REVIEW

The clinical relevance of KRAS gene mutation in NSCLC

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Purpose of review
There are conflicting data on the potential prognostic and predictive role of mutant KRAS in nonsmall cell lung cancer.

Recent findings
KRAS is the most frequently mutated oncogene in lung adenocarcinoma patients of non-Asian ethnicity. Novell data also revealed that allelic variants of mutant KRAS are different concerning their biochemistry, which may influence their prognostic and predictive role in nonsmall cell lung cancer (NSCLC). Though mutant KRAS is not the target of molecular therapy yet, molecular diagnostic algorythm involving KRAS determination can define a subgroup of tumors where no further diagnostic test is necessary due to the exclusivity of this driver oncogene mutation. Recent data indicated that the prognostic role of mutant KRAS in lung adenocarcinomas in Asian patients is evident, while more research is neccessary in non-Asian populations. Studies also suggest the potential predictive role of mutant KRAS in the context of chemosensitivity of NSCLC which may depend on the individual drug types. Recent data on the negative predictive role of KRAS mutation on the efficacy of EGFR tyrosine kinase inhibitor (TKI) therapies confirm previous findings.

Summary
Studies on the prognostic and predictive role of mutant KRAS in lung adenocarcinoma must be extended to the analysis of the potential role for allelic variants.

Keywords
allelic variants, KRAS mutation, lung adenocarcinoma, prediction, prognosticator

INTRODUCTION

KRAS protein is a member of the small GTPase (guanin triphosphate-ase) protein family and serves as a binary switch in signal transduction for most growth factor receptors including EGFR, MET or ALK. The human KRAS gene is located on chromosome 12.p12.1 encoded by six exons and is the most frequently mutated oncogene in humans: more than 80% of pancreatic cancers, more than 40% of colorectal cancers and around 30% of lung adenocarcinomas harbor activating mutations of the KRAS gene as one of the founder carcinogenic mutations of the genome [1]. The complexity of the function and regulation of KRAS protein may explain its significance as oncogenic driver (Fig. 1).

Mutation of the KRAS gene in lung- as well as colorectal or pancreatic cancers mostly occurs in exon 2 at codon 12, less frequently at codon 13 (3–5%) and more rarely at exon 3 codon 61 (less than 1%). The frequently mutated exon 2 codon 12/13 mutations affect the structure of the guanine nucleoside phosphate loop of the G-domain, resulting in stabilization of the interaction with GTP locking in the active state and inhibiting GTPase activity. The codon 61 mutation inhibits the GTPase potential of the protein resulting in the accumulation of the GTP-bound RAS protein. These mutations render KRAS protein constitutively active to stimulate effector proteins independent of the upstream growth factor receptor activity [2,3▪,4].

Interestingly, smoking carcinogenesis leaves a molecular fingerprint behind in KRAS, as G to

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Curr Opin Oncol 2014, 26:000–000
DOI:10.1097/CCO.0000000000000051

KEY POINTS

- Novel data demonstrate the differential functional consequences of the various amino acid substitutions in KRAS.
- The prognostic role of mutant KRAS in NSCLC must be reanalyzed based on mutation types (base changes and exon involvement).
- The predictive role of mutant KRAS in NSCLC must also be reanalyzed based on mutation types.

T transversion mutations are characteristic, unlike transition mutations (G to A) induced by other types of carcinogens. As a consequence, the base changes in KRAS in lung adenocarcinoma as compared with colorectal cancer are different, therefore, in lung cancers of smokers G12C, G12V and G12A amino-acid changes are the predominant in KRAS, while in nonsmokers G12D, G13D and G12S mutations [5].

This review systematically analyses recent literature on KRAS and lung cancer from molecular pathology to medical oncology, revealing key unresolved issues which collectively result in the dismail situation of KRAS-mutated NSCLC patients.

