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Targeting the KRAS variant for treatment of non-small cell lung cancer: potential therapeutic applications

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Lung cancer is the leading cause of cancer deaths worldwide, with non–small cell lung cancer (NSCLC) accounting for 80% of all lung cancers. Kirsten rat sarcoma viral oncogene homolog (KRAS) is one of the deadliest cancer-related proteins and plays a pivotal role in the most aggressive and lethal human cancers, including lung adenocarcinoma where it represents one of the most frequently mutated oncogene. Although therapeutic progresses have made an impact over the last decade, median survival for patients with advanced lung cancer remains disappointing, with a 5-year worldwide survival rate of <15%. For more than 20 years it has been recognized that constitutively active signaling downstream of KRAS is a fundamental driver of lung tumorigenesis. However, years of pursuit have failed to yield a drug that can safely curb KRAS activity; up to now no approved therapies exist for KRAS-mutant NSCLC. The aim of this review is to discuss the current knowledge of KRAS-mutated NSCLC, touching upon KRAS clinical relevance as a prognostic and predictive biomarker, with an emphasis on novel therapeutic approaches for the treatment of KRAS-variant NSCLC.

KEYWORDS: NSCLC • KRAS • signal transduction • selumetinib • synthetic lethality • mutant allele-specific imbalance • prognosis • prediction • targeted therapy

RAS biology and its clinical relevance in human tumors

The RAS genes are members of a multigene family with high-sequence homology and highly conserved amino acid sequences that suggest their essential cellular functions. The human RAS genes family consists of three members: HRAS, KRAS and NRAS. These genes encode four closely related proteins of about 190 amino acids and a molecular weight of 21,000 Da (HRAS, KRAS4A and KRAS4B resulting from alternative spicing, NRAS).[1]

In the primary structure of Ras proteins, four domains can be clearly identified: the first two, encompassing the first N-terminal 160 amino acids, represent the catalytic domain and are almost completely conserved among mammalian RAS gene products. The third domain constitutes the so-called hypervariable region, which is the less conserved part of the protein and presumably responsible for the different functions of RAS proteins; the fourth domain, at the COOH-terminal extremity, is constituted by the highly conserved four amino acid motif CAAX (C stands for cysteine, A for any aliphatic amino acid and X for any uncharged residue) responsible for membrane trafficking and anchorage.[2]

The mature RAS are GTPase membrane-bound proteins that operate as cellular switch conducting the signals from cell membrane receptors to the nucleus through the activation of different signaling pathways. They are physiologically involved in fundamental cellular processes such as cell proliferation, differentiation and survival, so their function is subject to a strictly regulatory system that acts through the control of its catalytic function. Similarly to other G proteins, RAS swing from the GTP-bound active form to the GDP-bound quiescent form and vice versa. These transitions are associated with conformational change of the
protein structure and a modification of its position with respect to the cell membrane.[3,4] Two main classes of proteins regulate RAS activity: the guanine nucleotide exchange factors (GEFs) and the GTPase activating protein (GAPs). GEFs facilitate GDP dissociation and GTP binding, thus promoting the active GTP-bound form of RAS proteins. GAPs, with opposite functions, stimulate the GTPase activity of RAS favoring the rapid conversion from the active to the inactive GDP-bound RAS.[5,6]

The relation between RAS mutation and cancer is clearly recognized. The most frequent activating point mutations of RAS mammalian genes involve codons 12, 13 or 61. These mutations are linked to an aberrant constitutively activated RAS protein that induces malignant cell transformation.[7–9] Indeed, RAS mutations are responsible for the abolishment of the GAP-induced GTP hydrolysis of RAS, thereby leading to the maintenance of the GTP-bound active form of the protein [10,11] or for the impairment of the active site conformation and consequently of the intrinsic hydrolysis activity.[12]

The early analyzes of a variety of human tumor samples revealed that all the three RAS genes are frequently mutated at critical positions. Moreover, these analyzes also showed that the incidence of RAS mutation varies in different tumor types and that different cancers are associated with specific mutated RAS member.[1,9]

The Catalog of Somatic Mutations in Cancer (COSMIC) is the broader database of cancer somatic mutations developed from large-scale tumor profiling. Through this database, Prior et al. recently reanalyzed the mutational spectra of RAS in human tumors. Although they confirmed previously data indicating that KRAS is the most frequently mutated gene (22%), followed by NRAS (8%) and HRAS (3%),[13] they did not confirm the overall mutation rate of RAS, which ranged approximately 30% in the COSMIC data set. Of note, this value was distorted by a screening bias that is particularly evident for KRAS as colorectal cancer represented the larger sample group in the COSMIC data set. In fact, when all cancers were counted in equal weighting, the average pan-RAS mutation incidence was only 16%.[11] Besides the well-known mechanism of activating mutations, it should be worth mentioning the role of amplified wild-type (WT) RAS genes as mechanism of malignant transformation using cell lines and murine models.[14] Recently, copy number gain of WT RAS genes has been associated with the development of different epithelial cancers, namely testicular germ cell tumors, lung, kidney, stomach, nasopharynx and ovarian cancer.[15]

**KRAS mutation in non-small cell lung cancer**

KRAS accounts for 90% of RAS mutations in lung adenocarcinomas and represents the most commonly mutated oncogene in non-small cell lung cancer (NSCLC), affecting nearly 25–30% of western patients.[9]

In 97% of cases, KRAS mutations involve exon 2 at codon 12; the rest of them involve codon 13 and occasionally exon 3 at codon 61.[16] KRAS mutations at the exon 2 codons 12 and 13 (G12, G13) result into a conformational change of the 3D structure of the guanine nucleoside phosphate loop of the catalytic domain, which in turn stabilizes the interaction with GTP, consequently maintaining KRAS-dependent downstream pathways constitutively activated.[9] In addition, G12 and G13 oncogenic substitution prevent the formation of van der Waals bonds between KRAS and the GAPs through a steric interference, thus disturbing the correct orientation of the catalytic glutamine (Q61) in KRAS protein, which contributes to the inhibition of GTP hydrolysis.[17] Similarly, exon 3 codon 61 mutation constitutively activates KRAS downstream effectors through the impairment of GTP hydrolysis reaction by hindering the coordination of a water molecule that is required for the nucleophilic attack on the GTP γ-phosphate.[18] Importantly, even though all KRAS mutations involve the same 3D domain in the folded protein, molecular modeling studies of KRAS protein have recently highlighted that not all mutant KRAS proteins affect patient survival and downstream signaling in a similar way.

