Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

REVIEW ARTICLE BIOSCIENCE RESEARCH, 202219(2):1139-1147. **OPEN ACCESS**

RAS gene mutations and their prevalence in non-small Cell lung cancer : A Review

Siraj B. Alharthi[1], Islam M. Tayeb[2], Romar Pascual[2], Salman Aloufi [3], Rasha A. Mutabbaqani [4], Dalia Alharthi[5], Ahmed S. Al-bariqi[4], Basem J. Almutiri[6] and Abdullah S. Alsulaiman[4]

[1]Department of Biological Sciences, King Abdulaziz University, Jeddah, **Kingdom of Saudi Arabia**
[2]Department of Sciences, Shorouq Al Mamlakah International School, Taif, **Kingdom of Saudi Arabia**
[3]Department of Biotechnology, Taif University, Taif, **Kingdom of Saudi Arabia**
[4]Molecular Diagnostic Unit, Alhada Armed Forces Hospital, Taif, **Kingdom of Saudi Arabia**
[5]PT Department, Alhada Armed Forces Hospital, Taif, **Kingdom of Saudi Arabia**
[6]Medical Diagnostic Laboratory, Al-Habib Medical Group, Riyadh, **Kingdom of Saudi Arabia**

***Correspondence:** sirajj1411@gmail.com **Received 30-04-2022, Revised: 03-06-2022, Accepted: 04-06-2022 e-Published: 05-06-2022**

Lung cancer has recently become one of the most common and lethal malignancies; the prevalence of non-small cell lung cancer (NSCLC) is also increasing. Most colorectal and pancreatic cancers as well as NSCLCs are caused by RAS oncogene mutations. These mutations are split into three subtypes: the neuroblastoma RAS viral oncogene homolog (NRAS), Harvey rat sarcoma viral oncogene homolog (HRAS), and Kirsten rat sarcoma viral oncogene homolog (KRAS). The GTPase activity of RAS proteins is largely affected by these mutations, causing them to remain activated, resulting in uncontrolled cell growth. In this review, three of the most common RAS gene variants—KRAS G12C, KRAS G12V, and KRAS G12D—are described. Together, these variants account for three-quarters of all KRAS mutations. KRAS G12C is found predominantly in smokers and lung adenocarcinoma patients. Nevertheless, a plethora of potent inhibitors such as sotorasib, adagrasib, GDC-6036, JNJ-74699157, and D-1553, are used, with the most effective being sotorasib and adagrasib. Furthermore, unlike other KRAS mutations, KRAS G12C signaling preferentially activates downstream Ral A/B and RAF/MEK/ERK pathways, resulting in lower levels of phosphorylated AKT, a trait shared with KRAS G12V mutations. The KRAS G12V mutation has a significant impact on failure-free survival and overall survival. Finally, when combined with TP53 co-mutations, the KRAS G12D mutation could be a potential immunotherapy biomarker. It is also worth noting that KRAS G12D mutations are associated with a low tumor mutational burden. Owing to the severity and prevalence of NSCLC, a thorough investigation into these findings is warranted, making treatment innovation all the more critical.

Keywords: RAS Gene Mutations, Non-Small Cell Lung Cancer, KRAS G12C, KRAS G12V, KRAS G12D, Lung Adenocarcinoma, Squamous Cell Carcinoma, NRAS, HRAS, KRAS.

INTRODUCTION

Lung cancer is the most prevalent type of cancer worldwide, accounting for 1.6 million deaths each year (Khaltaev & Axelrod, 2020; Kim et al. 2018; Torre et al. 2015). The two most common subtypes of non-small cell lung cancer (NSCLC) are lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), which together affect 85% of NSCLC patients. Interestingly, the number of NSCLC cases in Africa and the Middle East remains far lower than that in the West and East, but still remains a predominant issue (Yang et al. 2014; Alshammari et al., 2021). To better understand the mechanisms and pathways associated with lung cancer, it is imperative to realize how cancers emerge and develop in a broad context.

Cancer is caused by a multitude of factors, including environmental conditions and individual characteristics such as obesity; nonetheless, mutations in cancer susceptibility genes remain the most common cause. Cancer susceptibility genes can generate over- or under-expression of certain proteins, resulting in various adverse effects, the most notable being the uncontrollable proliferation of certain cells (i.e., cancer) (Faubert et al. 2020). Proto-oncogenes serve as initial regulators of this biological process, transmitting information and acting as growth factors. Mutations in these genes, which are subsequently called oncogenes, result in the formation of cancer cells. The activation pathways that lead to proto-

oncogenes include chromosomal translocations, point mutations, and gene amplification. According to the clonal theory of on cogenesis, a tumor begins with a single cell (Kontomanolis et al. 2020).

Understanding tumor immunity and developing effective cancer immunotherapy requires knowledge of how the host immune system interacts with tumor cells in the tissue microenvironment. In malignancies, the presence of lymphocytes is closely associated with better prognosis. T cells have a collection of cell surface receptors called immune checkpoints, which suppress T cell function when activated. Levels of immune checkpoint receptors, such as the programmed cell death 1 (PD-1) and the cytotoxic T lymphocyte-associated protein 4 (CTLA-4), are increased during T cell activation to protect the body from damage caused by an excessive immune response. As such, immune checkpoint inhibitors allow for an improved adaptive immune system response to cancer (Barrueto et al. 2020; Gatti-Mays et al. 2017; Rogers et al. 2015).

The oncogenes KRAS and EGFR, as well as the tumor suppressors TP53, KEAP1, STK11, and NF1, are among the most frequently mutated genes in LUAD patients. However, in LUSC patients, the most frequently mutated genes are the tumor suppressor genes TP53 and CDKN2A (Herbst et al. 2018). Several of these genes have been associated with various malignancies. Given the previously discussed accumulation of genetic and epigenetic mutations, it is important to assess how tumors grow in response to a combination of tumor suppressor and oncogenic gene mutations. Furthermore, although TP53 mutations have been identified in 50–60% of human malignancies, this review focuses on the RAS oncogene and its mutations (Baugh et al. 2017).