DIAGNOSTIC SIGNIFICANCE OF KRAS IN NONSMALL CELL LUNG CANCER

Molecular classification of adenocarcinoma of the lung is an emerging paradigm in pathology practice [6] and as national surveys have indicated, KRAS mutation is the most frequent genetic alteration in adenocarcinoma especially in patients with a smoking history among the non-Asian population [6,7]. However, this is the opposite in Asian patients, in case of whom EGFR mutation frequencies are higher as compared to KRAS [7]. Meanwhile, the debate seems to be continuing on two issues: association with a special histology and association with other driver mutations. In the past, several articles reported on KRAS mutations, though at much lower frequency, in squamous cell lung cancer. Detailed and proper analysis of this issue using up-to-date differential diagnostic criteria indicated that KRAS mutation does not occur in lung cancer of squamous histology [8▪▪]. In case it is detected, it represents an adenocarcinoma component in squamous cancer.

The other outstanding issue is KRAS mutations in combination with other mutations. This issue was also studied in the past and no novel data are available other than those indicating that double mutants (KRAS and EGFR or KRAS and ALK or EGFR and ALK) are extremely rare in lung

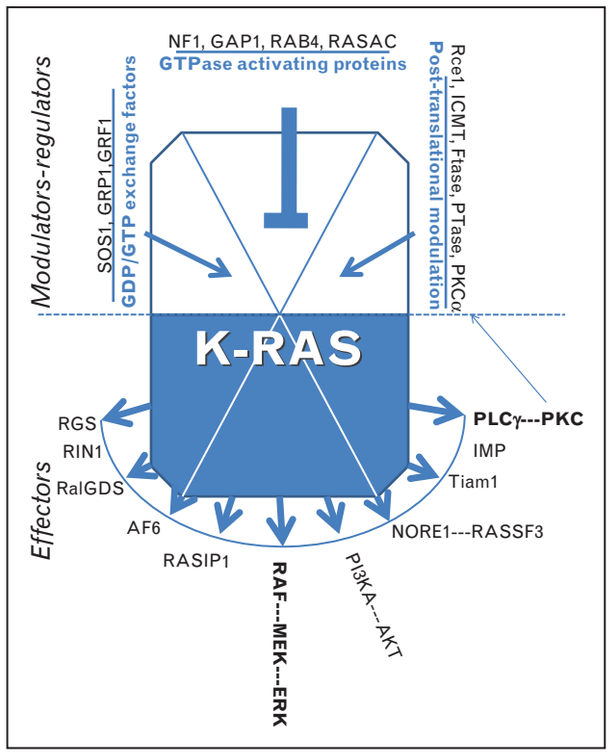

FIGURE 1. Schematic representation of KRAS functional modulators and effectors. Unlike other signal transducers, KRAS has close to a dozen various effector proteins, which are engaged in almost all important signaling pathways including MAPK-, AKT-, PLCγ-PKC- and several other less recognized ones suggesting that (k)-RAS protein may also serve as a signal distributor in growth factor receptor pathways [1]. It is less recognized that KRAS activity is regulated and modulated by another dozen of proteins. As activity of the KRAS protein is linked to its lipid membrane association, it is facilitated by proteolytic processing (Rce1), farnesylation/geranylation [farnesyl-/geranyl transferases, (FTase)], methylation (ICMT) or phosphorylation (PKCα). Splice variation also affects another membrane localization potential, since the unique cys179 in KRAS4A is palmitoylated [by palmytoil transferase, (PTase)]. Although most of the so-called posttranslational modifications of KRAS promote lipid membrane interactions, its phosphorylation by PKCα seems to be the only one as a negative regulator [1]. KRAS protein is a GDP/GTP binding protein, where only the GTP-RAS is active in signal transduction as well as a GTPase. In this case GTPase activity serves as an automatic delayed off-switch in this protein. Accordingly, positive and negative regulators of this activity are extremely important for the function of the wild type (and most probably for the mutant) protein. The activators of KRAS enhancing GDP/GTP conversion or GTP binding are called GEF (guanine exchange factors) proteins, which include SOS1, GRP1 and GRF1 while the physiological inhibitors are GTPase activator proteins (GAPs), with the best known being NF1 (mutation of which is responsible for neurofibromatosis), and others are GAP1, RAB4 and RASAC [1].