Transcriptome microarray studies of patient tumor samples and reverse-phase protein array studies of a panel of 67 NSCLC cell lines with known substitutions in KRAS and in immunolated human bronchial epithelial cells expressing different mutant KRAS proteins revealed that different conformations forced upon by mutant KRAS bring about to a different association with downstream signaling transducers.[19] For instance, KRAS mutation G12C and G12V preferentially bind to the Ral guanine nucleotide dissociation stimulator, while KRAS G12D has a stronger affinity for PI3K. As a consequence, NSCLC cell lines harboring KRAS G12D mutation predominantly activate PI3K and MEK signaling, whereas those harboring KRAS G12C or KRAS G12V alternatively activate Ral downstream pathway with a decreased growth factor-dependent Akt activation. Moreover, in vitro studies showed that KRAS-variants G12V and G12R exhibit greater transforming ability.[20] Nonetheless, once occurred, particular amino acid substitutions are more aggressive than others. For instance, mice with KRAS-variant G12V, G12R and G12D presented higher-stage lung tumors compared with KRAS G12C and WT KRAS.[19] More recently, Garassino et al. generated KRAS-overexpressing clones harboring the three most common KRAS amino acid substitutions in NSCLC (G12C, G12V and G12D) from human NSCLC cell line NCI-H1299 with WT EGFR. Interestingly, they reported that G12C variant was associated with a reduced response to cisplatin and an increased sensitivity to taxol and pemetrexed. Conversely, the expression of G12D mutant resulted in resistance to taxol treatment and sensitivity to sorafenib. The same authors also showed that G12V mutant yielded a strong sensitivity to cisplatin compared with WT clones and a slight resistance to treatment with pemetrexed. [21] Moving to clinical impact of the different KRAS mutations, Ihle et al. reanalyzed data form the BATTLE trial (a randomized phase II, single-center, open-label study in patients with advanced NSCLC refractory to prior chemotherapy, randomly assigned to oral treatment with erlotinib, vandetanib, erlotinib
plus bexarotene or sorafenib) to explore the associations between mutant KRAS with specific amino acid substitutions and patient survival, showing that either mutant KRAS G12C or mutant KRAS G12V were associated with a shorter progression-free survival (PFS) compared with other mutant KRAS or WT KRAS, 1.84, 3.35 and 1.95 months, respectively (p = 0.046). [22] Not surprisingly, the decrease in PFS was most pronounced in patients harboring either Cys or Val substitution at codon 12 in the sorafenib treatment arm (p = 0.026), as preclinical data indicate that G12C and G12V mutations preferentially activate the Ral downstream signaling rather than MEK or PI3K/AKT. [19,22] However, in vitro and in vivo data suggested a greater oncogenic potential for KRAS mutant G12V (present in approximately 20% of KRAS-mutated NSCLC) compared with other mutations.[23] Worth mentioning is another but unusual KRAS mutation, namely Q22K, which have been detected only in few other cancers such as colon, pancreas, CNS and hematopoietic/lymphoid with a reported incidence lower than 1% for each of these malignancies.[24] Recently, the authors’ group described for the first time a novel KRAS Q22K mutation with co-existing KRAS polysomy in a patient affected by enteric-like lung adenocarcinoma.[25] KRAS Q22K mutation consists of a C > A transversion substituting lysine (AAG) for normal glutamine (CAG) codon and stabilizing KRAS into a constitutively activated GTP-bound form.[26]

As previously mentioned, KRAS mutations develop more frequently in adenocarcinoma histology (20–30%) and only occasionally in squamous cell carcinoma (7%).[27] Mutations in the KRAS gene also display ethnicity imbalance, being more frequent in white populations rather than Asians (25–50% vs 5–15%, respectively). Furthermore, most patients are current or former cigarette smokers. Smoking-associated KRAS mutations (G12C, G12V, G12A) are transversion mutations (G>T or G>C), whereas KRAS transitions mutations (G>A) are more common in lung adenocarcinomas from never-smoker patients.[28–30] Specifically, cigarette smoking-associated carcinogens leave behind a molecular signature, presumably as a consequence of exposure to tobacco’s polycyclic aromatic hydrocarbons, thus explaining the higher prevalence of transversion mutation in NSCLC smoker patients compared with NSCLC never-smoker and other cancers, such as colon and pancreatic. [31] It should be underlined that despite KRAS mutations having always been supposed to be linked to tobacco exposure they occur in nearly 15% of lung adenocarcinomas from never-smokers patients; therefore, KRAS mutational status cannot be thoroughly predicted on the basis of smoking history alone.[28] Of note, by genotyping 3026 lung adenocarcinomas for the major EGFR (exon 19 deletions and L858R) and KRAS (G12, G13) mutations and examining correlations with demographic, clinical and smoking history data, Dogan et al. reported a higher percentage of the female patients with tumors harboring KRAS G12C transversion. Interestingly, the younger age at diagnosis and the fewer pack-years of smoking in women with this KRAS mutation, compared with men with the same mutation, provide evidence that women are more susceptible to tobacco carcinogens.[32] This enhanced susceptibility to tobacco carcinogenesis probably reflects the constitutive variation in genes encoding tobacco carcinogen-metabolizing enzymes, such as the GSTM1 polymorphism of cytochrome P450 phase I detoxifying enzyme CYP1A1, which shows higher expression in the normal lung tissue of female smokers than male smokers.[32,33]

Going beyond the aforementioned activating point mutation, a number of studies recently unveiled a novel potential oncogenic mechanism of KRAS in NSCLC, which resides in an increased copy number of the KRAS gene.[34] KRAS gene amplification frequently occurs in a subset of patients harboring KRAS point mutation and is associated with increased mutant allele transcription and gene activity. The combination of these molecular events results in an imbalance between the mutant allele and the WT allele. Of note, when mutant allele prevails over the WT one, this scenario is defined as mutant allele-specific imbalance, namely mutant allele-specific imbalance (MASI).[35] The incomplete dominance of M over WT is most frequently a result of selective amplification of M, although it may also represent a consequence of the selective contribution of M in the absence of WT, likewise an acquired uniparental disomy, which commonly leads to complete MASI.[36]

As opposed to KRAS point mutation, the significance of KRAS gene amplification is still relatively unknown. Although considered rather uncommon, current studies employing high-resolution techniques to map human genome reported KRAS gene amplification in 15% of NSCLCs and found that amplification was more likely in patients harboring KRAS point mutation.[34–38] Moving to the clinicopathological implication, Wagner et al. reported that KRAS amplification is associated with larger and less differentiated tumors and angiolymphatic invasion. Importantly, these differences reached the statistical significance in the subgroup of adenocarcinomas with KRAS point mutation, thus supporting the hypothesis of a synergistic relationship between point mutation and copy gain. [38] Whether KRAS amplification has a prognostic impact, affecting NSCLC patients survival is still controversial and will be handled further along.