RAS gene family

RAS gene mutations are responsible for 30% of human malignancies and 90% of pancreatic, lung, and colon cancers. The three types of RAS proteins—namely RAS, H-RAS, and N-RAS—function as molecular switches that are activated by binding to GTP, a key nucleotide in cell process control (Zinatizadeh et al. 2019). When GTP is converted to GDP, the GTPase in the RAS gene becomes activated, leaving the protein inactive. However, RAS carcinogenic mutations include alterations that result in the loss of internal GTPase action, leading to a persistently active protein (Donninger et al. 2007). Furthermore, GTP-bound RAS stimulates RAS-RAF-MEK-ERK signaling, which regulates cell proliferation and cell cycle, as well as the PI3K-AKT-mTOR pathway, which controls cell survival. RAS proteins have been assumed to be "non-druggable" because of their mysterious inhibitory potential; that is, until the Shokat Laboratory discovered the Switch II pocket in 2013 (Malapelle et al. 2021; Vasta et al. 2022).

In this section, we will review all RAS proteins and possible mutations, with special attention to the most prevalent mutations that occur on codons 12, 13, and 61.

Neuroblastoma RAS Viral Oncogene Homolog (NRAS)

The neuroblastoma RAS viral oncogene homolog (NRAS) gene is found on chromosome 1p13.2 and is responsible for 8% of all malignancies in humans. The most prevalent NRAS mutations are found on codon 61. NRAS was the first oncogene to be identified in melanoma, a type of cutaneous malignancy considered the deadliest form of skin cancer, and NRAS mutations are found in 20% of all melanomas (Garcia-Alvarez et al. 2021; O'Neill & Scoggins, 2019). NRAS is frequently linked to BRAF mutations, and both variants are known to create numerous melanomas. BRAF and NRAS mutations were detected in African American superficial melanoma (25% and 29%, respectively), nodular melanoma (29% and 28%, respectively), and lentigo maligna melanoma (25% and 29%, respectively) patients (15% and 16%, respectively) (Akslen et al. 2008). Only two BRAF mutations (8%) were found in a group of 26 Black African melanomas, both of which deviated from the common T1799A mutation (Akslen et al. 2008). Furthermore, many pathways have been linked to the etiology of cutaneous melanoma.

The RAS–RAF–MEK–extracellular signal-regulated kinase–mitogen-activated protein kinase pathway and the EGFR–NRAS–BRAF pathway are two pathways that are particularly associated with NRAS mutations. The frequency of NRAS mutations in lung cancer patients, especially those with NSCLC, is not as high as in those with melanoma. To date, no licensed medicines that target NRAS specifically are available. However, novel targeted therapy strategies, particularly MEK inhibitor mono-therapy and combination therapy, will likely soon provide viable treatment alternatives (Johnson & Puzanov, 2015).

Harvey Rat Sarcoma Viral Oncogene Homolog (HRAS)

The Harvey rat sarcoma viral oncogene homolog (HRAS) is found on chromosome 11p15.5 and is responsible for 3% of all human cancers. HRAS is commonly associated with the Costello syndrome (CS), a complex developmental condition that manifests itself through various symptoms, including failure to thrive, developmental delay, cardiac and skeletal defects, and susceptibility to benign and malignant neoplasia. CS is often caused by activating germline mutations in HRAS or by perturbation of function through the RAS pathway (Rauen, 2007). Moreover, it has been determined by multiple studies that the HRAS mutations leading to CS often result in the dysregulation of the RAS/mitogen-activated protein kinase pathway (Dunnett-Kane et al. 2020; Gripp & Lin, 2012; Kiuru et al. 2020). Germline mutations in codons 12 and 13 have been found in CS patients; however, no mutations have been found in codon 61. Somatic point mutations in HRAS account for approximately 1% of RAS mutations observed in sporadic malignancies, with bladder cancer (BC) being the most

frequently observed (Zhang & Zhang, 2015). Additionally, MAPK pathway inhibitors and statins have been proven to be effective in impeding the effects of CS (O'Bryan, 2019). HRAS mutations are detected more frequently in younger patients than in older patients. Interestingly, the same mutations that were frequent in the lower age groups (20) were extremely rare in the older age groups. Furthermore, investigations have suggested that HRAS-related BC and CS may be linked. For example, certain germ line mutations in HRAS, specifically p. (Gly12Ser/Ala), induce CS. Furthermore, HRAS mutations were extremely prevalent in BC patients under the age of 20 years (Beukers et al. 2014).

In the context of NSCLC, HRAS is commonly overexpressed in smokers or in individuals with LUSC subtypes (Pązik et al. 2020). HRAS has been demonstrated to have a considerable impact on the development of malignancies, such as head and neck squamous cell carcinomas (HNSCCs), when mutated in combination with NRAS or TP53 (Lyu et al. 2019); however, it is normally irrelevant in NSCLC (Zhao et al. 2021). Patients with HRAS-related NSCLC were found to be 69 years old on average, which is the same age as those with NRAS-related NSCLC (Tamiya et al. 2021). The RAS gene is considered uncommon. In contrast, HRAS T81C genetic polymorphisms have been shown to cause high susceptibility to severe NSCLC (Lee & Shih, 2020). Similar to NRAS, studies on the link between HRAS mutations and NSCLC are scarce. This is due to the high frequency of HRAS in other syndromes, such as CS and BC, resulting in HRAS receiving more research attention than NSCLC.

Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS)

The Kirsten rat sarcoma viral oncogene homolog (KRAS) gene is located on chromosome 12p12.1 and is the most frequently mutated oncogene in human cancers in general; LUAD in particular accounts for 22% of all human cancers (Aredo et al. 2019). The two primary splice variants of KRAS are KRAS4A and KRAS4B, with varying C-terminal sequences. The main type of KRAS4 is KRAS4B, whereas KRAS4A expression is enhanced in the presence of a tumor (Salgia et al. 2021). Furthermore, it has been demonstrated that this is essential for the development of lung cancer in mice (To et al. 2008). KRAS mutations have also been identified as indicators of poor outcomes in patients with EGFR-mutant disease who are receiving EGFR tyrosine kinase inhibitor therapy (Lindsay et al. 2018). Owing to its relatively smooth protein structure, KRAS has been considered "undruggable", prompting scientists to focus on downstream inhibitors to target it. Fortunately, scientists can now make use of direct-targeting strategies (e.g., sotorasib) to reduce or prevent KRAS overexpression; however, nothing is known regarding the primary or acquired resistance to such an approach (Reita et al. 2022). Differences in patient characteristics and other baseline demographics make it challenging to explain these discrepancies.