adenocarcinoma [9]. The exception is B-RAF mutation, which can occur together with KRAS in NSCLC unlike in colorectal cancer [9]. Based on these assumptions there are recent molecular pathologic recommendations for sequential testing of lung adenocarcinoma, [7,10,11▪▪] unfortunately none adjusted to the ethnicity of the patient population. Here, we propose an ethnicity-based testing algorithm in which the first step is to define the KRAS mutant and the larger molecular subgroup of non-Asian patients in which no further testing is required, followed by testing for a less frequent but predictive mutation as EGFR, then looking for ALK rearrangement. In case of Asian patients the rational alternative is to start with EGFR mutation testing as the larger molecular subgroup (Fig. 2). Although novel whole genome-sequencing technologies are available which can achieve parallel determination of several driver mutations, these have not yet been introduced into daily practice. In less developed countries or poorly reimbursed health systems the rational and cost-saving sequencial molecular testing is recommended.

Technology issues

The KRAS mutation status can be determined by various molecular technologies, which vary significantly in sensitivity from 0.1% (next-generation sequencing) to 20% (Sanger sequencing) [6]. Today no reliable data are at hand regarding the threshold, which could rationally be used to diagnose a KRAS mutant NSCLC. The presence of a minute mutant subpopulation in an otherwise wild type tumor population may have a questionable immediate clinical or biological significance.

On the contrary, some technologies allow the proper determination of the mutant/wild type ratio of KRAS alleles [12]. However, this ratio must be corrected for the T/N cell ratio as well, as this is also highly variable in NSCLC [10,11▪▪].

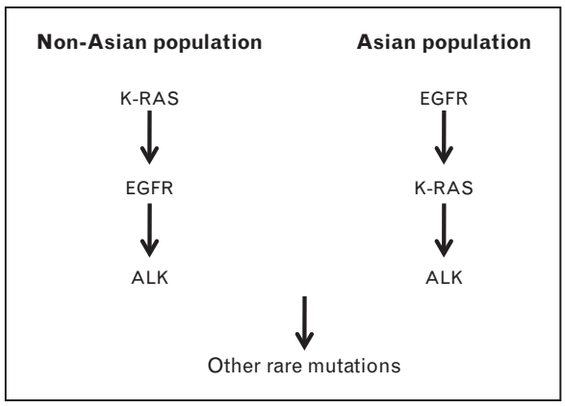

FIGURE 2. Ethnicity-based diagnostic algorithms for molecular pathology of nonsmall cell lung cancer (NSCLC) patients.

Heterogeneity by histology, by genetics

Lung adenocarcinoma represent a morphologically heterogenous class of lung cancer, in which the tumor is rarely monotonous, but more frequently heterogenous, accordingly recent guidelines recommend to report precisely the percentage of various histological types [6]. The question emerged if these histological variants are distinct genetically or not. This issue was analyzed in a small patient cohort in which KRAS and EGFR mutation statuses were determined in heterogenous tumors. Results indicated that despite the heterogenous morphology these subclones were identical genetically in respect to EGFR or KRAS mutations [13]. Whole genome sequencing was used to determine clonality status of primary lung adenocarcinoma with the finding that 50% of primary tumors were genetically biclonal [14]. Secondary tumors in lung cancer patients are relatively frequent and a clonality analysis was performed in a relatively small patient cohort. Molecular analysis indicated that 20% of EGFR wild type tumors were followed by a KRAS mutant secondary tumor and 15% of EGFR mutant tumors were followed by a KRAS mutant secondary tumor, indicating the emergency of a molecularly distinct clone in these patients [15]. There are no novel data at hand concerning the clonality fidelity of progressing lung adenocarcinoma: previous reports suggested a 20% alteration rate at metastatic sites [16]. In conclusion, clonality assessment of multiple areas in heterogenous lung adenocarcinoma is not recommended, but secondary tumors or metastatic sites are suggested to be retested for molecular classification due to a relatively high rate of possible alterations.

