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# KRAS and the Reality of Personalized Medicine in Non-Small Cell Lung Cancer

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Lung cancer is the leading cause of mortality among all cancer types worldwide. The latest available global statistics of the World Health Organization report 1.59 million casualities in 2012. Worldwide, 1 in 5 cancer deaths are caused by lung cancer. In 2016, in the United States alone, there are an estimated 224,390 new cases of lung cancer, of which 158,080 are expected to result in death, as reported by the National Cancer Institute. Non-small cell lung cancer (NSCLC), a histological subtype, comprises about 85% of all cases, which is nearly 9 out of 10 lung cancer patients. Efforts are under way to develop and improve targeted therapy strategies. Certain mutations are being clinically targeted, such as those in *EGFR* and *ALK* genes. However, one of the most frequently mutated genes in NSCLC is the Kirsten rat sarcoma viral oncogene homolog (*KRAS*), which is currently not targetable. Approximately 25% of all types of NSCLC tumors contain *KRAS* mutations, which remain as an undruggable challenge. These mutations are indicative of poor prognosis and show negative response to standard chemotherapy. Furthermore, tumors harboring *KRAS* mutations are unlikely to respond to currently available targeted treatments such as tyrosine kinase inhibitors. Therefore, there is a definitive, urgent need to generate new targeted therapy approaches for *KRAS* mutations. Current strategies have major limitations and revolve around targeting molecules upstream and downstream of *KRAS*. Direct targeting is not available in the clinic. Combination therapies using multiple agents are being sought. Concentrated efforts are needed to accelerate basic research and consecutive clinical trials to achieve effective targeting of *KRAS*.

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## INTRODUCTION

Statistics from 2013 onward indicate more than 200,000 new lung cancer cases in the United States alone, of which more than 150,000 are estimated to result in death. Worldwide statistics show lung cancer as the third most common cancer type. (1–4) In 2012, approximately 1,825,000 new cases were diagnosed globally. Incidence rates are shown to be highest in North America. (2,4) In Europe, lung cancer is the fourth most common cancer type, and 410,000 new cases were diagnosed in 2012. (2,4)

Lung cancer is divided into 2 major histological subtypes: small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). This review focuses on NSCLC. Early diagnosis for NSCLC has been difficult. Cancer Research UK reports the following diagnosis percentages: in stage I, 14.5%; in stage II, 7.3%; in stage III, 31.8%; and in stage IV, 35.8%. (2) This is mostly due to symptoms not immediately providing clues to the disease, such as persistent cough, which is already present in most smokers. This can lead to misdiagnosis of infection. For patients diagnosed at stage IV, where NSCLC is highly metastatic, the median overall survival (OS) is less than a year. (2,3)

Recently, there has been considerable progress in the survival of patients who are able to receive targeted therapy based on molecular profiling of their tumors. For such cases, the median OS was measured to be more than 3 years. Examples include erlotinib targeting *EGFR* mutations and crizotinib targeting *ALK* mutations. (1) Lung cancers very frequently harbor somatic *KRAS* mutations. (5) Mutations in this particular gene comprise about 25% of NSCLC tumors. (6,7) However, these mutations are not targetable and are indicative

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of poor prognosis. (8) In addition, tumors harboring *KRAS* mutations are unlikely to respond to any currently available targeted treatment. (1,3,7,9) In this review, *KRAS* genotypes in NSCLC are discussed with respect to the lack of and the need for novel molecular agents as targeted therapies.

## Types of NSCLC

NSCLC consists of subtypes grouped according to their histological distinctions: lung adenocarcinomas (LACs), which arise in cells lining the alveoli, account for about 40%; squamous cell carcinomas (SCCs) account for about 25% to 30%; and large-cell carcinomas (LCCs) account for about 10% to 15% of NSCLC cases. (3,10) SCC is reported to start in the early stages of squamous cells, which are mostly found near the bronchus in the middle of the lungs. Carcinoma starting in the early stages of squamous cells is generally linked to smoking history. (3) Adenocarcinoma is the most common subtype and is characterized by cells that normally secrete mucus. This type is mostly seen in current or former smokers. Interestingly, it is also the most common type in nonsmokers. (3) LCC can start in any part of the lungs. In comparison with other types, it tends to grow and spread faster, thus it becomes harder to follow and apply treatment. Aside from histological distinctions, molecular subtypes of NSCLC are continually expanding and are distinct per patient. Importantly, each individual case could result from various different molecular profiles, which is a challenge for treatment. (11) In this regard, tumor sequencing becomes useful.