Table 1: Table illustrating the differences in pathways, associated diseases, general distribution in cancers, inhibitors and therapies, and the relevant references for three primary RAS genes, namely NRAS, HRAS, and KRAS.

RAS Gene name	Pathways	Most prevalent associated diseases	Distribution Throughout all cancers (in %)	Inhibitors and therapies	Ref.
NRAS	RAS–RAF–MEK–extracellular signal-regulated kinase–mitogen-activated protein kinase + EGFR–NRAS–BRAF	Cutaneous Melanoma	8%	MEK inhibitor monotherapy	(Akslen et al. 2008; Aredo et al. 2019; Garcia-Alvarez et al. 2021; O'Neill & Scoggins, 2019)
HRAS	Ras/mitogen activated protein kinase	Costello Syndrome + Bladder Cancer	3%	MAPK pathway inhibitors + Statins	(Aredo et al. 2019; Dunnett-Kane et al. 2020; Gripp & Lin, 2012; Kiuru et al. 2020; Rauen, 2007; Zhang & Zhang, 2015)
KRAS	RAF/MEK/ERK + PI3K/AKT/mTOR	Non-small cell Lung Cancer	22%	Sotorasib + Adagrasib	(Aredo et al. 2019; Ou et al. 2022; Skoulidis et al. 2021; Tomasini et al. 2016)

Two separate studies in metastatic patients and patients with surgically resected NSCLC found that different codon variants may activate unique transcriptional networks that affect prognosis and/or therapeutic susceptibilities. More specifically, KRAS G12C or KRAS G12V positivity was associated with lower disease-free and overall survival (OS) when compared to other KRAS variants or wild-type proteins. This was at least partly due to increased ratios of epithelial to mesenchymal transition genes and reduced levels of genes, suggesting KRAS dependence. As a result, metastatic KRAS-related CRC and NSCLC have been linked (Ihle et al. 2012; Kurishima et al. 2018; Nadal et al. 2014). In NSCLC, KRAS is involved in the RAF/MEK/ERK and PI3K/AKT/mTOR pathways (Tomasini et al. 2016). Finally, although this will be discussed in detail at a later stage in this review, it is important to note that the two most effective inhibitors of KRAS in NSCLC are sotorasib and adagrasib (Ou et al. 2022; Skoulidis et al. 2021). The RAS genes and their associated pathways, diseases, cancers, and inhibitors are detailed in Table 1.

Common RAS mutations in NSCLC

KRAS mutations are detected in 20–40% of adenocarcinoma patients and the most prevalent KRAS mutations are found at codons 12 and 13 (Gauthaman & Moorthy, 2021; Passiglia et al. 2020). A study found KRAS mutations in 26.29% of 567 LUAD patient samples, with a KRAS/TP53 co-mutation rate of 9.7%. KRAS G12C was the most common mutant subtype in this study, accounting for 6.7% of the total mutants, making it the most common mutation in KRAS. Following KRAS G12C, G12V and G12D were the most common mutations with rates of 3.7% and 2.47%, respectively. The remaining samples had predominantly wild-type configurations, indicating that the majority of LUAD was caused by other mutations (e.g., TP53 or EGFR mutations) (Araujo et al. 2021; Gao et al. 2020). Finally, research has shown linkages between KRAS mutations and EGFR regulation and resistance (del Re et al. 2017; Kalikaki et al. 2008; Karachaliou et al. 2013b; Offerman et al. 2021).

The GTP/GDP binding site is the only deep surface pocket found in RAS proteins, making it challenging to develop high-affinity antagonists. As a result, RAS-related NSCLC is regarded as untreatable, creating serious complications for patients suffering from it (Roskoski, 2021). Furthermore, RAS mutations have on average been shown to affect Western populations more than Eastern populations. For example, one study compared a Chinese cohort with the COSMIC database and found a significant difference in the percentage of RAS-caused lung cancer (11.7% for the Chinese cohort and 17.3% for the COSMIC data) (Loong et al. 2020). Despite this, the literature is divided on the predictive power provided by using RAS genes to determine lung cancer survival (Mascaux et al. 2005).

In this section of the review, various aspects of RAS gene mutations will be discussed in detail.

KRAS G12C Mutation

The KRAS G12C trans version mutation accounts for 41% of KRAS mutations and is almost exclusively found in LUAD, with an occurrence rate in smokers of almost 90% (Addeo et al. 2021; Cai et al. 2020; Cannataro et al. 2018; Salem et al. 2022). When compared to other KRAS mutations, KRAS G12C signaling preferentially activates downstream Ral A/B and RAF/MEK/ERK pathways, as well as reduces phosphorylated AKT, a factor also seen with KRAS G12V mutations. Due to the widespread prevalence of this mutation, it has been intensively studied and targeted, leading to the development of tyrosine kinase inhibitors (TKIs) such as sotorasib, adagrasib, GDC-6036, JNJ-74699157, and D-1553. The most popular TKI used for the KRAS G12C mutation is sotorasib, a medication classified as an irreversible inhibitor with a half-life of approximately 6 hours, which functions by locking KRAS in an inactive, GDP-bound form. Adagrasib functions similarly, while other TKIs are still being studied and evaluated in clinical trials.

Studies have also assessed the median progression-free survival (PFS), or the duration between the initial treatment and the onset of cancer, of various KRAS mutations, revealing a median PFS for KRAS G12C of 15.57 weeks and an OS of 18.64 weeks (Hong et al. 2020). Notably, recent studies have reported KRAS mutation status not to be associated with patient sex, while strong associations with region and patient age were reported. Furthermore, the average age of KRAS G12C NSCLC patients was 67 years, with half of them being female. In addition, the South/Southeast parts of the world were reported to be the most frequently affected (8.2% and 8.1%, respectively) when compared to the North/Northeast parts of the world (5.1% and 3.6%, respectively). Finally, patients younger than 50 years showed a lower frequency of KRAS G12C mutations compared to those older than 50 years (≤ 2.0% and≥ 7.2%, respectively).

