Review Article

K-Ras Mutations in Non-Small-Cell Lung Cancer: Prognostic and Predictive Value

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Non-small-cell lung cancer (NSCLC) is a heterogeneous disease due to the presence of different clinically relevant molecular subtypes. Until today, several biological events have been identified in lung adenocarcinoma, including epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) translocations, offering new hopes to patients with metastatic disease. Unfortunately, in approximately 50% of adenocarcinoma and for those harbouring K-RAS mutations, the most frequent mutation in Caucasian lung adenocarcinoma, so far no specific drug demonstrated efficacy. The rat sarcoma (RAS) genes, including H-RAS, K-RAS, and N-RAS, encode a family of proteins regulating cell growth, differentiation, and apoptosis. K-RAS mutations are present in 20–30% of NSCLC and occur most commonly, but not exclusively, in adenocarcinoma histology and life-long smokers. Although in colorectal cancer patients K-RAS mutations represent a validated negative predictive biomarker for treatment with anti-EGFR monoclonal antibodies, their role in selecting specific treatment for NSCLC patients remains undefined. Aim of the present paper is to critically analyze the prognostic and predictive value of K-RAS mutations in NSCLC.

1. Introduction

In 2011 non-small-cell lung cancer (NSCLC) remains the principal cause of cancer-related death worldwide, accounting for more than one million deaths per year [1]. Therapeutic progresses have signed out the last decade, but median survival for patients in advanced stage is still disappointing [2]. NSCLC accounts for 80% of lung tumors, including adenocarcinoma in 35–40% of cases, squamous cell carcinoma in 25–30%, and large cell carcinoma in 10–15%. For many years we treated metastatic NSCLC with the same regimens, irrespective of any clinical or biological characteristics. Today, histology seems a relevant parameter for defining the best regimen, with new agents, such as pemetrexed and bevacizumab, effective and safe only in nonsquamous populations [3, 4]. During the last few years, improvement in the knowledge of lung cancer biology led to identification of molecular events crucial for tumor cell survival. Cancer cell survival might depend on the expression of a single-mutant oncogene according to a model called “oncogene addiction” [5, 6]. In NSCLC a number of driving mutations have been identified, including Epidermal growth factor receptor (EGFR) mutations, KRAS mutations, HER2 mutations and EML4-ALK translocations. Since their identification in 2004, activating EGFR gene mutations have emerged as the most relevant predictor of response to a class of compounds, the EGFR tyrosine kinase inhibitors (EGFR-TKIs) gefitinib and erlotinib. Six phase III randomized trials demonstrated that patients harboring activating EGFR mutations benefit more from EGFR-TKIs than from standard platinum-based chemotherapy at least in terms of response rate (RR), progression-free survival (PFS), toxicity profile and quality of life [7–12]. Randomized phase III trials in the maintenance setting (SATURN and ATLAS), in second-line versus chemotherapy (INTEREST and TITAN) and versus placebo (BR21) confirmed the high efficacy of EGFR-TKIs in the presence of activating EGFR mutations [13–17]. Today in patients harbouring an EGFR mutation, gefitinib or erlotinib represent the best therapeutic option irrespectively of treatment line. Nevertheless, large randomized clinical
trials demonstrated that erlotinib could produce a modest benefit even in the EGFR wild-type population [13, 14].

Therefore, a relevant issue in clinical practice is the identification of EGFR wild-type patients that could benefit or that could be excluded from an EGFR-TKI therapy. Unfortunately, at present, there is no single biomarker that could be used for precluding the treatment to any patient, including K-RAS mutations [14]. Although in colorectal cancer K-RAS mutations are the most useful biomarker for selecting patients who are candidate for treatment with anti-EGFR monoclonal antibodies, cetuximab or panitumumab, its role in NSCLC as prognostic or predictive marker is less defined [18]. The aim of the present paper is to analyze the role of K-RAS mutations in NSCLC.