**Prognostic significance of KRAS mutation in NSCLC**

Although initial studies indicated that KRAS mutations are a negative prognostic factor in NSCLC, their role remains hardly debated. In the early 1990s, Slebos et al. first evaluated KRAS mutational status as a prognostic factor in 69 early-stage (stage I and II) NSCLC patients who underwent complete resection. The presence of KRAS point mutation identified a subset of patients (19/69, 27.5%) with poor prognosis despite a complete resection, achieving a significantly shorter disease-free survival (DFS) (p = 0.038) and overall survival (OS) (p = 0.002). Worthy of note, the difference was consistent after adjustment for disease stage, tumor size and differentiation, thereby upholding the presence of KRAS mutation as the single most important prognostic factor in patients harboring the mutation.[39] A later study assessed the mutational status of KRAS in 184 complete resected patients with stage II and IIIA NSCLC who were...
randomly assigned to adjuvant radiotherapy with or without chemotherapy. Patients with KRAS mutation 44 (23.9%) experienced a shorter OS (30 vs 42 months; p = 0.38) but no differences in DFS were observed. Interestingly, mutated KRAS patients with N1 disease performed worse OS compared with KRAS WT patients (23.6 vs 45.2 months; p = 0.02).[40] More recently, in a prospective study of 365 patients with radical resected early-stage NSCLC, KRAS point mutation was strongly related with a decreased patient survival (p = 0.009). Notably, stratified analysis by pathological staging classes unveiled that this association was statistically significant for stage I disease only (p = 0.002) and multivariate analysis confirmed that the KRAS mutation was a statistically significant independent predictor of poor outcome after adjustment for the effects of age, sex and disease stage.[41] Woo et al. examined 190 cases of stage I lung adenocarcinomas for KRAS mutations and Ki-67 expression reporting a significantly higher risk of recurrence for KRAS-mutated cases than cases without mutations (p = 0.004). Of note, patients with both c high-expression and KRAS mutations achieved an additional higher risk of recurrence compared with Ki-67 low expressers patients without KRAS mutations (p < 0.001).[42] A further study in Japanese patients established that KRAS mutations represent a poor prognostic factor for OS in patients with resected stage I adenocarcinomas (5-year OS: 70.0 vs 86.6%; p < 0.01).[43] Remarkably, recent additional investigations confirmed that the KRAS-mutated patients have a poorer survival compared with KRAS-negative group in Asian population, thus suggesting a differential negative prognostic effect of KRAS mutation in Asian patients versus non-Asian, especially in early-stage adenocarcinomas.[24–44] Conversely, one of the most recent studies on radically resected early-stage NSCLC showed that the presence of KRAS point mutations did not carry a prognostic value, even though patients harboring concurrent TP53 mutation and KRAS experienced a poorer survival [hazard ratio (HR): 3.26; 95% CI: 1.07–9.90; p = 0.038].[45] Similarly, another study on Japanese patients with surgically resected lung adenocarcinomas did not find any prognostic significance for TP53 and KRAS mutations.[46] Again, Shepherd et al. reported no statistically significant prognostic value for KRAS mutations in 300 completely resected early-stage NSCLC patients form four trials of adjuvant chemotherapy.[47] When it comes to advanced disease, KRAS mutational status has been widely investigated as a negative prognostic factor with inconsistent results. In 1991, Mitsudomi et al. reported that KRAS mutations were associated with shortened survival in 45 patients with advanced NSCLC (p = 0.0103).[27] Bonanno et al. subsequently found that KRAS mutations was an independent negative prognostic factor in multivariate analysis in advanced stage lung adenocarcinoma (HR: 3.52; 95% CI: 1.39–8.9; p = 0.008).[48] Johnson et al. evaluated 1036 patients with stage IV lung adenocarcinomas for KRAS and EGFR mutational status. Among them, 275 had EGFR mutations (27%) and 241 harbored KRAS mutations (23%), whereas 520 were KRAS/EGFR WT (50%). In multivariate analysis, KRAS mutations were associated with a shorter OS (p = 0.048).[49] These findings confirmed and expanded upon those of two previous studies showing that KRAS mutant stage IIIb/IV NSCLC patients experienced a poor OS.[50,51] In contrast, Keohavong et al. found no association of KRAS mutation and survival in 173 lung adenocarcinomas and adenosquamous tumors.[52] Consistently, another study evaluating the prognostic role of a six-biomarker panel, including KRAS mutations, showed no influence of KRAS mutational status on OS (p = 0.517) in NSCLC patients.[53] Therefore, in light of the variable results from individual studies, Mascaux et al. performed a meta-analysis of 28 studies with the aim to assess the prognostic significance of the KRAS mutational status on survival in NSCLC. In this study, the presence of KRAS mutations was a negative prognostic factor for OS (p = 0.01). Furthermore, in lung adenocarcinoma KRAS mutation was confirmed to be significantly prognostic for a shorter OS (HR: 1.52; 95% CI: 1.30–1.78; p = 0.02) but not in squamous histology (HR: 1.49; 95% CI: 0.88–2.52; p = 0.48).[54] Beyond the prognostic significance of KRAS mutational status, a growing body of evidence is bringing out the specific contribution of subtype-specific KRAS mutations on survival and treatment response. In 1996, Keohavong et al. described differences of survival rates in 173 NSCLC patients according to the presence and type of KRAS mutation. Among them, 44 harbored a KRAS mutation (24.9%). Although KRAS mutational status did not correlate to a poorer survival (p = 0.96), by examining the different types of KRAS mutation these authors found an unexpected trend towards a poorer prognosis for patients with the substitution G12V and G12R compared with WT KRAS or other amino acid substitutions (p = 0.07). Conversely, patients carrying the G12D substitution experienced a better outcome than the G12V or G12R substitution (p = 0.06).[52] However, a later study has shown a significant prognostic effect on DFS (p = 0.004) and OS (p = 0.008) for G12V mutations, contradicting the previous finding.[47] Similarly, Cserepes et al. reported a modestly longer median PFS in the G12V mutant patient compared with other amino acid substitution.[55] Analogous findings have been reported in resected stage I lung adenocarcinoma by Izzard et al., who observed better DFS associated with G12C/G12V mutations compared with other amino acid-specific KRAS mutations (p = 0.0271) with a trend towards improved OS (p = 0.0636).[56] In the relapsed metastatic setting, the analysis of KRAS amino acid substitutions in patients enrolled in the BATTLE trial demonstrated that G12C or G12V substitutions at codon 12 were associated with significantly worse PFS compared with the other KRAS substitutions or WT KRAS.[19] Moreover, emerging data suggest that KRAS mutations at codon 13 seem to confer a poor prognosis in advanced NSCLC.[19,47,55,57] Recently, the authors found that among patients with KRAS mutation those harboring a mutation at codon 13 perform worse in terms of OS and PFS whether they received first-line platinum-based chemotherapy or EGFR tyrosine kinase inhibitors (TKIs).[58,59] However, data are still inconclusive and larger case series are needed to address the difference in clinical
outcome according to KRAS subtype mutations. More interestingly, recent studies also unveiled an unexpected prognostic value of KRAS mutant MASI in NSCLC. In a large series of KRAS mutated lung adenocarcinomas, Chioscea et al. showed that patients harboring KRAS mutant MASI had a worse OS. Of note, the subgroup analysis revealed that the negative prognostic significance of KRAS mutant MASI was independent of disease stage and confirmed in stage I patients. Accordingly, Villaruz et al. showed that KRAS mutant MASI was associated with a markedly inferior DFS compared with the absence of MASI, thus mirroring the adverse OS with MASI observed in the aforementioned study. Consistently with these findings, Sasaki et al. reported a significantly worse prognosis for KRAS polysomy or amplified patients compared with KRAS disomy patients.