## Tumor Sequencing and Mutations

Revolution in sequencing technologies has had major impact on identification and understanding of molecular variations within tumor genomes. Current developments in next-generation sequencing (NGS) enable scientists to analyze whole genomes in less than 2 wks. (12,13) The steadily decreasing cost of NGS is making tumor sample sequencing more accessible. Comparison of tumor genome and normal genome sequences of patients is in clinical use and is expected to become a standard clinical tool in identifying genome-wide cancer variations. Based on sequencing results, a better understanding of the molecular underpinnings of the tumor becomes possible, which is significant in guiding patients for mutation-specific treatment.

There are several challenges in NGS application. First, sequencing DNA from tumor tissue is challenging in itself. Tumor tissue samples are often heterogeneous, which leads to further difficulty in interpretation of driver mutation(s). (12–14) NSCLC tumors display high heterogeneity. However, as noted by Alsdorf *et al.* in 2013, in NSCLC tumor heterogeneity regarding the *KRAS* mutation is rare. *KRAS* mutation appears to be an early event in tumorigenesis and is a true driver mutation. (15) Furthermore, in most cases, biopsy is performed only once, leading to a single tumor sample from a specific body part. However, cancer keeps evolving and the tumor molecular profile keeps changing, presenting an additional challenge in identifying mutation-specific therapy options. (14)

Additionally, there are technical challenges due to elementary sequencing platform differences and error rates. This accuracy problem of NGS presents a challenge and can be overcome by resequencing the same genome several times. (12,13) Moreover, NGS coverage is also of importance. Simply put, higher levels of coverage indicate higher degrees of confidence. Differences in testing methods have been linked with identification of different molecular findings within the same NSCLC patient. (13) Comprehensive studies need to be designed to cover multiple platforms in cross-comparative analyses to improve detection rates overall.

Finally, rapid evolution of high-throughput technology has greatly moved forward the field of cancer biology. However, the main challenge in tumor sequencing is bioinformatics analysis. Without bioinformatics interpretation, molecular findings will not be of clinical use. Translation of NGS into clinics will only be plausible by validation and assembly of data through computational tools. (12,13,16) Future work is likely to shift in a direction to integrate pathway data, showing gene interactions specific to patients. Pathway knowledge is expected to prove useful for targeted treatment strategies. (17)

## Mutations and Personalized Targeted Treatment

Nowadays, genotype-driven therapy is a standardized treatment method for patients diagnosed with a prominent subtype of NSCLC. (18) A significant amount of attention has been paid to developing precision treatment strategies targeting *EGFR* and *ALK* mutations for adenocarcinoma. NSCLC driver mutations occur in many other oncogenes, such as *AKT1, BRAF, HER2, KRAS, MEK1, MET, NRAS, PIK3CA, RET* and *ROS1.* Figure 1 shows the distribution of driver mutation frequencies in NSCLC tumors. As can be seen, *KRAS* mutations comprise a very large proportion of the driver genotypes.

Thus far, mutations in *EGFR* and translocations and rearrangements in *ALK* are the most effectively targeted oncogenes using specific tyrosine kinase inhibitors (TKIs) such as afatinib, crizotinib, erlotinib and gefinitib. (1) However, currently there is no targeted drug treatment for *KRAS* mutations.

***KRAS* oncogene.** *KRAS* functions by propagating signal transduction pathways upon activation and is involved in cell differentiation, cell growth, apoptosis, cell survival and cell proliferation. *KRAS* is one of the initially defined oncogenes and belongs to the Ras family with other genes including *HRAS* and *NRAS*. Different Ras proteins are involved in different cancer types. Ras molecules are downstream of growth factor receptors and upstream of pathways such as RAF-MEK-ERK, critical for cellular proliferation, and PI3K-AKT-mTOR, critical for cell survival (1,5) (Figure 2).

KRAS is a GTPase, which interacts with a set of activators and effectors. KRAS is activated with the binding of GTP and begins transmitting signals.