Other KRAS Mutations

As previously stated the KRAS G12V transition mutation accounts for 19% of KRAS mutations and is linked to lower levels of phosphorylated AKT in cell lines. Recent studies have linked these findings to suggest that cancers with the KRAS G12V mutation are more reliant on the RAS/RAF/MEK/ERK signaling pathway for survival, ultimately making MEK inhibitors effective against them (Biernacka et al. 2016; Tang et al. 2021). KRAS G12V mutations have also been shown to have a significant predictive effect on failure-free survival ($P = 0.004$) and OS ($P = 0.008$) rates. The KRAS G12V mutation was found to have a median PFS of 23.28 weeks and a median OS of 27.57 weeks. Finally, studies have revealed

that KRAS G12V mutations generate more aggressive metastasis when compared to KRAS G12D mutations. This is due to modulation of the KRAS/RhoA/Wnt regulatory pathway during NSCLC metastasis (Jänne et al. 2015).

Along with TP53 co-mutations, the KRAS G12D mutation accounts for 13% of KRAS mutations and is considered a biomarker for negative immunotherapy outcomes. KRAS G12D is most prevalent in CRC, accounting for 13.4% of all patients. A comprehensive analysis of 404 well-represented CRC cases showed that patients with tumors containing KRAS G12D mutations had a poor prognosis, as opposed to patients with similar KRAS mutations that affect CRC (e.g., KRAS G13D) (Zlobec et al. 2010).Furthermore, the presence of KRAS G12D mutations has been linked to reduced tumor mutational burden (TMB), a measure of the number of gene mutations in tumor tissue (Sha et al. 2020). In addition, when KRAS G12D mutations are detected, the expression of PD-L1, an important immunotherapy biomarker, is significantly reduced. Studies have also shown that KRAS G12C and TP53 co-mutations suppress immune cell infiltration, leading to further tumor progression (Zuo et al. 2020). KRAS G12D also has a median PFS of 11 weeks and an OS of 21.35 weeks, and as such, KRAS G12D is regarded as inferior to the G12V and G12C mutations in terms of PFS. The three KRAS mutations and their associated genomic distributions (GD), PFS, and OS are detailed in Table 2.

Table 2: Table illustrating the differences in genomic distribution (GD), progression-free survival (PFS), and overall survival (OS) among three different KRAS mutations, namely KRAS G12C, KRAS G12V, and KRAS G12D.

Mutations	GD	PFS	OS	Ref.
$KRAS^{G12C}$	41%	15.57 weeks	18.64 weeks	(Hong et al. 2020)
$KRAS^{G12V}$	19%	23.28 weeks	27.57 weeks	(Jänne et al. 2015)
$KRAS^{G12D}$	13%	11.00 weeks	21.35 weeks	(Jänne et al. 2015)

Notably, when comparing never-smoker patients to those with a history of cigarette smoking, trans-versions (substitution of a pyrimidine for a purine or purine for a pyrimidine) were more prevalent than transitions (substitution of a purine for purine or pyrimidine for pyrimidine). Most variants identified in mutation profiling studies have been found to be too rare to warrant further investigation; this is in contrast to the previously mentioned mutations which are more common (i.e., KRAS G12C, KRAS G12V, and KRAS G12D) (Karachaliou et al. 2013a; Riely et al. 2008; Roberts & Stinchcombe, 2013). This has resulted in a paucity of research on variants other than KRAS G12C, with only a few dozen studies examining the impact of the KRAS G12V and G12D mutations.

CONCLUSION

This review examined several types of RAS genes, focusing primarily on NRAS, HRAS, and KRAS. Based on the literature assessed herein, NRAS mutations are accountable for 8% of human cancers and are commonly found in codon 61. Furthermore, changes in specific codons associated with CRC have no effect on NSCLC (codons 59, 117, and 146). HRAS mutations are responsible for 3% of all human cancers and their overexpression is common in smokers and LUSC patients. Finally, KRAS mutations are responsible for 22% of all human cancers, making them the most common and lethal.

In this review, we thoroughly explored prevalent RAS mutations (particularly of the KRAS subtype). Only patients with LUAD and smokers had KRAS G12C, a transversion mutation that accounts for 41% of the GD of KRAS variants. The ubiquitous nature of KRAS G12C had led to extensive studies on it and numerous related TKIs have been evaluated, including sotorasib, adagrasib, GDC-6036, JNJ-74699157, and D-1553. However, unlike other KRAS mutations, KRAS G12C activates downstream Ral A/B and RAF/MEK/ERK pathways, as well as decreases phosphorylated AKT levels. The second mutation assessed, KRAS G12V, was found in 19% of the overall KRAS mutations. Finally, we discussed the KRAS G12D mutation, which accounted for 13% of all KRAS mutations. When combined with TP53 co-mutations, KRAS mutations have shown promising potential as biomarkers for unfavorable immunotherapy outcomes and have been linked to low TMB. It also had a median PFS of 11 weeks, which was the shortest among the KRAS G12C and KRAS G12V mutations, with median PFSs of 15.57 weeks and 23.28 weeks, respectively.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

We appreciate Alhada Armed Forces Hospital's cooperation and input on this review.

AUTHOR CONTRIBUTIONS

All authors contributed equally and have been involved in the writing of the manuscript at draft, any revision stages, and have read and approved the final version.