2. RAS Mutations in NSCLC

The RAS gene family includes H-RAS, K-RAS and N-RAS and encodes for membrane-bound 21-kd guanosine-triphosphate-(GTP-) binding proteins regulating cell growth, differentiation and apoptosis by interacting with multiple effectors including mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) and signal transducer and activator of transcription (STAT) cascades (Figure 1). RAS proteins acquire transforming potential when a point mutation in the gene replaces an amino acid at position 12, 13 or 61 [19]. These mutations lead to forms of RAS with impaired GTPase activity, causing a constitutive activation of RAS signalling pathway. Mutations in K-RAS gene occur frequently in NSCLC [20], more frequently (20–30%) in adenocarcinoma and less frequently (about 7%) in squamous-cell carcinoma [21]. In NSCLC the vast majority of K-RAS mutations involve codons 12 or 13 and are usually associated with a history of tobacco use [22]. K-RAS mutations frequency varies among different ethnic groups, with lower frequency observed among Asians and higher frequency among African Americans compared to white Caucasians [23]. Recently K-RAS mutations have been detected in a significant proportion of never smoker NSCLC patients, with an incidence up to 15% [23]. Thus, unlike EGFR mutations, which occur more frequently in never smokers, presence of a K-RAS mutation cannot be predicted on the basis of smoking history alone.

3. K-RAS Mutation as Prognostic Biomarker

The role of K-RAS mutations as a prognostic factor in NSCLC remains controversial. Although some studies suggested a potential negative prognostic effect, other studies did not confirm any negative impact on survival for individuals harbouring a K-RAS mutation. More than 50 studies have been published, using different methods for K-RAS testing and with conflicting results (Table 1). In an ancillary study of JBR.10 trial, a phase III trial of adjuvant chemotherapy versus observation in resected NSCLC, among the 450 analyzed cases, 26% harboured a K-RAS mutation [24]. In the group of patients not treated with chemotherapy, K-RAS mutations were not prognostic for survival (P = 0.4). In the E4592 trial, another phase III trial of adjuvant chemotherapy versus observation in resected NSCLC, 24% of 184 assessable tumors were positive for K-RAS mutations [25]. The median survival of mutated and wild-type patients was not statistically different (30 and 42 months, resp. P = 0.38). Graziano et al. investigated the prognostic effect of K-RAS mutations in stage I and II resected NSCLC [26]. In the whole population, no statistical difference was found in OS for K-RAS-mutations-positive and negative patients (P = 0.33). Keohavong et al. found no association of K-RAS mutation and survival in 173 adenocarcinoma and adenosquamous NSCLC patients [27]. In another study, Lu et al. evaluated the prognostic role of a panel of six biomarkers including K-RAS mutations, in completely resected stage I NSCLC [28]. Patients were followed up for a minimum of 5 years; K-RAS mutations were detected in 34% of samples and were not associated with overall survival (P = 0.517). Conversely, Slebos reported a series of 69 surgically treated adenocarcinomas of the lung in which K-RAS codon-12 point mutations resulted in a negative prognostic factor for disease-free survival (P = 0.038) and overall survival (P = 0.002) [29]. This difference was consistent also after adjustment for factors such as stage, tumor size and differentiation. In a prospective series of 365 patients with resected early stage NSCLC treated at Massachusetts General Hospital, K-RAS mutations were found only in smokers and were associated with worse survival (P = 0.009, log-rank test) only in stage I disease, but not in the whole population [30]. In a Japanese study, Fukuyama et al. examined 159 cases of NSCLC for mutation at codon 12 of K-RAS gene and found 6.9% of mutated patients [31]. The K-RAS mutation positive group had a worse survival than the K-RAS negative group (P < 0.05). In another Japanese study, K-RAS mutations were detected in 8.3% of 144 patients [32]. The OS rate of NSCLC patients with wild-type K-RAS was better than that of patients whose tumours harboured mutations of K-RAS (P = 0.033). Miyake et al. analysed tumor tissue from 187 NSCLC patients, among which 8% harboured a K-RAS mutation [33]. In this study, patients with wild-type K-RAS had a significantly better survival rate than those with mutant K-RAS (P = 0.0369). In another study Marks et al. evaluated the prognostic role of EGFR and K-RAS in 296 resected lung adenocarcinomas [34]. Patients were stratified on EGFR and K-RAS mutation, K-RAS mutation or absence of EGFR and K-RAS mutation. In the absence of targeted therapies, 3-years OS was 90%, 76%, and 66% for patients with EGFR mutations, EGFR/K-RAS wild type and K-RAS-mutations, respectively. The difference in survival between EGFR-mutated group and K-RAS mutated group was statistically significant (P = 0.009). In 2005 a systematic review and meta-analysis of 28 studies including a total of 3620 patients showed that presence of K-RAS mutations confers a significantly worse prognosis, with a combined HR of 1.35 for OS in the random effect model [35]. In a subgroup analysis according to histology, K-RAS mutation resulted in a statistically significant prognostic factor for survival only for adenocarcinoma (HR 1.59).
Available data suggest that K-RAS mutations represent a negative prognostic factor particularly in patients populations with high incidence of EGFR mutations, such as in adenocarcinoma and in Asiatic patients. A possible explanation is that in adenocarcinoma and in Asiatic patients there is a high incidence of EGFR mutations that are considered a positive prognostic factor. In fact, in the study conducted by Marks et al., OS was significantly worse in lung adenocarcinomas with K-RAS mutations when compared to patients harbouring EGFR mutations [34].