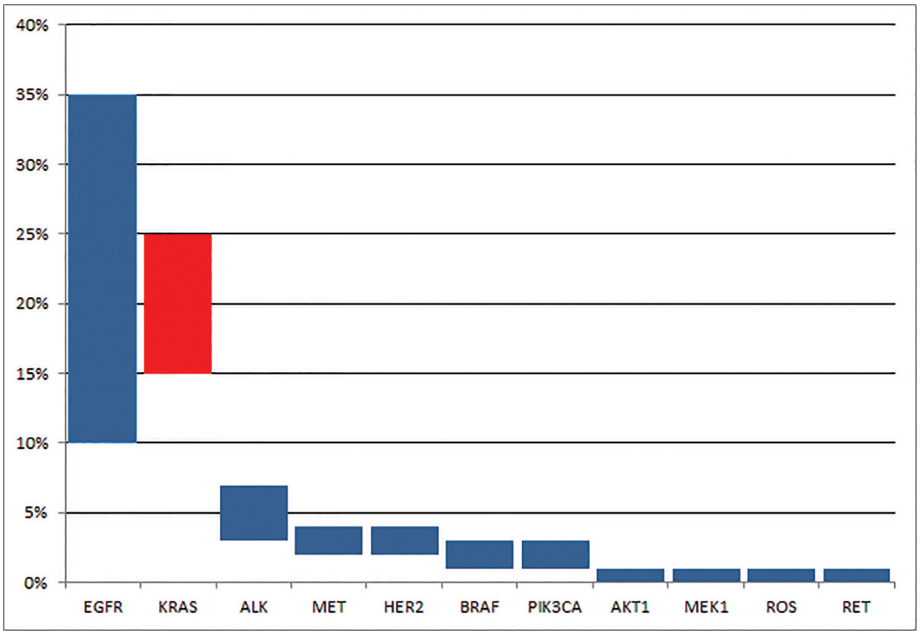


**Figure 1.** Frequency of driver mutations in NSCLC. Red: *KRAS* genotypes; blue: other genotypes. (Data obtained from https://mycancergenome.org/ on 2 June 2016.)

*KRAS* is inactivated when GTP is converted to GDP, and stops signaling. (19) When mutated, *KRAS* becomes constitutively active, which results in continuous cellular proliferation independent from its upstream effector epidermal growth factor receptor (EGFR) (5,19) (Figure 2).

***KRAS* genotypes in NSCLC.** In addition to lung cancer, *KRAS* mutations are frequently found in colon and pancreatic cancers, which are among the most aggressive, deadliest forms of cancer. (1,3,10) *KRAS* mutations most often arise in smokers or former smokers. However, they are not exclusive to smokers. Recent studies document substantial *KRAS* mutation cases in patients who have never smoked. (7)

The prognostic value of *KRAS* mutations in NSCLC has been controversial. (20) However, there are accumulating studies reporting poor prognosis and worse OS of patients with *KRAS* mutations compared with other NSCLC patients. A recent meta-analysis carried out by Meng *et al.* showed that *KRAS* mutations have an inverse association with OS in adenocarcinoma patients. (21) In addition, these mutations are negatively associated with response to standard chemotherapy, as well as certain available targeted therapies such as EGFR TKIs. (22)

Specific *KRAS* genotypes include those in the 12th and 13th codons. Mutations are mainly missense mutations with a single nucleotide change. The most commonly found genotypes are G12C, G12D and G12V (Table 1). *KRAS* mutations are generally mutually exclusive of other driver mutations and genetic anomalies such as *BRAF* and *EGFR* mutations and *ALK* and *ROS1* rearrangements. (1) However, there are exceptional cases showing co-mutations. *EGFR* and *KRAS* coexistence as well as *PIK3CA* and *KRAS* coexistence have recently been reported. (23)

## Targeting *KRAS*

*KRAS* is a mandatory target for NSCLC as one of the most frequently mutated genes. *KRAS* targeting strategies could include targeting upstream and/or downstream molecules, as well as direct targeting. Currently, a direct target molecule for *KRAS* is not available in the clinic. Earlier efforts proved farnesyltransferase inhibitors as direct targets to be of minimal use. (9)

Most of the signaling cascades downstream of KRAS have been described as shown in Figure 2. Targeting KRAS indirectly has been inefficient. (24) The focus was mainly on inhibiting the activities of RAF-MAPK and PI3K-AKT pathways. Yet many of the attempts to target these cascades did not provide major improvement in treatment. Figure 2 shows KRAS-related pathways and available targeted treatment agents for NSCLC driver mutations. It should be noted that inhibitors specifically designed for a certain gene are also being clinically investigated to target other mutations.