**KRAS as a predictive factor in NSCLC**

A great improvement in treatment strategies has been obtained since the discovery of specific molecular alterations that paved the way to personalized medicine. Thus, activating mutations of the EGFR or gene rearrangement of anaplastic lymphoma kinase (ALK) have radically changed the way to approach patients with advanced disease as they allow the use of specific TKIs. Unfortunately, the majority of NSCLC patients carry an EGFR and ALK WT genotype; therefore, platinum-based chemotherapy still represents the standard of care.

The role of KRAS mutations as a predictive factor of treatment response has been extensively studied, but no conclusive data have been obtained.

**KRAS and chemotherapy**

The role of KRAS mutation as biomarker of sensitivity to chemotherapy is still debated. Historically, patients with KRAS-mutated NSCLC were considered rather resistant to anticancer treatment compared with KRAS WT patients. However, early studies failed in demonstrating a predictive value of KRAS mutation, probably because of the retrospective nature of these studies, the lack of control groups and the heterogeneity of samples.

A recently published meta-analysis of 10 studies, involving 1677 patients with advanced NSCLC treated with chemotherapy, showed that patients harboring KRAS mutation had a statistically significant lower objective response rate (ORR) compared with WT patients (25.1 vs 34.4%; odds ratio: 0.67; 95% CI: 0.50–0.88; p = 0.004), whereas no significant differences were observed in terms of PFS.

Interestingly, the authors’ group recently reported a significant lower ORR in KRAS-mutated NSCLC patients with advanced disease receiving first-line platinum-based doublet therapies compared with KRAS WT/EGFR WT group, although no differences in terms of disease control rate (DCR), PFS and OS were obtained. However, significantly shorter PFS and OS were found when the KRAS-mutated group was compared with the KRAS WT/EGFR WT/ALK WT group. Nevertheless, the multivariate analysis for PFS showed significant shorter PFS in KRAS mutant group along with well-known poor prognostic factor (poor performance status and stage IV at diagnosis). Moreover, treatment outcome was not associated with the type of KRAS mutation (mutated codon, common mutation variant at codon 12 and type of DNA substitution).

The TAILOR trial is a prospective randomized trial that aimed to evaluate the role of KRAS as a predictive factor in NSCLC treated with erlotinib or docetaxel in second-line setting. The results of this study showed that the presence of KRAS mutation did not adversely affect PFS and OS, thus not showing a predictive value for either docetaxel or erlotinib. Similarly, Kalikaki et al. evaluated the clinical outcome of stage IIIIB–IV NSCLC patients receiving both platinum and non-platinum-based first-line chemotherapy according to KRAS mutational status. They found no differences in terms of ORR, PFS, time to progression and OS between KRAS-mutated and WT patients. Besides, no differences were noticed on the basis of the type of therapy received. Another study failed to demonstrate differences in terms of ORR, DCR and PFS between mutated and nonmutated KRAS NSCLC patients receiving platinum-based treatment for advanced disease. Again, no differences were observed according to the chemotherapy regimen received. Additionally, another retrospective analysis conducted by Sun et al. demonstrated a limited predictive role of KRAS mutation on the clinical efficacy of different cytotoxic chemotherapy regimens.

In the adjuvant setting, two studies have tried to define the role of KRAS mutational status and response to chemotherapy. The JBR.10 clinical trial was a phase III prospective study for adjuvant chemotherapy versus observation in resected stage IB–II NSCLC patients. A retrospective analysis from this trial showed no benefit from adjuvant chemotherapy in KRAS-mutated patients (HR: 0.95; p = 0.87). Shepherd et al. recently evaluated the potential role of KRAS as predictive biomarker from four randomized trials of adjuvant platinum-based treatment in NSCLC patients, showing no differences in terms of OS and DFS according to the presence of KRAS mutation.

When it comes to the predictive role of the different KRAS mutations, a preclinical study demonstrated differences in sensitivity to chemotherapy according to different KRAS mutations in KRAS overexpressing NSCLC clones. In this study, KRAS G12C mutant was associated to a reduced sensitivity to cisplatin but increased responsiveness to pemetrexed and taxol, whereas the G12V-expressing clones exhibit an increased sensitivity to cisplatin. This study has supported the intriguing idea of a differential sensitivity pattern to currently available anticancer treatments according to different KRAS mutations. In clinical setting, several studies have investigated this issue, but no statistical differences were observed for codon 12 mutations, despite Garassino et al. having reported a worse trend in PFS on platinum-based chemotherapy for G12V variant. Finally, recent findings indicate that patients with codon 13 KRAS mutation experience a worse response to chemotherapy, both in early-stage and advanced NSCLC.
KRAS and EGFR TKIs

The role of mutated KRAS as a predictive factor for EGFR TKIs has been an area of great interest among researchers. Early retrospective studies published on this issue analyzed the role of mutated KRAS in patients treated with EGFR TKIs compared with placebo or best supportive care.[67–72] Although these studies found a lack of response to EGFR TKIs in KRAS-mutated patients, these results could not be considered conclusive because these were achieved from a retrospective trial and with a lack of data regarding PFS and OS.

More recently, two meta-analyses have explored this relationship. Linardou et al. considered 17 studies where 165 of 1008 patients had mutated KRAS. In this study, the presence of KRAS mutations was significantly associated with an absence of response to EGFR-targeted therapy in both colorectal cancer and NSCLC.[73] On the other hand, Mao et al. conducted a meta-analysis of 22 studies consisting of 1470 NSCLC patients, of whom 231 had KRAS mutations and reported a poorer outcome for KRAS mutant patients treated with EGFR-TKIs.[30] Both meta-analyses suggest that KRAS mutation may act as a negative predictive biomarker for tumor response in NSCLC patients treated with EGFR-TKIs. It should be emphasized that in both studies the group of KRAS WT patients incorporates EGFR-mutated patients. However, it was not possible to determine the relation between KRAS mutation status and PFS and OS because of insufficient data.