REFERENCES

Addeo, A., Banna, G. L., & Friedlaender, A. (2021). KRAS G12C Mutations in NSCLC: From Target to Resistance. *Cancers 2021, Vol. 13, Page 2541*, *13*(11), 2541. https://doi.org/10.3390/CANCERS13112541

Akslen, L. A., Puntervoll, H., Bachmann, I. M., Straume, O., Vuhahula, E., Kumar, R., & Molven, A. (2008). Mutation analysis of the EGFR-NRAS-BRAF pathway in melanomas from black Africans and other subgroups of cutaneous melanoma. *Melanoma Research*, *18*(1), 29–35. https://doi.org/10.1097/CMR.0B013E3282F32517

Araujo, L. H., Souza, B. M., Leite, L. R., Parma, S. A. F., Lopes, N. P., Malta, F. S. V., & Freire, M. C. M. (2021). Molecular profile of KRAS G12C-mutant colorectal and non-small-cell lung cancer. *BMC Cancer*, *21*(1), 1–8. https://doi.org/10.1186/S12885-021-07884-8/TABLES/2

Aredo, J. v., Padda, S. K., Kunder, C. A., Han, S. S., Neal, J. W., Shrager, J. B., & Wakelee, H. A. (2019). Impact of KRAS mutation subtype and concurrent pathogenic mutations on non-small cell lung cancer outcomes. *Lung Cancer (Amsterdam, Netherlands)*, *133*, 144–150. https://doi.org/10.1016/J.LUNGCAN.2019.05.015

Barrueto, L., Caminero, F., Cash, L., Makris, C., Lamichhane, P., & Deshmukh, R. R. (2020). Resistance to Checkpoint Inhibition in Cancer Immunotherapy. *Translational Oncology*, *13*(3), 100738. https://doi.org/10.1016/J.TRANON.2019.12.010

Baugh, E. H., Ke, H., Levine, A. J., Bonneau, R. A., & Chan, C. S. (2017). Why are there hotspot mutations in the TP53 gene in human cancers? *Cell Death & Differentiation 2018 25:1*, *25*(1), 154–160. https://doi.org/10.1038/cdd.2017.180

Beukers, W., Hercegovac, A., & Zwarthoff, E. C. (2014). HRAS mutations in bladder cancer at an early age and the possible association with the Costello Syndrome. *European Journal of Human Genetics*, *22*, 837–839. https://doi.org/10.1038/ejhg.2013.251

Biernacka, A., Tsongalis, P. D., Peterson, J. D., de Abreu, F. B., Black, C. C., Gutmann, E. J., Liu, X., Tafe, L. J., Amos, C. I., & Tsongalis, G. J. (2016). The potential utility of re-mining results of somatic mutation testing: KRAS status in lung adenocarcinoma. *Cancer Genetics*, *209*(5), 195. https://doi.org/10.1016/J.CANCERGEN.2016.03.001

Cai, D., Hu, C., Li, L., Deng, S., Yang, J., Han-Zhang, H., & Li, M. (2020). The prevalence and prognostic value of KRAS co-mutation subtypes in Chinese advanced non-small cell lung cancer patients. *Cancer Medicine*, *9*(1), 84–93. https://doi.org/10.1002/CAM4.2682

Cannataro, V. L., Gaffney, S. G., Stender, C., Zhao, Z. M., Philips, M., Greenstein, A. E., & Townsend, J. P. (2018). Heterogeneity and mutation in KRAS and associated oncogenes: evaluating the potential for the evolution of resistance to targeting of KRAS G12C. *Oncogene 2018 37:18*, *37*(18), 2444–2455. https://doi.org/10.1038/s41388-017-0105-z

Del Re, M., Tiseo, M., Bordi, P., D'Incecco, A., Camerini, A., Petrini, I., Lucchesi, M., Inno, A., Spada, D., Vasile, E., Citi, V., Malpeli, G., Testa, E., Gori, S., Falcone, A., Amoroso, D., Chella, A., Cappuzzo, F., Ardizzoni, A., … Danesi, R. (2017). Contribution of KRAS mutations and c.2369C > T (p.T790M) EGFR to acquired resistance to EGFR-TKIs in EGFR mutant NSCLC: a study on circulating tumor DNA. *Oncotarget*, *8*(8), 13611. https://doi.org/10.18632/ONCOTARGET.6957

Donninger, H., Vos, M. D., & Clark, G. J. (2007). The RASSF1A tumor suppressor. *Journal of Cell Science*, *120*(18), 3163–3172. https://doi.org/10.1242/JCS.010389

Dunnett-Kane, V., Burkitt-Wright, E., Blackhall, F. H., Malliri, A., Evans, D. G., & Lindsay, C. R. (2020). Germline and sporadic cancers driven by the RAS pathway: parallels and contrasts. *Annals of Oncology*, *31*(7), 873–883. https://doi.org/10.1016/J.ANNONC.2020.03.291

Falah Alshammari, R., Safaq Alshammari, N., Alreshidi, F., Alharbi, S., Galdlkarim Ahemd, H., hamid Alrashidi, S., & Ghali Alanazi, F. (2021). *Role of demographic characteristics in attitude towards cancer prevention some measures in Hail region, Saudi Arabia*. Retrieved June 2, 2022, from www.isisn.org

Faubert, B., Solmonson, A., & DeBerardinis, R. J. (2020). Metabolic reprogramming and cancer progression. *Science*, *368*(6487). https://doi.org/10.1126/SCIENCE.AAW5473/ASSET/686116D0-E501-42AE-991A-BA9FC51159B0/ASSETS/GRAPHIC/368_AAW5473_FA.JPEG

Gao, G., Liao, W., Ma, Q., Zhang, B., Chen, Y., & Wang, Y. (2020). KRAS G12D mutation predicts lower TMB and drives immune suppression in lung adenocarcinoma. *Lung Cancer*, *149*, 41–45. https://doi.org/10.1016/J.LUNGCAN.2020.09.004

Garcia-Alvarez, A., Ortiz, C., & Muñoz-Couselo, E. (2021). *Current Perspectives and Novel Strategies of NRAS-Mutant Melanoma*. https://doi.org/10.2147/OTT.S278095

Gatti-Mays, M. E., Redman, J. M., Collins, J. M., & Bilusic, M. (2017). Cancer vaccines: Enhanced immunogenic

modulation through therapeutic combinations. *Https://Doi.Org/10.1080/21645515.2017.1364322*, *13*(11), 2561–2574. https://doi.org/10.1080/21645515.2017.1364322

Gauthaman, A., & Moorthy, A. (2021). Prevalence of K-ras Codon 12 Mutations in Indian Patients with Head and Neck Cancer. *Indian Journal of Clinical Biochemistry*, *36*(3), 370–374. https://doi.org/10.1007/S12291-020-00882-W/TABLES/1

Gripp, K. W., & Lin, A. E. (2012). Costello syndrome: a Ras/mitogen activated protein kinase pathway syndrome (rasopathy) resulting from HRAS germline mutations. *Genetics in Medicine 2012 14:3*, *14*(3), 285–292. https://doi.org/10.1038/gim.0b013e31822dd91f