A novel approach being sought is to inhibit multiple agents in the pathways KRAS utilizes, with the main idea being parallel inhibition of different agents linked in different pathways. (24) In this respect, the linkage of EGFR-RAS-RAF-MAPK cascades is critical due to their function in cell proliferation, especially when their frequent mutations in cancers are considered.

## Direct Targeting of *KRAS*

Recently, direct targeting of the *KRAS* G12C genotype with irreversible inhibitors has been reported to show activity in preclinical models. Ostrem *et al.* developed a small molecule that irreversibly binds to mutated KRAS (G12C) and does not have any effect on wild-type KRAS. When these inhibitors are bound to KRAS (G12C), GDP is preferred over GTP, inhibiting RAF binding. These cysteine-reactive small molecules selectively bind to mutant KRAS, providing promising results *in vitro.* (25)

Hunter *et al.* developed a molecule called SML-8-73-1, which irreversibly inhibits KRAS, specifically the G12C driver mutation. It was observed that SML-8-73-1 binds to G12C-mutated KRAS irreversibly and selectively, even under circumstances where GDP and GTP were found in high levels. The significance of SML-8-73-1 is that

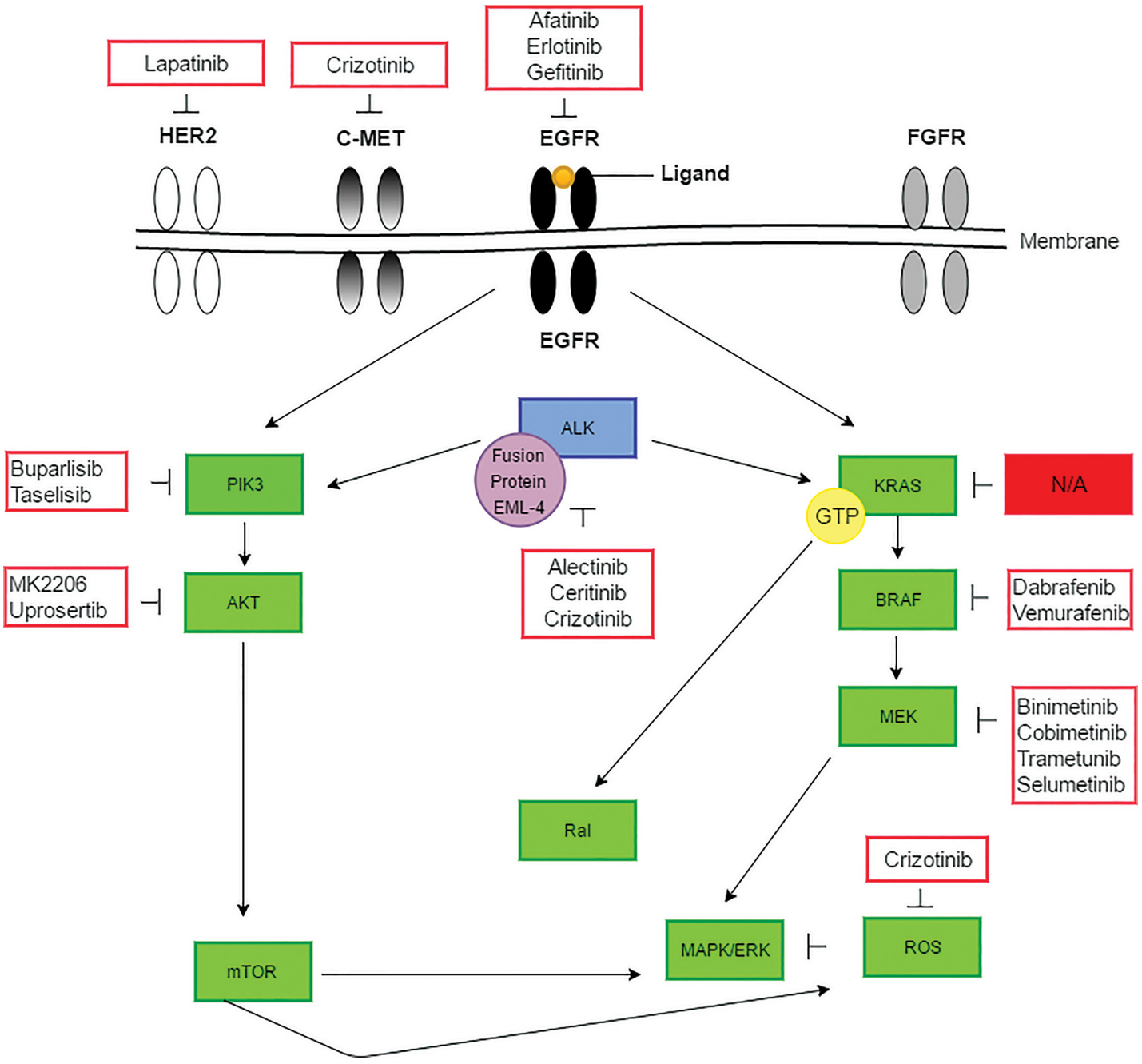


**Figure 2.** KRAS-related pathways. Targeted agents for pathogenic mutations involved in NSCLC are shown. (Comprehensive information available at: https://mycancergenome.org.)

it is the first GTP-competitive inhibitor discovered so far. (26) The main challenge in targeting KRAS directly has been its constant activation; it binds to GTP as long as there is no other agent to take GTP's place. SML-8-73-1 provides inactivation of KRAS by competing with GTP and binding to KRAS irreversibly. It was shown that SML-8-73-1 is highly selective for the KRAS G12C genotype, regardless of the GTP concentration. (26)

On the other hand, previous findings by Sunaga *et al.* in a knockdown study suggested that targeting *KRAS* directly and alone does not provide successful results for treatment. The authors concluded that direct targeting combined

**Table 1.** *KRAS* mutations reported to date. (Data obtained from https://mycancergenome.org on June 2, 2016.)

| Gene Level | Protein Level | Frequency in NSCLC |
|---|---|---|
| 34G > T | G12C | 42% |
| 35G > T | G12V | 20% |
| 35G > A | G12D | 17% |
| 35G > C | G12A | 7% |
| 34G > A | G12S | 5% |
| 37G > T | G13C | 3% |