It is well recognized that EGFR mutations and KRAS mutations are usually mutually exclusive[67,74] and that the main predictor of response to EGFR TKIs is represented by the activating EGFR mutations. Whether KRAS mutations play as role in predicting response to EGFR TKIs in the group of EGFR WT patients, where the magnitude of the effect played by these drugs is not yet defined, is an area of great interest. In this regard, the authors recently reported that the clinical benefit achieved with EGFR TKIs in EGFR WT patients is strongly connected with a KRAS WT genotype, as patients harboring KRAS mutations experience a shorter PFS.[58]

The role played by specific KRAS mutation on clinical outcome with EGFR TKIs has also been investigated. In a preclinical setting, Garassino et al. showed that specific amino acid substitution may portray a role in response patterns to standard chemotherapy as well as targeted therapies.[21] In the aforementioned study, no differences were found in terms of ORR and DCR when comparing the response to EGFR TKIs among codon 12 and codon 13-mutated KRAS. However, codon 13-mutated patients experienced a significantly shorter PFS and OS compared with codon 12-mutated patients and KRAS WT group.[58]

Targeting KRAS in NSCLC

In the era of personalized medicine, cancer research is focusing on genomic alterations of protein-coding genes that may be predictive of greater or lesser response to anticancer treatments. Although the discovery of specific genetic alterations such as EGFR mutations, ALK and ROS1 rearrangements has enormously improved the clinical outcome of patients harboring such mutations, targeting KRAS variant in NSCLC remains an unsolved issue.

KRAS mutations were identified in lung cancer >30 years ago, thenceforth no effective treatments for patients harboring this type of disease have been developed and KRAS mutant NSCLC still remains an elusive target for cancer therapy despite the efforts made so far. Initially, researchers developed farnesyl transferase inhibitors (FTIs) (e.g. lonafarnib, tipifarnib) with the aim to avoid a pivotal post-transcriptional modification in immature KRAS protein impeding isoprenylation. Considering that farnesyl residues are necessary to connect KRAS to inner cell membrane, these inhibitors have been hypothesized to be effective in blocking KRAS activity.[75] Unfortunately, although in vitro studies and preclinical animal models showed a significant inhibition of KRAS oncogenic activity, in clinical setting only a little efficacy was reported, probably because of the rise of compensatory post-transcriptional alteration.[76] Similarly, a novel class of FTIs (e.g. salirasib) containing farnesylcysteine failed to induce radiographic response or increase survival in a phase II NSCLC trial.[77] The failure of FTIs-based therapy paved the way for preclinical studies of the other enzymes involved in the RAS processing pathway, namely Rec1, Icmt and Pdeo. Again, several inhibitors have been evaluated in in vitro models of KRAS-driven tumors, without achieving meaningful preclinical results.[78] However, these strategies failed mainly because these enzymes have many other substrates besides RAS family and specifically KRAS can be modified by geranylgeranylation in the presence of FTIs. Nevertheless, despite initial breakdown, in recent years novel therapeutic approaches to hit KRAS-mutated NSCLCs have been developed and numerous clinical trials are currently ongoing (Table 1).

Direct targeting of KRAS protein

Although different potential binding sites have been identified using computational approaches, no hydrophobic pockets that may allow a stable binding of small molecules on the surface of KRAS have been found. Moreover, KRAS protein binds GTP with high affinity, which range in picomolar levels.[78] Nonetheless, several studies explored the feasibility of a direct targeting of KRAS. Patagiri et al. designed a cell-permeable synthetic α-helix based on the GEF Sos that prevents RAS–Sos interaction, thus leading to RAS downstream signaling inhibition.[79] Two further studies identified, through integrated approaches of fragment-based small-molecule screening and high-resolution structural analyzes, a group of small compounds that bind to a common site on KRAS. In both studies, tested inhibitors bind at a hydrophobic pocket between the switch 2 and core beta sheet region of the protein with micromolar affinity. Notably, the binding site is partially overlapping with the SOS binding site. As a consequence, SOS is unable to activate KRAS protein.[80,81] Ostrem et al. developed an irreversible inhibitor of KRAS
G12C variant, which blocks with submicromolar affinity, the SOS-mediated nucleotide exchange, thereby maintaining KRAS protein switched off. Of note, this study took advantage from the unique nucleophilicity of the mutant cysteine-12 thiol, thus adding the benefit of a selective inhibition of mutant KRAS over WT.[82]

Targeting MEK pathway

Having established that KRAS direct targeting could be challenging, most of the efforts turned to KRAS downstream effectors. Till date, MEK inhibitors represent the most promising therapeutic approach for patients with advanced KRAS mutant NSCLC. Remarkably, the Ras/Raf/MEK/ERK cascade is frequently upregulated in NSCLC, especially in the presence of KRAS and BRAF point mutations. As previously reported, KRAS mutation occurs in approximately one-fourth of NSCLC, while BRAF mutation is detectable in 5% of lung adenocarcinomas. Therefore, it is expected that nearly 30% of patients affected by NSCLC might benefit from a therapy targeting the Ras/Raf/MEK/ERK pathway.

Selumetinib (AZD6244, ARRY-142886) is an orally bioavailable, selective, non-ATP-competitive, potent inhibitor of MEK1/MEK2 kinases (IC50: 14 nM for MEK1). Selumetinib activity and specificity have been well characterized in multiple cell lines from different tumors types. Its IC50 against purified constitutively active MEK1 range 10–14 nmol/l. Of note, the IC50 values against Raf-activated MEK1 and MEK2 are comparable, underscoring that selumetinib does not discriminate between MEK1 and MEK2.[83,84] Preclinical studies demonstrated that selumetinib significantly suppressed tumor growth in KRAS mutant NSCLC xenografts.[85,86] Early-phase clinical studies showed that selumetinib monotherapy exhibits tumor responses, manageable safety and tolerability profile and identified a suitable dose for clinical trials that results in targets inhibition (100 mg orally, twice daily continuously).[87,88] Nevertheless, in a randomized phase II study selumetinib alone as second- or third-line therapy did not appear to be superior in terms of ORR and PFS over standard treatment with pemetrexed in an unselected NSCLC population.[89] On the other hand, data from preclinical in vivo studies provided evidence that the combination of selumetinib with cytotoxic drugs and novel targeted therapies may be more effective in advanced NSCLC. In particular, selumetinib plus docetaxel

<table>
<thead>
<tr>
<th>ClinicalTrials.gov identifier</th>
<th>KRAS mutational status</th>
<th>Drug</th>
<th>Stage</th>
<th>Phase</th>
<th>Primary end point</th>
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<tr>
<td>NCT00890825</td>
<td>KRAS mutant</td>
<td>Docetaxel + selumetinib vs docetaxel alone</td>
<td>Advanced</td>
<td>II</td>
<td>OS</td>
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<td>NCT01229150</td>
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<td>Erlotinib vs selumetinib Erlotinib vs erlotinib + selumetinib</td>
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<td>II</td>
<td>PFS, ORR</td>
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<tr>
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<td>II</td>
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<td>Advanced</td>
<td>IB/II</td>
<td>ORR and toxicity</td>
</tr>
<tr>
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<td>II</td>
<td>PFS</td>
</tr>
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<td>IV</td>
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<tr>
<td>NCT02079636</td>
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<td>Abemaciclib in combination with multiple single agents</td>
<td>Advanced</td>
<td>IV</td>
<td>Toxicity</td>
</tr>
<tr>
<td>NCT01021748</td>
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<td>Advanced</td>
<td>I</td>
<td>Toxicity</td>
</tr>
<tr>
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</tr>
<tr>
<td>NCT01427946</td>
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<td>IB/II</td>
<td>ORR</td>
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<td>II</td>
<td>DCR</td>
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<td>ORR</td>
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<td>PFS</td>
</tr>
<tr>
<td>NCT02022982</td>
<td>KRAS mutant</td>
<td>Palbociclib + PD-0325901</td>
<td>Advanced</td>
<td>VII</td>
<td>Toxicity</td>
</tr>
<tr>
<td>NCT01833143</td>
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<td>II</td>
<td>ORR</td>
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<td>GI-4000</td>
<td>Early</td>
<td>II</td>
<td>Immune response</td>
</tr>
</tbody>
</table>