Herbst, R. S., Morgensztern, D., & Boshoff, C. (2018). The biology and management of non-small cell lung cancer. *Nature Publishing Group*. https://doi.org/10.1038/nature25183

Hong, D. S., Fakih, M. G., Strickler, J. H., Desai, J., Durm, G. A., Shapiro, G. I., Falchook, G. S., Price, T. J., Sacher, A., Denlinger, C. S., Bang, Y.-J., Dy, G. K., Krauss, J. C., Kuboki, Y., Kuo, J. C., Coveler, A. L., Park, K., Kim, T. W., Barlesi, F., … Li, B. T. (2020). KRAS G12C Inhibition with Sotorasib in Advanced Solid Tumors . *New England Journal of Medicine*, *383*(13), 1207–1217. https://doi.org/10.1056/NEJMOA1917239/SUPPL_FILE/NEJMOA1917239_DATA-SHARING.PDF

Ihle, N. T., Byers, L. A., Kim, E. S., Saintigny, P., Lee, J. J., Blumenschein, G. R., Tsao, A., Liu, S., Larsen, J. E., Wang, J., Diao, L., Coombes, K. R., Chen, L., Zhang, S., Abdelmelek, M. F., Tang, X., Papadimitrakopoulou, V., Minna, J. D., Lippman, S. M., … Powis, G. (2012). Effect of KRAS Oncogene Substitutions on Protein Behavior: Implications for Signaling and Clinical Outcome. *JNCI: Journal of the National Cancer Institute*, *104*(3), 228–239. https://doi.org/10.1093/JNCI/DJR523

Jänne, P. A., Smith, I., McWalter, G., Mann, H., Dougherty, B., Walker, J., Orr, M. C. M., Hodgson, D. R., Shaw, A. T., Pereira, J. R., Jeannin, G., Vansteenkiste, J., Barrios, C. H., Franke, F. A., Crinò, L., & Smith, P. (2015). Impact of KRAS codon subtypes from a randomised phase II trial of selumetinib plus docetaxel in KRAS mutant advanced non-small-cell lung cancer. *British Journal of Cancer 2015 113:2*, *113*(2), 199–203. https://doi.org/10.1038/bjc.2015.215

Johnson, D. B., & Puzanov, I. (2015). Treatment of NRAS-Mutant Melanoma. *Current Treatment Options in Oncology*, *16*(4), 1–12. https://doi.org/10.1007/S11864-015-0330-Z/FIGURES/1

Kalikaki, A., Koutsopoulos, A., Trypaki, M., Souglakos, J., Stathopoulos, E., Georgoulias, V., Mavroudis, D., & Voutsina, A. (2008). Comparison of EGFR and K-RAS gene status between primary tumours and corresponding metastases in NSCLC. *British Journal of Cancer 2008 99:6*, *99*(6), 923–929. https://doi.org/10.1038/sj.bjc.6604629

Karachaliou, N., Mayo, C., Costa, C., Magrí, I., Gimenez-Capitan, A., Molina-Vila, M. A., & Rosell, R. (2013a). KRAS mutations in lung cancer. *Clinical Lung Cancer*, *14*(3), 205–214. https://doi.org/10.1016/J.CLLC.2012.09.007

Karachaliou, N., Mayo, C., Costa, C., Magrí, I., Gimenez-Capitan, A., Molina-Vila, M. A., & Rosell, R. (2013b). KRAS Mutations in Lung Cancer. *Clinical Lung Cancer*, *14*(3), 205–214. https://doi.org/10.1016/J.CLLC.2012.09.007

Khaltaev, N., & Axelrod, S. (2020). Global lung cancer mortality trends and lifestyle modifications: Preliminary analysis. *Chinese Medical Journal*, *133*(13), 1526–1532. https://doi.org/10.1097/CM9.0000000000000918

Kim, J., Peters, C. E., Arrandale, V. H., Labrèche, F., Ge, C. B., McLeod, C. B., Song, C., Lavoué, J., Davies, H. W., Nicol, A. M., Pahwa, M., & Demers, P. A. (2018). Burden of lung cancer attributable to occupational diesel engine exhaust exposure in Canada. *Occupational and Environmental Medicine*, *75*(9), 617–622. https://doi.org/10.1136/OEMED-2017-104950

Kiuru, M., Urban, J., Zhu, G., Rybak, I., Terrell, J. R., Qi, L., McPherson, J. D., Marghoob, A. A., & Rauen, K. A. (2020). RAS pathway influences the number of melanocytic nevi in cardiofaciocutaneous and Costello syndromes. *Journal of the American Academy of Dermatology*, *82*(5), 1091–1093. https://doi.org/10.1016/J.JAAD.2020.01.038

Kontomanolis, E. N., Koutras, A., Syllaios, A., Schizas, D., Mastoraki, A., Garmpis, N., Diakosavvas, M., Angelou, K., Tsatsaris, G., Pagkalos, A., Ntounis, T., & Fasoulakis, Z. (2020). Role of Oncogenes and Tumor-suppressor Genes in Carcinogenesis: A Review. *Anticancer Research*, *40*(11), 6009–6015. https://doi.org/10.21873/ANTICANRES.14622

Kurishima, K., Miyazaki, K., Watanabe, H., Shiozawa, T., Ishikawa, H., Satoh, H., & Hizawa, N. (2018). Lung cancer patients with synchronous colon cancer. *Molecular and Clinical Oncology*, *8*(1), 137. https://doi.org/10.3892/MCO.2017.1471

Lee, Y.-L., & Shih, C.-M. (2020). Association between H-RAS T81C genetic polymorphism with non-small cell Lung cancer. *European Respiratory Journal*, *56*(suppl 64), 1760. https://doi.org/10.1183/13993003.CONGRESS-2020.1760

Lindsay, C. R., Jamal-Hanjani, M., Forster, M., & Blackhall, F. (2018). KRAS: Reasons for optimism in lung cancer. *European Journal of Cancer*, *99*, 20–27. https://doi.org/10.1016/J.EJCA.2018.05.001