4. K-RAS Mutation as Predictive Biomarker

4.1. Chemotherapy. Recent data suggested that K-RAS mutations may affect the outcome of NSCLC patients receiving chemotherapy (Table 2). In the adjuvant setting, data from the JBR10 trial suggested no benefit from adjuvant chemotherapy in K-RAS mutated patients (HR 0.95, \( P = 0.87 \)) [24, 36]. In the LACE-BIO pooled analysis the prognostic and predictive role of K-RAS mutations was investigated in 1751 patients treated with adjuvant chemotherapy [37]. Among evaluable patients, 304 (19.7%) harboured K-RAS mutations with no effect on survival (HR 1.18, \( P = 0.09 \)).

Several studies investigated the influence of K-RAS mutations on sensitivity to chemotherapy in advanced NSCLC. Camps et al. analyzed K-RAS status in plasma samples from 308 advanced NSCLC patients treated with cisplatin and docetaxel. No difference in PFS (5.4 versus 5.7 months, \( P = 0.2 \)) or OS (10.0 versus 9.0 months, \( P = 0.5 \)) was detected between K-RAS wild-type and K-RAS mutant patients [38]. Another study retrospectively analyzed 162 chemotherapy-naive patients with locally advanced/metastatic NSCLC who received first-line chemotherapy [39]. Presence of K-RAS mutations did not affect response to chemotherapy (RR, 26.5% for K-RAS wild type versus 25% for K-RAS mutant; \( P = 0.87 \)) nor time to progression (TTP, 4.2 months for K-RAS mutant versus 4.7 months for K-RAS wild type; \( P = 0.42 \)). Furthermore, no significant difference in survival was detected between K-RAS wild type and K-RAS-mutated patients (14.5 versus 18.5 months for mutations positive and wild-type K-RAS patients, respectively; \( P = 0.52 \)).

Overall, these data indicate that K-RAS mutations have no role in response prediction to standard chemotherapy in NSCLC and, therefore, such test should not be used in clinical practice.
4.2. EGFR-TKIs. K-RAS is a critical downstream effector of the EGFR pathway (Figure 2). Therefore, there is a biologic rationale supporting the hypothesis that NSCLC tumors with K-RAS mutations are intrinsically resistant to EGFR-directed therapies. In fact, mutations in this gene may produce constitutive activation of the kinase that may overrule the inhibition of EGFR signaling. Initial studies in small cohorts of NSCLC showed lack of response to EGFR-TKIs in patients harboring K-RAS mutations [40–43]. Giaccone et al. analyzed K-RAS status in patients treated with frontline erlotinib and found that none of 10 mutated patients responded to anti-EGFR treatment [40]. Absence of response to erlotinib was reported in another phase II trial in elderly patients. In this study tissue samples from 41 patients were analyzed for K-RAS mutations and all the 6 mutated patients identified were refractory to erlotinib [41]. Pao et al. investigated the role of K-RAS mutations in 60 lung adenocarcinomas treated with gefitinib or erlotinib; K-RAS mutations were identified in 9 (24%) of 38 patients refractory to either drug, whereas no mutation was detected in 21 sensitive patients [42]. A retrospective analysis of K-RAS mutations in patients treated with EGFR-TKIs was conducted by Massarelli et al. In this study 16 (22.8%) of 70 patients had a K-RAS mutation and all of them (100%) had progressive disease during the treatment [43]. These studies suggested an association between K-RAS mutations and an absence of response to EGFR-TKIs. More recently, two meta-analyses showed that the presence of K-RAS mutations was associated with lack of response to EGFR-TKIs in NSCLC patients [44, 45]. Nevertheless, both meta-analyses were insufficient to determine the association between K-RAS status and PFS and OS.