PROGNOSTIC SIGNIFICANCE OF KRAS IN NONSMALL CELL LUNG CANCER

There is an ongoing debate in the literature concerning the potential prognostic role of KRAS mutation in NSCLC. In the referred period, four original studies were published from Asian and non-Asian NSCLC patient populations. In non-Asian patients the KRAS mutant status was associated with a nonsignificant trend for progressive disease [17], while in another study [12] only c13 mutant tumors were associated with a nonsignificantly increased OS. On the contrary, in Asian patients two studies concluded that KRAS mutant status was found to be a poor prognostic factor for

overall survival [18,19]. The differential prognostic effect of KRAS mutation in Asian versus non-Asian NSCLC patient populations was also discovered by a recent meta-analysis, indicating that among Asian patients, unlike in non-Asians, KRAS mutant status is a significant poor prognostic factor [20▪▪]. The fourth study performed was a meta-analysis of four trial cohort NSCLC patients (nonselected for ethnicity) in which KRAS mutant status was again proved to be a neutral prognostic factor for survival [21], but was however, associated with a higher risk for secondary cancers. Based on these novel data it can be concluded that ethnicity of NSCLC patients is a significant factor meaning a controversy over KRAS mutation status. It can be concluded that among Asian NSCLC patients the KRAS mutant status is unquestionably a poor prognostic factor, while in non-Asian patients further retrospective and prospective studies are needed to discern the potential role in cancer progression.

PREDICTIVE VALUE OF KRAS IN NONSMALL CELL LUNG CANCER: CHEMOTHERAPY

A long-lasting debate, similar to the prognostic one concerns the potential predictive role of KRAS mutation status in case of lung adenocarcinoma treated with chemotherapy. Two studies analysed a heterogenously treated NSCLC patient population for the potential predictive role of the mutant KRAS status and found no association [12,22], though a potential negative effect of c13 mutations was suggested in one study [12]. Another study performed in Asian patients separated chemotherapies based on the individual protocols used [19] and revealed that in case of pemetrexed or gemcitabine protocols KRAS mutation status was associated with a significantly poorer response rate and progression free survival (PFS), which was not the case in protocols containing taxanes. Another meta-analysis was performed on a large trial-cohort of NSCLC patients treated with ACT protocol, revealing similarly to another study [12] that in patients with c13 mutations therapy response was significantly poorer, resulting in lower disease-free survival (DFS) and overall survival (OS) [21]. Collectively, these data suggest that KRAS mutant status could be a predictive factor for chemotherapy in NSCLC patients but further studies are need in two directions. Clearly, the type of chemotherapy as well as the exact type of KRAS mutations (codon involvement or specific amino acid substitutions) may play significant role, both of which must be rigorously evaluated before drawing conclusions.

PREDICTIVE VALUE OF KRAS IN NONSMALL CELL LUNG CANCER: TARGET THERAPY

The major debate over the predictive role of KRAS mutant status of NSCLC patients takes place in the field of EGFR target therapies. In the early years, this issue seemed to be simple as two studies and a meta-analysis suggested that KRAS mutant status is a significant negative predictor for EGFR tyrosine kinase inhibitor (TKI) therapy [23–25]. However, two recent studies have challenged these data; one performed in Asian patients exclusively [19] and the TAILOR trial performed on EGFRwt patients [26]. Among the Asian NSCLC patients, RR and PFS were found to be significantly poorer in EGFR TKI treated KRAS mutant patients as compared with KRASwt [19], but subgroup analysis of the EGFRwt patients found only a trend for better PFS in KRASwt patients compared with mutants. The TAILOR trial was designed to analyse the efficacy of Erlitinib + Docetaxel regime in EGFRwt NSCLC patients as compared with Docetaxel alone based on KRAS mutation or EGFR amplification statuses [26]. Influenced by 2011'ASCO recommendations [27▪▪] and interim analysis, KRAS and EGFR copy number determinations were discontinued at interim analysis therefore their predictive roles cannot be properly assessed. A recent analysis of the German Extended Access Program of IRESSA for the molecular characteristics of long-term responder patients revealed that EGFR activating mutation was a positive predictor, while KRAS mutation was a negative predictor for Gefitinib LTR [28]. A recent Phase-II trial of Dacotinib (an irreversible EGFR TKI) versus Erlotinib on advanced NSCLC patients contributed to this debate as well [29]. PFS of Dacotinib treated patients was superior in comparison to Erlotinib: in the EGFR mutant population, however, there was no difference in efficacy [29]. Further analysis of the molecular subgroups revealed that in KRAS wild type or KRAS and EGFR double wt populations Dacotinib significantly outperformed Erlotinib as far as the PFS was concerned, suggesting a positive predictive role for wild type KRAS status for EGFR TKI sensitivity. A commentary review of this trial summarized this debate, suggesting that the EGFR mutant group of NSCLC patients has the longest PFS and KRAS mutant (EGFRwt) patients are characterized by the worst PFS upon EGFR TKI treatment, while EGFRwt/KRASwt patients are between these two ends [30▪] (Fig. 3) A recent retrospective analysis of EGFR TKI treatment in various molecular subgroups of NSCLC patients further supported this opinion [31] but opened a novel area of research, revealing that the KRAS mutant resistant group of NSCLC patients can be further subdivided into