Loong, H. H. F., Du, N., Cheng, C., Lin, H., Guo, J., Lin,

G., Li, M., Jiang, T., Shi, Z., Cui, Y., Jin, X., Yao, J., Xing, Y., Yao, M., Wang, K., Mok, T. S. K., & Liu, L. (2020). KRAS G12C mutations in Asia: a landscape analysis of 11,951 Chinese tumor samples. *Translational Lung Cancer Research*, *9*(5), 1759. https://doi.org/10.21037/TLCR-20-455

Lyu, H., Li, M., Jiang, Z., Liu, Z., & Wang, X. (2019). Correlate the TP53 Mutation and the HRAS Mutation with Immune Signatures in Head and Neck Squamous Cell Cancer. *Computational and Structural Biotechnology Journal*, *17*, 1020–1030. https://doi.org/10.1016/J.CSBJ.2019.07.009

Malapelle, U., Passiglia, F., Cremolini, C., Reale, M. L., Pepe, F., Pisapia, P., Avallone, A., Cortinovis, D., de Stefano, A., Fassan, M., Fontanini, G., Galetta, D., Lauricella, C., Listì, A., Loupakis, F., Pagni, F., Pietrantonio, F., Pilotto, S., Righi, L., … Novello, S. (2021). RAS as a positive predictive biomarker: focus on lung and colorectal cancer patients. *European Journal of Cancer*, *146*, 74–83. https://doi.org/10.1016/J.EJCA.2021.01.015

Mascaux, C., Iannino, N., Martin, B., Paesmans, M., Berghmans, T., Dusart, M., Haller, A., Lothaire, P., Meert, A. P., Noel, S., Lafitte, J. J., & Sculier, J. P. (2005). The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. *British Journal of Cancer*, *92*(1), 131–139. https://doi.org/10.1038/SJ.BJC.6602258

Nadal, E., Chen, G., Prensner, J. R., Shiratsuchi, H., Sam, C., Zhao, L., Kalemkerian, G. P., Brenner, D., Lin, J., Reddy, R. M., Chang, A. C., Capellà, G., Cardenal, F., Beer, D. G., & Ramnath, N. (2014). KRAS-G12C mutation is associated with poor outcome in surgically resected lung adenocarcinoma. *Journal of Thoracic Oncology*, *9*(10), 1513–1522. https://doi.org/10.1097/JTO.0000000000000305/ATTACHMENT/297BB80F-83B1-4223-B1C6-A56F7491DD85/MMC2.PPT

O'Bryan, J. P. (2019). Pharmacological Targeting of RAS: Recent Success with Direct Inhibitors. *Pharmacological Research*, *139*, 503. https://doi.org/10.1016/J.PHRS.2018.10.021

O'Neill, C. H., & Scoggins, C. R. (2019). Melanoma. *Journal of Surgical Oncology*, *120*(5), 873–881. https://doi.org/10.1002/JSO.25604

Offerman, S., Prinsen, C. F., Knol, A., Methorst, N., Kamphorst, J., & Niemantsverdriet, M. (2021). Short report: Performance evaluation of the Idylla™ KRAS and EGFR mutation tests on paraffin-embedded cytological NSCLC samples. *Diagnostic Pathology*, *16*(1), 1–9. https://doi.org/10.1186/S13000-021-01121-3/FIGURES/2

Ou, S.-H. I., Jänne, P. A., Leal, T. A., Rybkin, I. I., Sabari, J. K., Barve, M. A., Bazhenova, L., Johnson, M. L., Velastegui, K. L., Cilliers, C., Christensen, J. G., Yan, X., Chao, R. C., & Papadopoulos, K. P. (2022). First-in-Human Phase I/IB Dose-Finding Study of Adagrasib (MRTX849) in Patients With Advanced KRASG12C Solid Tumors (KRYSTAL-1). *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology*, JCO2102752. https://doi.org/10.1200/JCO.21.02752

Passiglia, F., Malapelle, U., del Re, M., Righi, L., Pagni, F., Furlan, D., Danesi, R., Troncone, G., & Novello, S. (2020). KRAS inhibition in non–small cell lung cancer: Past failures, new findings and upcoming challenges. *European Journal of Cancer*, *137*, 57–68. https://doi.org/10.1016/J.EJCA.2020.06.023

Pązik, M., Michalska, K., Żebrowska-Nawrocka, M., Łochowski, M., & Balcerczak, E. (2020). *Clinical signicance of HRAS and KRAS genes expression in patients with non-small-cell lung cancer*. https://doi.org/10.21203/rs.3.rs-108191/v1

Rauen, K. A. (2007). HRAS and the Costello syndrome. *Clinical Genetics*, *71*(2), 101–108. https://doi.org/10.1111/J.1399-0004.2007.00743.X

Reita, D., Pabst, L., Pencreach, E., Guérin, E., Dano, L., Rimelen, V., Voegeli, A.-C., Vallat, L., Mascaux, C., & Beau-Faller, M. (2022). Direct Targeting KRAS Mutation in Non-Small Cell Lung Cancer: Focus on Resistance. *Cancers 2022, Vol. 14, Page 1321*, *14*(5), 1321. https://doi.org/10.3390/CANCERS14051321

Riely, G. J., Kris, M. G., Rosenbaum, D., Marks, J., Li, A., Chitale, D. A., Nafa, K., Riedel, E. R., Hsu, M., Pao, W., Miller, V. A., & Ladanyi, M. (2008). Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *14*(18), 5731–5734. https://doi.org/10.1158/1078-0432.CCR-08-0646

Roberts, P. J., & Stinchcombe, T. E. (2013). KRAS mutation: Should we test for it, and does it matter. *Journal of Clinical Oncology*, *31*(8), 1112–1121. https://doi.org/10.1200/JCO.2012.43.0454

Rogers, L. M., Suresh Veeramani, •, & Weiner, G. J. (2015). *Complement in monoclonal antibody therapy of cancer*. https://doi.org/10.1007/s12026-014-8542-z

Roskoski, R. (2021). Blockade of mutant RAS oncogenic signaling with a special emphasis on KRAS. *Pharmacological Research*, *172*, 105806. https://doi.org/10.1016/J.PHRS.2021.105806