Table 2 reports data on K-RAS mutational status and its relationship with survival in phase III trials with anti-EGFR therapy. In the TRIBUTE study, comparing chemotherapy and chemotherapy plus erlotinib, patients with K-RAS mutations had significantly shorter survival when treated with chemotherapy plus erlotinib, suggesting a possible detrimental effect of TKIs in patients harbouring such mutations [46]. The BR.21 trial, evaluating erlotinib versus placebo in second- and third-line setting, showed a survival advantage for erlotinib in the overall population (6.7 versus 4.7 months, HR 0.70; P < 0.001) [13]. Two hundred and six samples were available for K-RAS analysis and in 16% of cases a K-RAS mutation was detected. In the Cox model, the interaction between K-RAS mutation status and treatment suggested a lack of benefit from erlotinib in patients with mutations (P < 0.09). Importantly, on multivariate analysis, the presence of K-RAS mutation was not predictive for a differential treatment effect (P = 0.13) [47].

A potential benefit in survival produced by erlotinib in K-RAS mutated NSCLC has been reported in the SATURN trial, a large phase III trial randomizing 889 patients who did not progress after first-line chemotherapy, to receive erlotinib or placebo as maintenance treatment [14, 48]. Four hundred ninety-three (55.4%) tumor samples were analyzed for K-RAS mutations. Patients treated with erlotinib experienced longer PFS irrespectively of K-RAS mutational status, with a marginal even if not significant survival improvement in the K-RAS mutant population (HR 0.79). Another maintenance study, the ATLAS trial, evaluated maintenance treatment with bevacizumab plus placebo or erlotinib in metastatic NSCLC patients not progressing after 4 cycles of platinum-based chemotherapy. The addition of erlotinib significantly reduced the risk of progression (HR 0.72), with the highest benefit observed in the EGFR mutated patients [15]. Analysis of K-RAS mutations highlighted longer PFS for K-RAS wild type patients treated with bevacizumab/erlotinib (HR 0.66, log-rank P = 0.0105) but no difference for K-RAS-mutant patients (HR 0.92, log-rank P = 0.76) between the two arms. Finally, in the INTEREST study, a large phase III trial comparing gefitinib and docetaxel as second-line therapy in metastatic NSCLC, 18% of patients harboured K-RAS mutations [16, 49]. No differences in PFS and response rates were detected in both treatment arms according to K-RAS status, with no evidence of any differential survival effect (P = 0.51).

Therefore, although patients harbouring a K-RAS mutation do not respond to EGFR-TKIs, a minimal survival effect cannot be excluded. For such reason, at present, K-RAS testing is not recommended for precluding an EGFR-TKI therapy to any NSCLC patient.

4.3. Anti-EGFR Monoclonal Antibody. A second strategy aimed at inhibiting EGFR signalling is the use of monoclonal antibodies binding the extracellular domain of the receptor. Two large phase III trials investigated the combination of cetuximab, a human-murine chimeric anti-EGFR IgG monoclonal antibody, with chemotherapy versus chemotherapy alone [50, 51]. In the FLEX trial, 1125 patients with EGFR expressing advanced NSCLC were randomized to receive first-line cisplatin/vinorelbine with or without cetuximab [50]. The addition of cetuximab to chemotherapy led to a significant but clinically marginal survival improvement (11.3 versus 10 months, HR 0.87, P = 0.044) with an increased risk of toxicity, in particular febrile neutropenia. Similar
Table 3: KRAS and sensitivity to anti-EGFR agents in phase III trial.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Anti-EGFR agent</th>
<th>Total number of patients (n)</th>
<th>Patients tested for KRAS (n)</th>
<th>KRAS mutant n (%)</th>
<th>Survival in KRAS mutant (HR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIBUTE [46]</td>
<td>Gefitinib</td>
<td>1079</td>
<td>264</td>
<td>55 (21)</td>
<td>2.1*</td>
</tr>
<tr>
<td>BR. 21 [47]</td>
<td>Erlotinib</td>
<td>731</td>
<td>206</td>
<td>30 (15)</td>
<td>1.67</td>
</tr>
<tr>
<td>SATURN [48]</td>
<td>Erlotinib</td>
<td>889</td>
<td>493</td>
<td>90 (18)</td>
<td>0.79</td>
</tr>
<tr>
<td>ATLAS [15]</td>
<td>Erlotinib</td>
<td>768</td>
<td>NR</td>
<td>NR</td>
<td>0.92</td>
</tr>
<tr>
<td>INTEREST [49]</td>
<td>Gefitinib</td>
<td>1466</td>
<td>275</td>
<td>49 (18)</td>
<td>0.91</td>
</tr>
<tr>
<td>FLEX [57]</td>
<td>Cetuximab</td>
<td>1125</td>
<td>379</td>
<td>72 (19)</td>
<td>1.0</td>
</tr>
<tr>
<td>BMS099 [56]</td>
<td>Cetuximab</td>
<td>676</td>
<td>202</td>
<td>35 (17)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