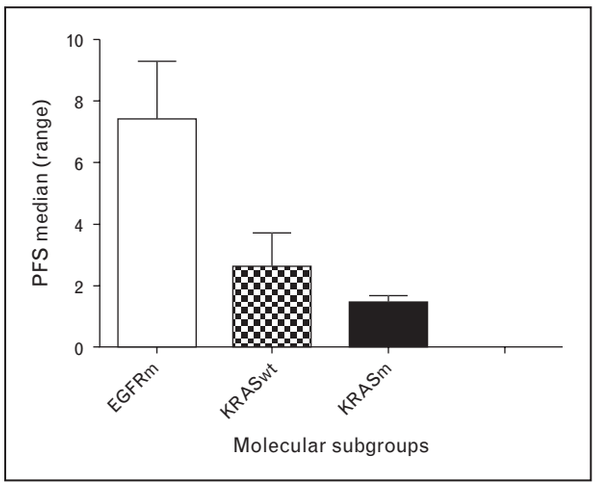

FIGURE 3. Progression-free survival of EGFR-TKI treated advanced nonsmall cell lung cancer (NSCLC) patients according to molecular subgroups [30▪]. Data are mean of published individual studies (+/− ranges) expressed in months.

heterogenous responders to EGFR TKI based on the KRAS codons involved and the type of amino acid substitutions. This is a novel area of research which may affect future studies and which may explain the controversy over the role of mutant KRAS in NSCLC.

As compared to EGFR TKIs, anti-EGFR antibody therapy is not a success story for the target therapy of NSCLC. Cetuximab is weakly active in NSCLC and mostly in the adenocarcinoma subgroup and its effect is independent of the molecular status of the tumor including KRAS mutation [32]. These data are completely contradictory to the paradigm present in colorectal cancer in which case anti-EGFR monoclonal antibody therapies are the standard, in which mutant KRAS is the strongest (negative) molecular predictor [33].

NSCLC also seems to be different from colorectal cancer in terms of acquired resistance to EGFR target therapies. In colorectal cancer patients one dominant resistance mechanism is the emergence of the mutant KRAS subclone in the treated predominantly wild type tumor population [34▪,35▪]. In NSCLC various types of molecular mechanisms other than KRAS mutation seem to be operational during the development of resistance to EGFR TKI including development of novel EGFR resistance mutation (T790 M), HER2- or MET amplifications [36].

Studies from the late 90s suggested that various amino acid substitutions in KRAS induced by oncogenic mutations variably affect chemical and biological characteristics [1]. Using contemporary technologies, this issue was recently revisited [2,3▪,4]. KRAS is active in GTP-bound form, therefore the GDP/GTP exchange rate is key factor but oncogenic mutants are heterogeneous in this respect. Oncogenic mutations lock KRAS in the active state as the GTPase switch does not function properly, but this inactivity is also heterogenous in various oncogenic mutants. Accordingly, it did not come by surprise that sensitivity to GEF and GAP activators of the various KRAS mutants is also heterogenous. (Table 1.) In conclusion, based on the variability of the biochemical potentials of various KRAS mutants, it is irrational to accept homogenous functional activity in NSCLC or in other cancer types. Clinical data have already started to accumulate on the heterogeneous role of various KRAS mutants in NSLC patients, but these studies must be performed systematically. Since large NSCLC patient databases are now available in which the mutational statuses and chemo- and target therapy efficacies are documented, these must be analysed first before major prospective randomized marker studies are performed.