| 38G > A | G13D | 2% |
| 34G > C | G12R | 2% |
| 37G > C | G13R | < 1% |
| 37G > A | G13S | < 1% |
| 38G > C | G13A | < 1% |
| 181C > A | Q61K | < 1% |
| 182A > T | Q61L | < 1% |
| 182A > G | Q61R | < 1% |
| 183A > C | Q61H | < 1% |
| 183A > T | Q61H | < 1% |

with other strategies could potentially provide better results. (27)

### Targeting Upstream of *KRAS*

KRAS functions downstream of receptor tyrosine kinases (RTKs), some of which are growth factors, which provide targeted therapy options in NSCLC (Figure 2). In this section, some of the upstream molecules of KRAS implicated in NSCLC are detailed.

**Anaplastic lymphoma kinase.** Anaplastic lymphoma kinase (ALK) is an RTK that regulates multiple cellular processes. (1) *ALK* gene rearrangements cause fusion with the *echinoderm microtubule-associated protein-like 4 (EML4)* gene, which leads to its activation in cancer cells. About 2% to 5% of NSCLC cases, mostly young adenocarcinoma patients with no former smoking history, carry these rearrangements (Figure 1). *ALK* mutations are generally mutually exclusive of *EGFR* and *KRAS* mutations. (28) Several inhibitors for *ALK* mutations in NSCLC are in use, such as alectinib, ceritinib and crizotinib (Figure 2). *ALK*-positive NSCLC patients have high response rates of about 60% for crizotinib. However, most patients with *ALK* mutations develop drug resistance and undergo relapse after a few years. Based on these limitations about crizotinib, several other ALK inhibitors are under development. (29)

**Epidermal growth factor receptor.** Epidermal growth factor receptor (EGFR) is an RTK, activated by ligand binding, which in turn activates PI3K and MAPK/ERK pathways. (1) *EGFR* is frequently mutated in NSCLC (Figure 1) and is being clinically targeted with TKIs (Figure 2). Mutations in *EGFR* increase its activity, causing a chain hyperactivation of the RAS pathway downstream of it. *KRAS* mutations decrease the responsivity of the tumor cell to EGFR TKIs. (11) On the other hand, *EGFR* and *KRAS* co-mutations are reported to be rare, and such tumors are also unlikely to be responsive to EGFR TKIs. (23,30) The EGFR TKIs afatinib, erlotinib and gefitinib are being clinically used for NSCLC patients harboring specific *EGFR* mutations, either combined with chemotherapy drugs or alone. EGFR inhibitors decrease tumor formation for several months, but eventually patients stop responding most of the time. (3)