HR: hazard ratio; NR: not reported. *Statistically significant.

results were observed in the BMS099 trial, a phase III trial that randomly assigned 676 chemonaive NSCLC patients to carboplatin plus a taxane versus the same chemotherapy regimen plus cetuximab [51]. Notably, patients were enrolled into the study regardless of EGFR expression. Although a nonsignificant trend toward longer survival (9.6 versus 8.3 months HR 0.89, P = 0.17) was reported, the primary end point of improved PFS in the cetuximab arm was not met (4.4 versus 4.2 months, P = 0.2). Based on these studies results, European Medicine Agency (EMA) recently rejected cetuximab approval for advanced NSCLC. This decision clearly highlights the need for biomarkers useful in selecting patients potentially candidate to cetuximab therapy. A recent biomarker analysis of FLEX trial has highlighted a survival benefit in NSCLC patients overexpressing EGFR even in the absence of a PFS benefit [52]. This led to a new submission to EMA in March 2011.

The lack of benefit from anti-EGFR monoclonal antibodies in colorectal cancer patients with K-RAS mutations has been demonstrated [53–55]. The status of K-RAS gene has been investigated even in FLEX and BMS099 trials. In the BMS099 study K-RAS mutant patients treated with cetuximab plus chemotherapy had a trend toward improved PFS and OS than those treated only with chemotherapy [56].

Similarly, in the FLEX trial, K-RAS gene testing failed to identify patients not benefiting from cetuximab and showed similar survival between K-RAS mutant and wild type patients regardless of treatment [57].

These results demonstrate that, unlike colorectal cancer case, the negative predictive value of K-RAS mutations in NSCLC remains unclear. A possible explication of the different role of K-RAS mutation in lung and colorectal cancer has been recently proposed. Danenberg et al. analyzed K-RAS mutation status in 2693 colorectal and lung specimens [58]. Surprisingly, different types of K-RAS mutations were detected in lung and colorectal cancer, with a significant predominance of DNA K-RAS transversions in NSCLC, likely linked to tobacco exposure. The ratio of base transversions to transitions was 3.27 versus 0.77 (p < 0.001) in NSCLC and colorectal cancer, respectively. Tobacco-carcinogenesis-associated G>T transversions (codon 12 GGT>TGT plus GGT>GGT) represented 61% of K-RAS mutations in NSCLC and 39% in colorectal cancer (P < 0.001). It is possible that the distinct mutation pattern and biological function may contribute to differences in predictive value for cetuximab therapy between NSCLC and colorectal cancer.
5. Conclusion

*K-RAS* mutation testing is a validated biomarker in clinical practice to predict anti-EGFR treatment outcome in colorectal cancer. In a significant fraction of NSCLC, particularly adenocarcinoma and smokers, a *K-RAS* mutation is detectable, but its prognostic and predictive role remains unclear. Although this event is generally considered associated to a worse prognosis and resistance to several drugs, including EGFR-TKIs, available data are conflicting, not supporting the use of *K-RAS* testing in clinical practice for selection of NSCLC.

Unfortunately, although *K-RAS* mutations are one of the most commonly occurring oncogene aberrations in human cancer, no specific treatment is currently available. A new hope for *K-RAS* mutant patients is represented by novel drugs currently under investigation in phase II and III trials [59]. More recently, scientists uncovered a crack in the molecular armor of *RAS*, a binding pocket of functional significance that could provide the long-sought attack point for a therapeutic agent [60]. Twenty-five compounds with affinity for binding to *RAS* oncoproteins were identified by nuclear magnetic resonance spectroscopy. Although all these compounds demonstrated weak affinity for *RAS* protein and inability to completely knock out the oncoprotein, they represent the first generation of *RAS* inhibitors, opening a new notable way for research of other compounds able to prevent *RAS* activation. While waiting for new drugs, the continuous collaboration between basic scientists and clinical researchers is the most relevant way to give hope to our cancer patients.

Abbreviations

NSCLC: Non-small-cell lung cancer  
EGFR: Epidermal Growth Factor Receptor  
ALK: Anaplastic Lymphoma Kinase  
TKIs: Tyrosine kinase inhibitors  
RR: Response rate  
PFS: Progression-free survival  
OS: Overall survival  
GTP: Guanosine triphosphate  
MAPK: Mitogen-activated protein kinase  
PI3K: Phosphoinositide 3-kinase  
STAT: Signal transducer and activator of transcription  
TTP: Time to progression  
EMA: European Medicine Agency

Acknowledgment

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