DCR: Disease control rate; ORR: Objective response rate; OS: Overall survival; PFS: Progression-free survival.
combination therapy results in a greater tumor growth inhibition or regression and apoptosis induction, with a synergistic effect. [90,91] Once assessed the safety profile of selumetinib plus docetaxel in a previous phase I study, [92] a randomized, double-blind, phase II clinical trial combining docetaxel (75 mg/m² on day 1 of a 21-day cycle in the second-line treatment of KRAS mutant NSCLC) with or without oral selumetinib (75 mg twice daily in a 21-day cycle) in KRAS mutant NSCLC patients was performed and showed a statistically significant increase in median PFS (5.3 vs 2.1 months; HR: 0.58; 80% CI: 0.42–0.79; p = 0.014) and a promising trend for superior OS in patients treated in experimental arm (median OS 9.4 vs 5.2 months; HR: 0.80; 80% CI: 0.56–1.14; p = 0.21). In addition, an unexpected response rate of 37% in the combination group compared with 0% in the docetaxel alone arm was observed (p < 0.0001). Post hoc analyses also revealed improvements in disease-related symptoms, particularly with regard to occurrence of all grades dyspnea, which appear to be less frequent in the selumetinib group. Despite this, a higher rate of febrile neutropenia (18 vs 0%), diarrhea, vomiting and stomatitis occurred with selumetinib plus docetaxel. [93] Interestingly, a retrospective analysis of KRAS mutation types in this study revealed that patients receiving selumetinib plus docetaxel who harbored KRAS G12C or G12V mutations had trends towards a greater improvement in OS, PFS and ORR compared with other KRAS mutations. [94] This suggests that specific mutant forms of KRAS could exhibit differential sensitivity to selumetinib/docetaxel combination therapy. However, a larger phase III clinical trial of docetaxel with selumetinib or placebo in patients with advanced KRAS-positive NSCLC is currently ongoing (NCT01933932). Interestingly, wide genomic approaches revealed that KRAS mutation in NSCLC patients could coexist with additional mutations, such as TP53 and LKB1, thus conditioning response to treatment. To this regard, in mouse models with KRAS mutant lung cancer concomitant loss of either TP53 or LKB1 markedly impaired the response of KRAS mutant tumors to docetaxel monotherapy. Importantly, the addition of selumetinib provided significant benefit for mice with lung cancer caused by KRAS and TP53 mutations, whereas mice harboring KRAS and LKB1 mutations showed primary resistance to this combination therapy. [95]

Of note, preclinical data indicate that MEK inhibitors cause inhibition of cell proliferation, invasion, migration, anchorage-independent growth in vitro and of tumor growth in vivo in TKIs-resistant cell lines. [96] Owing to these findings, selumetinib has also been investigated in combination with erlotinib in two parallel randomized phase II clinical trials. Patients with NSCLC harboring KRAS mutation were randomized to receive selumetinib alone or selumetinib and erlotinib, on the other side KRAS-WT patients randomly received erlotinib alone or erlotinib plus selumetinib, having ORR and PFS as primary end points, respectively. Unfortunately, this study failed to show improvement for the combination therapy over single agent in both populations. However, the KRAS mutant group experienced a not statistically significant (p = 0.11) longer PFS with the selumetinib/erlotinib combination compared with KRAS-WT patients, which however came at the cost of increased toxicity. [97]

Trametinib is a reversible, allosteric MEK1/MEK2 inhibitor with an IC50 of 0.7 nM for MEK1, showing promising activity in KRAS mutant cell lines and xenograft models. [97] However, a randomized, multicenter, phase II study to assess efficacy and safety of trametinib compared with docetaxel in KRAS mutant advanced NSCLC failed to meet its primary end point of PFS (11.7 vs 11.4 weeks; HR: 1.14; p = 0.5197) in the second-line setting. [98] Besides, a multiarm phase I/II trial (NCT01192165) assessed trametinib activity in KRAS mutant NSCLC patients in combination with docetaxel. The ORR assessed by the investigator in KRAS mutant evaluable (n = 21) and KRAS-WT (n = 19) NSCLC patients was respectively 28 and 32%. Interestingly, a subgroup analysis showed that patients harboring KRAS G12C mutation (n = 8) had response rate of 40% with a DCR of 80%. [99] Of note, another phase I/II trial evaluating trametinib in combination with pemetrexed in KRAS mutant and KRAS WT advanced NSCLC patients revealed an ORR among evaluable KRAS mutant (n = 22) and KRAS-WT (n = 15) patients of 17 and 15%, respectively, with similar PFS. [100]

**PI3K/AKT/mTOR inhibitors**

Aberrant activation of the PI3K- AKT-mTOR signaling pathway plays a critical role in lung cancer carcinogenesis and progression. A number of molecular alterations involving the PI3K pathway have been detected in NSCLC, including PIK3CA amplification and mutation that have been reported in 2 and 12–17% of cases, respectively. [101,102] Importantly, PIK3CA mutations frequently occur in combination with other driver mutations such as EGF R or KRAS, often conditioning the development of acquired EGFR TKIs resistance. [103] Preclinical studies revealed that PI3K/AKT signaling is required for KRAS-driven lung tumorigenesis and that KRAS mutations are associated to primary resistance to PI3K inhibitors. [104–106] Similarly, treatment of KRAS mutant cancer cell lines with MEK inhibitors results in increased phosphorylation of the PI3K pathway effector AKT. Therefore, a potential therapeutic approach for KRAS mutant NSCLC should be focused on dual inhibition of PI3K and MEK/ERK pathways.

MK-2206 is an oral pan-AKT inhibitor that has shown preclinical activity in a panel of NSCLC lines, with the greatest activity in a PIK3CA-mutated model. [106,107] Combination therapy with selumetinib has proved to be highly synergistic in vitro and very effective in the treatment of lung cancer xenografts and is currently under clinical evaluation. [108] Of note, Tolcher et al. recently conducted a dose/schedule-finding study evaluating MK-2206 and selumetinib in patients with advanced treatment-refractory solid tumors, achieving durable responses in KRAS mutant cancers (NCT01021748). [109]

Ridaforolimus (AP23573; MK8669) is an investigational targeted small molecule that binds and inhibits the mTOR,
a serine/threonine kinase located downstream of the PI3K/AKT signaling pathway and involved in KRAS-mediated oncogenesis. Ridaforolimus has shown potent single-agent antitumor activity in preclinical models of NSCLC harboring KRAS mutations.[110] Moreover, a randomized discontinuation phase II trial of ridaforolimus in advanced NSCLC patients with KRAS mutations demonstrated that in patients with KRAS mutation who had stable disease after 8 weeks of treatment ridaforolimus continuation was associated with prolonged PFS versus observation (p = 0.013).[110]

Collectively, these findings suggest that dual inhibition of MEK/ERK and PI3K/AKT/mTOR pathways might be clinically effective in KRAS mutant NSCLCs and several clinical trials of combination regimens targeting MEK and PI3K signaling are currently ongoing (Table 2).