**Fibroblast growth factor receptor 1.** *FGFR1* is an RTK in the fibroblast growth factor receptor (*FGFR*) family (Figure 2). When activated, FGFR1 affects the dowstream effectors, influencing cell differentiation and mitogenesis. (11) It is mainly involved in cell proliferation, differentiation and migration and embryonic development regulation. It is involved in the activation of MAPK/ERK pathways. In different cancer types, mutated *FGFRs* are shown to be deregulated by translocation, amplification and point mutation. Mutated *FGFR1* is more frequently involved in SCCs (20%)than with adenocarcinomas (5%). Clinical trials for inhibitors of FGFRs are currently ongoing. (11)

**Human epidermal growth factor receptor 2.** Human epidermal growth factor receptor 2 *(HER2)* belongs to the ERBB RTK family (Figure 2). *HER2* amplifications are found in 2% to 4% of NSCLCs (Figure 1). Adenocarcinomas harbor *HER2* mutations more prevalently compared with other types. *HER2* amplifications have negative prognostic value, and several *HER2* targeted therapies are available in clinic and in trial. (11,31) An inhibitor developed for EGFR, afatinib, has also shown activity against *HER2* mutations. (32) A combination of an irreversible pan-HER inhibitor, neratinib, and an mTOR inhibitor, temsirolimus, provided clinical activity in patients with *HER2* mutations. (33) Initial clinical trials using available therapeutic agents, such as trastuzumab, together with chemotherapy to target *HER2* in NSCLC were unsuccesful, especially with amplified *HER2*. Some inhibitors under investigation to target HER2 are afatinib, dacomitinib and neratinib. (31) In addition, some HER2-directed antibodies such as pertuzumab or trastuzumab are being studied. (11)

**Mesenchymal-epidermal transition.** Mesenchymal-epidermal transition (*MET*) is a proto-oncogene RTK controlling tyrosine kinase activities. MET activates several signaling pathways such as RAS-RAF-MAPK and PI3K-AKT-mTOR by going through homodimerization and autophosphorylation (Figure 2). In NSCLC, specifically in adenocarcinomas, MET amplifications are found in around 2% to 5% of cases (11,34) (Figure 1). Crizotinib, an ALK/ROS1 inhibitor, was first developed for inhibiting MET and demonstrates clinical activity in tumors with *MET* amplification. (29) Erlotinib and tivantinib are being clinically evaluated for *MET* inhibition. (35) Other TKIs are still under clinical investigation for *MET* inhibition, such as cabozantinib and forenitib. (11,36)

### Targeting Downstream Effectors

In some NSCLC tumors, downstream effectors of KRAS are mutated, although not as frequently as KRAS itself (Figure 1). These effectors are potential target molecules for inhibition of constitutive KRAS activation (Figure 2). Many of the downstream effectors are kinases, presenting a targetable opportunity via kinase

inhibitors. (24) It has also been shown that some downstream effectors are frequently co-mutated with *KRAS*. For example, Wang *et al.* recently reported that *PIK3CA* and *KRAS* mutations co-occur in high frequencies. (37) In general, with co-mutations, targeted therapy becomes even more challenging and thus a more negative prognosis is expected. (37,38) In this section, some of the downstream effectors of KRAS implicated in NSCLC are detailed.

**v-Raf murine sarcoma viral oncogene homolog B.** RAF proteins are downstream effectors of the RAS protein cascade. They belong to a kinase family that transmit signals from growth factor receptors outside the cell to the nucleus. (11) v-Raf murine sarcoma viral oncogene homolog B (BRAF), found downstream of KRAS, is upstream of MEK-ERK and functions as a serine threonine kinase, which affects cell division, differentiation and secretion (Figure 2). *BRAF* mutations are involved in several cancer types. (1,6) Up to 5% of NSCLC adenocarcinomas contain *BRAF* mutations, specifically the V600E genotype (11,39) (Figure 1). Studies show that when mutated, BRAF can signal independent from its upstream effectors. Due to BRAF's constant activation, downstream molecules MEK and ERK become overactivated, leading to uncontrolled and excess cell growth independent from the growth factors, and resistance against apoptosis can arise. In preclinical studies, *BRAF* mutations were reported to be sensitive to MEK inhibitors. Moreover, combining BRAF and MEK inhibitors showed activity as well. (11)