Salem, M. E., El-Refai, S. M., Sha, W., Puccini, A., Grothey, A., George, T. J., Hwang, J. J., O'Neil, B., Barrett, A. S., Kadakia, K. C., Musselwhite, L. W., Raghavan, D., van Cutsem, E., Tabernero, J., & Tie, J. (2022). Landscape of KRAS G12C , Associated Genomic Alterations, and Interrelation With Immuno-Oncology Biomarkers in KRAS -Mutated

Cancers . *JCO Precision Oncology*, *6*. https://doi.org/10.1200/po.21.00245

Salgia, R., Pharaon, R., Mambetsariev, I., Nam, A., & Sattler, M. (2021). The improbable targeted therapy: KRAS as an emerging target in non-small cell lung cancer (NSCLC). *Cell Reports. Medicine*, *2*(1). https://doi.org/10.1016/J.XCRM.2020.100186

Sha, D., Jin, Z., Budczies, J., Kluck, K., Stenzinger, A., & Sinicrope, F. A. (2020). Tumor Mutational Burden (TMB) as a Predictive Biomarker in Solid Tumors. *Cancer Discovery*, *10*(12), 1808. https://doi.org/10.1158/2159-8290.CD-20-0522

Skoulidis, F., Li, B. T., Dy, G. K., Price, T. J., Falchook, G. S., Wolf, J., Italiano, A., Schuler, M., Borghaei, H., Barlesi, F., Kato, T., Curioni-Fontecedro, A., Sacher, A., Spira, A., Ramalingam, S. S., Takahashi, T., Besse, B., Anderson, A., Ang, A., … Govindan, R. (2021). Sotorasib for Lung Cancers with KRAS p.G12C Mutation . *New England Journal of Medicine*, *384*(25), 2371–2381. https://doi.org/10.1056/NEJMOA2103695/SUPPL_FILE/NEJMOA2103695_DATA-SHARING.PDF

Tamiya, Y., Matsumoto, S., Ikeda, T., Yoh, K., Kato, T., Nishino, K., Kodani, M., Nakamura, A., Furuya, N., Kuyama, S., Miyamoto, S., Toyozawa, R., Shingyoji, M., Naito, T., Oizumi, S., Tanaka, H., Udagawa, H., Nosaki, K., Zenke, Y., & Goto, K. (2021). Clinico-pathological and genomic features of NRAS- or HRAS-mutated non-small cell lung cancer (NSCLC) identified in large-scale genomic screening project (LC-SCRUM-Asia). *Https://Doi.Org/10.1200/JCO.2021.39.15_suppl.9054*, *39*(15_suppl), 9054–9054. https://doi.org/10.1200/JCO.2021.39.15_SUPPL.9054

Tang, D., Kroemer, G., & Kang, R. (2021). Oncogenic KRAS blockade therapy: renewed enthusiasm and persistent challenges. *Molecular Cancer 2021 20:1*, *20*(1), 1–24. https://doi.org/10.1186/S12943-021-01422-7

To, M. D., Wong, C. E., Karnezis, A. N., del Rosario, R., di Lauro, R., & Balmain, A. (2008). Kras regulatory elements and exon 4A determine mutation specificity in lung cancer. *Nature Genetics 2008 40:10*, *40*(10), 1240–1244. https://doi.org/10.1038/ng.211

Tomasini, P., Walia, P., Labbe, C., Jao, K., & Leighl, N. B. (2016). *Targeting the KRAS Pathway in Non-Small Cell Lung Cancer*. https://doi.org/10.1634/theoncologist.2015-0084

Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-Tieulent, J., & Jemal, A. (2015). Global cancer statistics, 2012. *CA: A Cancer Journal for Clinicians*, *65*(2), 87–108. https://doi.org/10.3322/CAAC.21262

Vasta, J. D., Peacock, D. M., Zheng, Q., Walker, J. A., Zhang, Z., Zimprich, C. A., Thomas, M. R., Beck, M. T., Binkowski, B. F., Corona, C. R., Robers, M. B., & Shokat, K. M. (2022). KRAS is vulnerable to reversible switch-II pocket engagement in cells. *Nature Chemical Biology 2022*, 1–9. https://doi.org/10.1038/s41589-022-00985-w

Yang, J., Lin, J., Liu, T., Chen, T., Pan, S., Huang, W., & Li, S. (2014). Analysis of lncRNA expression profiles in non-small cell lung cancers (NSCLC) and their clinical subtypes. *Lung Cancer*, *85*(2), 110–115. https://doi.org/10.1016/J.LUNGCAN.2014.05.011

Zhang, X., & Zhang, Y. (2015). Bladder Cancer and Genetic Mutations. *Cell Biochemistry and Biophysics 2015 73:1*, *73*(1), 65–69. https://doi.org/10.1007/S12013-015-0574-Z

Zhao, J., Zhang, X., He, M., Chen, X., Cui, X., Qin, T., Niu, X., & Zhao, L. (2021). Poor Prognosis of Pulmonary Adenosquamous Carcinoma with NRAS and HRAS Double Mutation. *OncoTargets and Therapy*, *14*, 1113. https://doi.org/10.2147/OTT.S295813

Zinatizadeh, M. R., Momeni, S. A., Zarandi, P. K., Chalbatani, G. M., Dana, H., Mirzaei, H. R., Akbari, M. E., & Miri, S. R. (2019). The Role and Function of Ras-association domain family in Cancer: A Review. *Genes & Diseases*, *6*(4), 378–384. https://doi.org/10.1016/J.GENDIS.2019.07.008

Zlobec, I., Kovac, M., Erzberger, P., Molinari, F., Bihl, M. P., Rufle, A., Foerster, A., Frattini, M., Terracciano, L., Heinimann, K., & Lugli, A. (2010). Combined analysis of specific KRAS mutation, BRAF and microsatellite instability identifies prognostic subgroups of sporadic and hereditary colorectal cancer. *International Journal of Cancer*, *127*(11), 2569–2575. https://doi.org/10.1002/IJC.25265

Zuo, S., Wei, M., Wang, S., Dong, J., & Wei, J. (2020). Pan-Cancer Analysis of Immune Cell Infiltration Identifies a Prognostic Immune-Cell Characteristic Score (ICCS) in Lung Adenocarcinoma. *Frontiers in Immunology*, *11*, 1218. https://doi.org/10.3389/FIMMU.2020.01218/BIBTEX