**CDK4/6 inhibitors**

Recent studies identified a synthetic lethal interaction between the presence of KRAS oncogene and genetic deletion of CDK4 in NSCLC. CDK4 and CDK6 alterations have been previously implicated in many human tumors including NSCLC.[111,112] Such alterations primarily include as point mutations that directly activate CDK 4/6, gene amplifications and genetic losses, which reduce expression of protein inhibitors such as p16. In preclinical setting, Puyol et al. showed a reduction in the growth of KRAS mutant lung adenocarcinoma following the administration of a selective pharmacological inhibitor of CDK4. Importantly, KRAS mutant xenografts exhibited greater sensitivity to LY2835219 compared with KRAS WT xenografts, thus further upholding a role for CDK4 as a therapeutic target in KRAS-driven NSCLC.[112]

Abemaciclib (LY2835219) is an orally available selective ATP-competitive CDK inhibitor that targets the CDK4 and CDK6 pathway. Abemaciclib specifically binds CDK4 and CDK6, consequently inhibiting retinoblastoma protein phosphorylation in early-phase G1.[113] In a phase I study of advanced NSCLC that progressed or relapsed after standard treatments, single-agent abemaciclib demonstrated acceptable safety and tolerability, also achieving an overall DCR of 51%, partly reflecting the higher DCR of 54% for KRAS mutant patients (n = 26) compared with KRAS-WT (DCR 37%, n = 19).[114]

Another CDK4/6 inhibitor, palbociclib is currently under clinical evaluation in a phase I/II clinical trial in combination with the MEK inhibitor PD-0325901 in patients with KRAS mutant NSCLC (NCT02022982).

**Expert commentary and five-year view**

KRAS is one of the most common mutated oncogenes in lung adenocarcinomas. Nevertheless, the prognostic and predictive role and consequently the clinical utility of KRAS oncogenic mutations in lung cancer are still highly controversial issues. However, a systematic review and meta-analysis of 28 studies assessed the negative prognostic significance of the KRAS mutational status on survival in advanced nonsquamous NSCLC.[54] More importantly is likely that different KRAS mutant alleles might contribute to differences in clinical outcome of this subset of NSCLC patients. Available data suggest that KRAS mutant G12C and G12V, codon 13 mutations and MASI are candidate biomarkers for poor prognosis and should be incorporated in prospective studies evaluating novel therapies targeting KRAS variant NSCLC.[19,21,37,47,57,58] On the other hand, even though data on KRAS predictive value in response prediction to standard chemotherapy are still inconclusive, two meta-analyses suggest that KRAS mutations may represent a predictive biomarker for nonresponsiveness to EGFR-TKIs in NSCLC.[30,73]

For more than 20 years, mutant KRAS has been considered “undruggable,” but novel therapeutic approaches may change this paradigm (Figure 1). KRAS direct targeting strategies turned up to be too challenging as KRAS binds rapidly and tightly to GTP, making it extremely difficult to block such interaction. However, despite being considered potentially effective for a long time, only recently a KRAS mutation-specific approach has been evaluated. In 2013, Ostrem et al. reported a compound that specifically targets the KRAS G12C mutation, which is found in 20% of lung cancers. Although this inhibitor requires additional investigation before it can enter in clinical setting, to date it is the first candidate drug that truly binds directly to KRAS.[82] Certainly this approach, as much as appealing, is still far from a real clinical application. As a consequence, in recent years most of the efforts focused on KRAS downstream signaling pathways and new drugs targeting KRAS downstream effectors, thus selectively hindering the viability of KRAS mutant cells, are currently under clinical evaluation. Among them, selumetinib, a potent inhibitor of MEK1/MEK2 kinases, has recently demonstrated to potentially improve ORR and PFS in advanced KRAS mutant NSCLC in combination with docetaxel. Moreover, recent insights showed that concurrent inhibition of both MEK1/MEK2 and PI3K/AKT signaling pathways may be more effective than single-agent treatment; therefore, drug combinatorial therapies are believed to be one of the most promising future directions. To this regard, novel alternative targeted strategies of enhancing apoptosis (MEK + BCL2/BCL-XL inhibition) or targeting PI3K upstream (MEK + IGF1R inhibition) have already entered the clinical setting.[115]

Long with the aforementioned potential targets, a number of KRAS-specific synthetic interactions have been recently described, generating a tremendous excitement among researchers. Using a lentiviral shRNA screening strategy, Barbie et al. identified suppression of TBK1 as a synthetic lethal interaction with KRAS. In this study, cell growth and proliferation was markedly reduced in cell lines with endogenous KRAS mutation following TBK1 knockdown. Moreover, growth of tumor xenografts was inhibited in KRAS mutant cells expressing TBK1 shRNAs, whereas TBK1 shRNA showed no effect on the tumor developing...
Table 2. Ongoing clinical trials evaluating combination therapy targeting MEK1/2 and PI3K in human cancers.