**MEK.** MEK proteins are a family of enzymes upstream of mitogen-activated protein kinases (MAPK), with mutations in about 1% of NSCLC cases (Figure 1). Together with conventional chemotherapy, inhibition of MEK, a downstream effector of KRAS, appears to be a promising approach to some extent (Figure 2). In a clinical trial, NSCLC patients with *KRAS* mutation were given the MEK inhibitor selumetinib in addition to the chemotherapy drug docetaxel. They showed increased overall response rate and improved median progression-free survival rates, along with improvement in OS. Yet the combination of docetaxel and MEK inhibitor also gave rise to side effects such as neutropenia and penumonia. (40) Another MEK inhibitor, trametinib, was reported to show activity in patients with *KRAS* mutation when given together with chemotherapy drugs, but failed to improve the therapy outcome when applied alone, in comparison with chemotherapy alone. (41)

**Phosphatidylinositol 3-kinase.** Phosphatidylinositol 3-kinase (PI3K) is a pathway involved in cell growth and survival (Figure 2). The PI3K-AKT-mTOR pathway is a very complicated signaling cascade and is frequently deregulated in several cancer types. (1,37,42) PI3K is involved in the phosphorylation of AKT when activated. Phosphorylated AKT affects the downstream signaling cascades, which have roles in cell proliferation, survival, motility and invasion. About 1% to 3% of all NSCLC cases harbor somatic *PIK3CA* mutations (1) (Figure 1). *PIK3CA* mutations frequently coexist with other driver mutations. This indicates that *PIK3CA* mutations are generally not driver mutations in NSCLC. These mutations also confer an acquired resistance against EGFR TKIs. (37,43,44) *PIK3CA* mutations provide prognostic value for *EGFR/KRAS* wild-type patients, therefore determining the *PIK3CA* mutation status could prove useful for personal therapeutic strategies. (37) Tumors with *PIK3CA* mutations are reported to be highly susceptible to PI3K inhibitors, as the preclinical data indicates. (42,43)

**RET.** *RET* is an RTK and an oncogene involved in several cancer types with gain of function mutations. *RET* translocations are found in 1.5% of NSCLC cases, specifically in adenocarcinomas (Figure 1). Translocations in *RET* do not coexist with other driver mutations. In NSCLC, alectinib, an inhibitor developed for ALK, has shown activity against RET alterations. Other inhibitors for RET, such as sorafenib, sunitinib and vandetanib, caused loss of cell viability. In a precilinical model, cabozantinib, a multityrosine kinase inhibitor, showed partial response. In addition, vandetanib has shown activity in 2 clinical trials of adenocarcinoma patients harboring *RET* translocations. Clinical trials on cabozantinib, vandetanib and other potential inhibitors to evaluate activity against RET are still ongoing.(11, 47)

**C-ros oncogene 1.** *C-ros oncogene 1 (ROS1)* belongs to the insulin receptor family and is an RTK (Figure 2). *ROS1* rearrangements are found in 0.7% to 1.7% of NSCLC cases (Figure 1), mainly in adenocarcinomas and mostly in light smokers or never-smoking young patients. Crizotinib, which was developed as a MET inhibitor, has also displayed activity against *ROS1* rearrangements. However, some novel mutations in *ROS1* showed resistance against crizotinib. (11) There is also a novel approach under investigation for patients with *ALK* or *ROS1* translocations to overcome resistance against crizotinib, using a combination of ALK/ROS1 and heat shock protein 90 inhibitors. (45) Other inhibitors under investigation include ceritinib, foretinib, AP26113 and PF-06463922. As an inhibitor of MET/RET/VEGFR pathways, cabozantinib revealed activity in alterations resistant to ROS1 inhibitor in a preclinical model. (36,46)

## DISCUSSION

There are several factors challenging treatment of NSCLC. One is the histological and molecular subtypes of NSCLC. (48) A 2015 article suggests that an *EGFR*-mutant lung adenocarcinoma can transform into SCC by formation of a secondary mutation, which presents a challenge for treatment with EGFR inhibitors. (49) Another recently published article accentuates a new potential subtype as adenosquamous carcinoma (ADSQ), which can also be a transition state between 2 predominant histological subtypes, SCC and adenocarcinoma. ADSQ accounts for 2% to 4% of NSCLC subtypes. It is not yet clear if ADSQ is a hybrid or a genuine type, owing to its morphological pattern consisting of both

SCC and adenocarcinoma characteristics. Yet ADSQ is found to be more aggressive compared with these subtypes. Patients with ADSQ could benefit from targeted therapy, due to infrequent *KRAS* mutation compared with *EGFR* mutation. (50) Even though adenocarcinoma is less aggressive than ADSQ, treatment remains challenging due to the highly frequent *KRAS* mutation, once again emphasizing the difficulty of *KRAS* mutation presence in targeted therapies.