THERAPY OF KRAS MUTANT LUNG CANCER: NOVEL AGENTS, FUTURE PERSPECTIVES

KRAS mutant NSCLC remains a clinical challenge as far as therapy is concerned, due to a relative

Table 1. Functional differences between RAS mutation types [1,3▪,4]

		Amino acid changes		
Codon	**G12V**	**G13D**	**Q61L**	**Q61L**
Functional consequences				
GDP/GTP exchange	Decreased	Extremely high	Increased	Increased
GEF sensitivity	Decreased	Maintained	Maintained	Maintained
GAP sensitivity	Lost	Increased	Increased	Increased
GTPase activity	Minimal	Decreased	Minimal	Minimal

GEF, GDP/GTP exchange factor; GAP, GTPase activating protein.

resistance to standard chemotherapeutic protocols and to an absolute resistance to EGFR targeted TKI therapies. This special patient group is now the target of various phase I-II-III clinical trials involving developmental therapeutics from immunotherapies to novel target therapies. In the absence of clinically available selective inhibitors of mutant KRAS, one feasible approach is to target the main signaling pathways controlled by the constitutively active mutant KRAS, including RAF-MEK-ERK or PI3K-AKT-mTOR. In the Battle trial chemorefractory, NSCLC patients were randomized for EGFR TKI, VEGFR TKI or B-RAF inhibitor [37] based on the molecular status (EGFR or KRAS mutation). Interestingly, the B-RAF inhibitor, Sorafenib, was proved to be clinically active in various molecular subgroups but most importantly in the KRAS mutant patient population [37]. Although selumetinib, a MEK inhibitor in monotherapy was clinically inactive in KRAS mutant NSCLC patients, in combination with docetaxel it exhibited an impressive clinical activity as second line treatment for Stage III-IV patients (significant improvement in PFS and OS) [38]. This encouraging result may facilitate the initiation of similar trial settings in which other RAS-effector pathway inhibitors, such as mTOR, will be the target arm of chemotherapy combinations for KRAS mutant NSCLC patients.

However, the future of KRAS mutant cancer patients remains largely in the hands of drug designers. Previously mutant RAS was considered as nondrugable owing to several unsuccessful chemical approaches from farnesyl transferases to GTP analogues. This trend seemed to be broken in the past years. Using structure based drug design technology, various RAS-binding proteins (SOS1, C-RAF1, PDEd) were used to design novel small molecular inhibitors of mutant RAS [39▪–41▪]. Some of these RAS-inhibitors (Kobe0065, deltarasin) exhibited impressive preclinical activity in KRAS mutant tumor models [40▪,41▪]. Meanwhile, the future of these agents will clearly depend on their clinical activity in KRAS mutant NSCLC patients.

CONCLUSION

All above data confirm that KRAS mutation is the hallmark of lung adenocarcinoma or the adenosquamous mixed variant. Recent data indicated that the prognostic role of mutant KRAS in lung adenocarcinomas in Asian patients is evident, while more research is necessary in non-Asian populations. Studies also suggest that the potential predictive role of mutant KRAS in the context of chemosensitivity of NSCLC may depend on the individual drug types. Recent data on the negative predictive effects of KRAS mutation on efficacy of EGFR TKI therapies confirm the previous findings. Determination of KRAS mutation status of NSCLC is useful in defining patient populations where no further molecular testing is necessary due to the exclusive driver role of this oncogene.

Acknowledgements

None.

Conflicts of interest

Grant support: Roche Hungary kft, Boehringer-Ingelheim kft. Honoraria: Roche Hungary kft, Boehringer-Ingelheim kft, Pfizer Hungary kft.

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<AQ2>	As per journal style, all annotated reference numbers must have bullets, and all reference numbers with bullets must have annotations. Please provide annotations for ref. [39,40].	Annotation at the end refers to all marked references 39,40,41