<table>
<thead>
<tr>
<th>ClinicalTrials.gov identifier</th>
<th>Drug</th>
<th>Target</th>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
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<td>NCT01510444</td>
<td>Selumetinib MK-2206</td>
<td>MEK1/2AKT</td>
<td>II</td>
<td>Selumetinib and Akt inhibitor MK2206 in treating patients with stage III or stage IV melanoma who failed prior therapy with vemurafenib or dabrafenib</td>
</tr>
<tr>
<td>NCT01333475</td>
<td>Selumetinib MK-2206</td>
<td>MEK1/2AKT</td>
<td>II</td>
<td>MK-2206 and AZD6244 in patients with advanced colorectal carcinoma</td>
</tr>
<tr>
<td>NCT01206140</td>
<td>Selumetinib Temsirolimus</td>
<td>MEK1/2 mTOR</td>
<td>II</td>
<td>Selumetinib with or without temsirolimus in treating patients with metastatic, recurrent or locally advanced soft tissue sarcoma that cannot be removed by surgery</td>
</tr>
<tr>
<td>NCT01166126</td>
<td>Selumetinib Temsirolimus</td>
<td>MEK1/2 mTOR</td>
<td>II</td>
<td>mTOR inhibitor temsirolimus combined with MEK inhibitor AZD 6244 in patients with BRAF mutant stage IV melanoma</td>
</tr>
<tr>
<td>NCT01248858</td>
<td>Trametinib GSK2126458</td>
<td>MEK1/2 PI3K</td>
<td>I</td>
<td>Dose-escalation study to investigate the safety, pharmacokinetics, pharmacodynamics and clinical activity of GSK2126458 and GSK1120212. Combination therapy in subjects with advanced solid tumors</td>
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<td>NCT01155453</td>
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<td>MEK1/2 PI3K</td>
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<td>Dose-escalation study of oral BKM120 in combination with oral GSK1120212 in adult patients with selected advanced solid tumors</td>
</tr>
<tr>
<td>NCT01476137</td>
<td>Trametinib GSK2110183</td>
<td>MEK1/2 AKT</td>
<td>I/II</td>
<td>Study of the safety and activity of the MEK inhibitor given together with the AKT inhibitor to patients with multiple myeloma or solid tumor cancers</td>
</tr>
<tr>
<td>NCT00955773</td>
<td>Trametinib Everolimus</td>
<td>MEK1/2 mTOR</td>
<td>I/II</td>
<td>Study of the GSK MEK inhibitor GSK1120212 and everolimus with expansion cohort in patients with KRAS-mutant NSCLC</td>
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<tr>
<td>NCT01376310</td>
<td>Trametinib Everolimus</td>
<td>MEK1/2 mTOR</td>
<td>II</td>
<td>Rollover study to provide continued treatment with GSK1120212 (Trametinib) plus second agent including (everolimus) to subjects with solid tumors and leukemia</td>
</tr>
<tr>
<td>NCT01378377</td>
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<td>MEK1/2 mTOR</td>
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<td>Dose-escalation trial of MEK1/2 inhibitor MSC1936369B combined with temsirolimus in subjects with advanced solid tumors</td>
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<td>NCT01390818</td>
<td>Pimasertib SAR245409</td>
<td>MEK1/2 PI3K/mTOR</td>
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<td>Dose-escalation trial of oral combination therapy with MSC1936369B and SAR245409 in subjects with locally advanced or metastatic solid tumors</td>
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<td>MEK1/2 PI3K</td>
<td>IB</td>
<td>Combination study of PI3K inhibitor BAY 80-6946 and allosteric-MEK inhibitor BAY 86-9766 in subjects with advanced cancer</td>
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<td>NCT01363232</td>
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<td>Dose-escalation and -expansion study of an orally administered combination of BKM120 plus MEK162 in adult patients with selected advanced solid tumors (including NSCLC) with KRAS, NRAS or BRAF mutations</td>
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<tr>
<td>NCT01449058</td>
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<td>Dose-escalation study evaluating the safety, tolerability and pharmacokinetics of GDC-0973 in combination with GDC-0941 when administered in patients with locally advanced or metastatic solid tumors</td>
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(continued)
Table 2. (continued).

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<tr>
<td>NTC01347866</td>
<td>PD-0325901</td>
<td>MEK1/2</td>
<td>IB</td>
<td>Dose-escalation study of the safety, pharmacokinetics and pharmacodynamics of the dual PI3K/mTOR inhibitors PF-04691502 and PF-05212384 in combination with experimental or approved anticancer agents in patients with advanced cancer</td>
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<tr>
<td></td>
<td>PF-04691502</td>
<td>PI3K/mTOR</td>
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</tbody>
</table>

NSCLC: Non-small cell lung cancer.
ability of cells harboring WT KRAS.\[116\] Similarly, Scholl et al. identified STK33 as a potential target that is selectively required for KRAS-driven carcinogenesis across different cancer cell lines. STK33 is a member of the calcium/calmodulin-dependent protein kinase involved in KRAS-dependent cells proliferation and survival through regulation of S6K1-mediated inhibition of the proapoptotic protein BAD.\[117\]

Unbiased shRNA screens revealed GATA-binding Factor 2 (GATA2) as a further potential target in KRAS variant NSCLC. In lung adenocarcinoma mouse models, genetic ablation led to tumor regression in KRAS-mutated cells, whereas WT cells were unaffected.\[118\] Although undruggable in itself, pharmacological inhibition of GATA2-mediated pathways with bortezomib and fasudil showed an impressive tumor inhibition in KRAS-driven lung tumors, providing evidence for a new potential treatment option to KRAS mutant NSCLC.\[119\] Finally, other potential KRAS effectors have been identified in lung cancer, such as RNA binding motif 5, enhancer of zeste homolog and Wilms tumor gene 1. Further elucidation of these mechanisms may afford us to develop novel effective therapeutic strategies for KRAS variant NSCLC in the coming years.

Certainly, the difficulties of targeting KRAS-mutated NSCLC still lie ahead, but new knowledge and strategies make us cautiously optimistic about the future prospect of defeating KRAS-driven lung cancer.

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**Key issues**

- **KRAS** represents one of the most common mutated oncogenes in lung adenocarcinomas. In advanced non-small cell lung cancer (NSCLC) patients, KRAS mutation is associated with poor prognosis. Conversely, its role as a negative predictive marker seems to be defined only for treatment with reversible EGFR tyrosine kinase inhibitors.
- Up to now, no specific treatments for patients with **KRAS** variant NSCLC have been developed and **KRAS** mutant NSCLC still last a slippery target for cancer therapy.
- **KRAS** direct targeting strategies turned up to be too challenging; therefore, most of the efforts focused on **KRAS** downstream effectors. In this regard, MEK1/2 inhibitors represent the most promising therapeutic approach for patients with advanced **KRAS** mutant NSCLC.
- Dual inhibition of MEK/ERK and PI3K/AKT/mTOR signaling pathways might be clinically effective in **KRAS** mutant NSCLCs and different clinical trials are currently addressing this issue.
- Genome-wide approaches revealed a number of targetable “synthetic KRAS interactions” (e.g. NF-kB, enhancer of zeste homolog, GATA-binding Factor 2, RNA binding motif 5, CDK4/69) that represent promising future targets in treating **KRAS** variant NSCLC.

**References**

Papers of special note have been highlighted as:

- of interest
- of considerable interest

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• This study showed that patients harboring KRAS mutant MASI experienced a worse overall survival. The negative prognostic significance of KRAS mutant MASI was independent of disease stage


• First study evaluating KRAS mutation as a prognostic factor in non-small cell lung cancer (NSCLC)


• This study revealed that patients harboring KRAS codon 13 mutations derived significant harm from adjuvant chemotherapy


• This meta-analysis first assessed the negative prognostic significance of KRAS mutation in NSCLC


57. Villaruz LC, Socinski MA, Cunningham DE, et al. The prognostic and predictive value of KRAS oncogene substitutions in


- This study showed that mutations in KRAS gene are associated with a lack of sensitivity to either gefitinib and erlotinib.


Page 68


• Clinical trial showing the clinical benefit with a selumetinib-based therapy in KRAS-mutant NSCLC


• Phase 2 clinical trial evaluating clinical activity of ridaforolimus in KRAS-variant NSCLC