Another challenge stems from cancer evolution. Cancer is a an evolving, multistep process, where tumor heterogeneity is a fact. (14) *KRAS* mutations occur early in cancer progression, thus targeting *KRAS* alone might not be a sufficient therapeutic approach, especially in advanced NSCLC. (51) Furthermore, sequencing cancer tissue is generally done only once. For improvement in molecular profiling, when possible, sequencing could be done several times during the course of treatment, to guide treatment and combat drug resistance.

Co-mutations present another challenge. As long as *KRAS* co-occurs with other driver mutations, treatment will not be as effective and will require inhibition of multiple agents (Figures 1 and 2). Resistance to current therapy can be a result of co-occuring driver mutations and the tumor can relapse. This was documented in a clinical trial, where knocking down of *KRAS* did not prevent uncontrolled cell proliferation, due to abnormalities in associated molecules and pathways. (27)

*KRAS* mutations also co-occur with mutations in *STK11* and *TP53* tumor suppressor genes. (52) Coexistence of these mutations has a critical effect on *KRAS*-driven lung cancers in the sense of tumor formation, immune surveillance and proliferative response. *TP53* and *STK11* can promote tumor progression with different mechanisms. *TP53* mutations have a role in increased cell proliferation, where *STK11* mutations were understood to be related to supression of immune response to the tumor. Mutated *STK11* is reported to increase KRAS signal transduction, both independent and dependent of the coexisting mutated *KRAS*. (52)

Recently, Zhu *et al.* suggested that multitargeted therapy is required to combat NSCLC. Combining a group of inhibitors in a novel fashion could provide better results. (24) However, in combination therapies, drug-drug interactions are a challenge and can cause resistance. As an alternative, a combination of therapies targeting *KRAS* directly and inhibiting other factors, either ones that have already provided considerable results or novel agents, in the cascades where KRAS is involved seems to be more promising. When putting these appraoches into clinical use, it should be noted that combining drugs with different mechanisms can result in either overlapping or nonoverlapping toxicities, negatively affecting the flow of the treatment. (24)

It should also be noted that recent developments in cancer immunotherapy could be promising in NSCLC, although these are not discussed here, as they are outside of this review's scope. Briefly, in 2015, 2 new immunotherapy agents, nivolumab and pembrolizumab, were approved for lung cancer treatment after standard chemotherapy has stopped working. These immune checkpoint inhibitor antibodies function by interfering with the programmed death protein 1 (PD-1) signaling, with the aim of improving immunity against tumor. (53)

## CONCLUSION

The majority of NSCLC cases are not yet addressed by any available targeted therapy. Specifically, as outlined in this review, *KRAS* genotypes, covering the majority of driver mutations, do not have a target match in clinic and are not responsive to standard chemotherapy. NSCLC consists of a large number of genomic anomalies, and the documented aberrations are increasing, making NSCLC harder to cope with.

The combined use of multiple signaling inhibitors targeting more than one pathway is conceptually appealing. Although clinical experience with this approach is limited, to date such combination therapies have not shown great promise. The reason for this lack of success is not clear, but it is thought to be due to cumulative toxicity of the combined agents and/or limited suppression of signaling by the inhibitors at the doses used. Despite the promise, it is possible that the extensive subclonal tumor heterogeneity of *KRAS*-driven NSCLC may make even the inhibition of multiple downstream effectors insufficient for long-term tumor control and prolonged survival.

Concentrated research efforts should be made in exploring and developing inhibitors to target KRAS as well as potential molecules in the associated signaling pathways. Combining the efforts of collaborative international groups and studying international patient data could facilitate and hasten discovery. More funding in this respect, as well as increasing the numbers and availability of international clinical studies, could prove useful. New efforts to ensure that even negative results of clinical trials are published will also help focus future efforts in new, potentially more successful directions.

## DEDICATION

Dedicated to the loving memory of an amazing father, A. Ertuğ Taneri.

## DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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