4, and TRIM33 (117, 119, 123, 124). Preclinical data demonstrated the activity of RET inhibitors in lung cancer cell lines harboring activating RET fusions (119, 122–124). The initial evidence for clinical efficacy of these agents in patients is mostly anecdotal, albeit with

positive and encouraging results (125). Cabozantinib, an inhibitor of multiple kinases including RET, is currently being studied in a prospective phase II trial. Experience with the first three patients with *RET* fusion-positive NSCLC enrolled on the study was reported by Drilon *et al*, with two of the three patients achieving a partial response and the third patient experiencing stable disease (126). Currently, multiple clinical trials are underway using the presence of RET fusion as a predictive biomarker to select NSCLC patients for prospective evaluation of the clinical efficacy of RET inhibitors such as cabozantinib (NCT01639508), lenvatinib (NCT01877083), ponatinib (NCT01813734) and vandetanib (NCT01823068).

4.0. Conclusions

NSCLC has evolved into a conglomerate of tumor subgroups characterized by specific molecular aberrations rather than by simple origination from the lung. Most of the genetic alterations described to date present valid targets for therapeutic intervention with varying success, as demonstrated by the FDA approval of agents targeting EGFR and ALK alterations. Other rarer mutations such as ROS1 and RET have also been successfully targeted whereas attempts to target RAS gene alterations remain a work in progress. While these genetic alterations meet the basic definition of predictive biomarkers, their prognostic value has not been well characterized. Challenges related to sequence of testing, i.e. single assay *vs* multiplex assay, reflex testing *vs* testing on request, assay performance, and the comparison of platforms for detecting genetic alterations between immunohistochemistry, FISH, targeted DNA sequencing and next generation sequencing assay, continue to evolve as technological capabilities advance.

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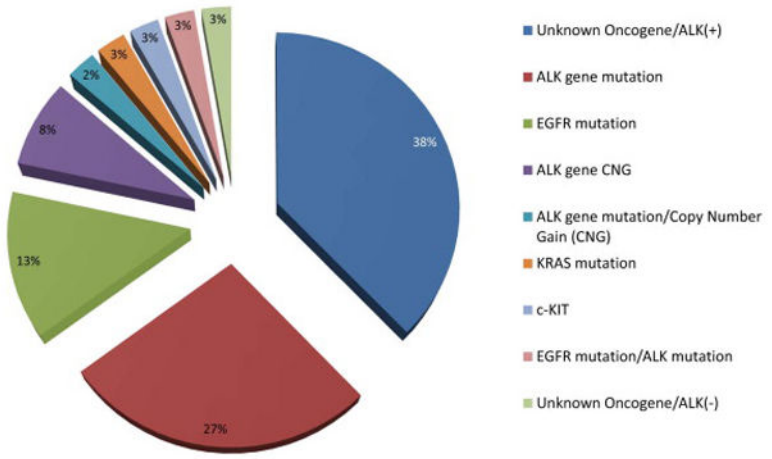

Figure 1.

Mechanisms of resistance to ALK inhibitor therapy reported in published data generated from tumor samples obtained from patients with acquired resistance to crizotinib (74, 78, 84)

Table 1

Mechanisms of acquired resistance to EGFR tyrosine kinase inhibitors in NSCLC

Pharmacological mechanisms		Inadequate CNS penetration Non-compliance/dose reduction or interruption Smoking Reduced absorption Increased hepatic metabolism
Biological mechanisms	Altered drug target ~ 60% (24)	T790M alone ~40–60% (32, 127) T790M with *EGFR* amplification ~10% (127) Other *EGFR* mutations ~1–2% (127)
	Bypass tracks ~ 20% (24)	MET amplification ~5% (32, 127) HER2 amplification ~ 8–13% (32) PIK3CA ~1–2% (128)
	Altered Phenotype	EMT ~1–2% (32, 127, 129) SCLC ~6% (32, 127)
	Unknown mechanism	Accounts for 18–20% of acquired resistance (32, 127)

Table 2

Active clinical trials of therapies targeting *KRAS* mutant lung cancer

Study	Treatment	Design	Primary Endpoint	Rationale and Strategy
SELECT-1 study; (NCT01933932)	Selumetinib + Docetaxel	Randomized phase III	PFS	Improved efficacy of the combination over docetaxel alone
Phase II Study of VS-6063 (NCT01951690)	VS-6063 (Defactinib)	Phase II	PFS	Demonstrate if VS-6063 improves PFS within each cohort
AZD6244 in Combination With Docetaxel, Compared With Docetaxel Alone, (NCT00890825)	AZD6244 + Docetaxel	Randomized, Phase II	OS	Compare the efficacy of the combination over docetaxel alone
Phase II Study of AZD6244 MEK-Inhibitor With Erlotinib (NCT01229150)	AZD6244 + Erlotinib	Randomized, Phase II	PFS	Compare the efficacy of combination over erlotinib alone
Erlotinib Plus Tivantinib (ARQ197) Versus Single Agent Chemotherapy (NCT01395758)	RQ 197 + erlotinib Vs. Pemetrexed, Docetaxel or Gemcitabine	Randomized, Phase II	PFS	Compare efficacy of combination erlotinib plus tivantinib over single agent chemotherapy.
Phase I/IB Trial of MEK162 in Combination With Erlotinib (NCT01859026)	MEK162 + Erlotinib	Non-Randomized, Phase I/IB	Maximum Tolerated Dose (MTD)	Safety of the combination MEK162 and erlotinib
Phase I/II Study of the CDK4/6 Inhibitor Palbociclib (PD-0332991) in Combination With the MEK Inhibitor PD-0325901 (NCT02022982)	Palbociclib + PD-0325901	Phase I/II	Safety and Maximum Tolerated Dose (MTD)	Evaluate safety and compare efficacy of palbociclib in combination with another experimental drug PD-0325901
Phase 1 Study of Trametinib in Combination With Chemoradiation (NCT01912625)	Trametinib + Chemoradiation	Phase I	DLT	Evaluate safety of trametinib in combination with chemotherapy and radiation therapy
JUNIPER (NCT02152631)	Abemaciclib + Best Supportive Care Vs. Erlotinib Plus Best Supportive	Randomized Phase III	PFS OS	Compare efficacy of abemaciclib over erlotinib