Scientific Research Publishing

Journal of Cancer Therapy, 2023, 14, 257-276
https://www.scirp.org/journal/jct
ISSN Online: 2151-1942
ISSN Print: 2151-1934

Exploration of Kras Mutations and Their Potential for Being a Target Molecule in Cancer Chemotherapy

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How to cite this paper: Chinthareddy, A. and Oroszi, T. (2023) Exploration of Kras Mutations and Their Potential for Being a Target Molecule in Cancer Chemotherapy. *Journal of Cancer Therapy*, **14**, 257-276.
https://doi.org/10.4236/jct.2023.146022

Received: April 18, 2023
Accepted: June 13, 2023
Published: June 16, 2023

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Abstract

The Rat sarcoma virus (RAS) family of proteins, which includes the Kristen Rat sarcoma virus (KRAS), is linked to nearly one-fourth of all human cancers. KRAS mutations, in particular, are associated with Non-Small Cell Lung Carcinoma (NSCLC), colorectal cancer, adenocarcinomas, ovarian carcinoma, and endometrial tumors. KRAS activates 80 different signaling pathways, including Mitogen-activated protein kinases (MAPK) and Phosphoinositide 3-kinase (PI3K), and up-regulates transcription factors such as ETS like Protein (ELK), Jun Proto-Oncogene (JUN), and Myelocytomatosis (MYC), which are involved in cell differentiation, proliferation, transformation, and survival. KRAS mutations are also known to cause autocrine function, which further exacerbates the situation. In NSCLC, KRAS mutations have a strong positive correlation with the disease, particularly in patients with a smoking history. In pancreatic cancer, KRAS mutations are a dominant pathological basis, with most mutations being G12D, G12V, G13D, G13C, G13S, and G13R. These mutations serve as initial markers in tumorigenesis and are associated with poor prognosis and high mortality rates. In colorectal cancer, KRAS mutations contribute to 4/5 of cases, with cellular mechanisms involving the MAPK pathway, which resists anti-epidermal growth factor antibodies. In Low-grade Serous Ovarian Cancer (LGSOC), KRAS mutations are associated with altered signaling in the MAPK pathway and drug resistance. However, treatments such as Selumetinib, a down regulator of RAS/Rapidly Accelerated Fibrosarcoma (RAF)/Mitogen-activated protein kinase (MEK) pathways, and a combination of trametinib and buparlisib have shown promise in managing LGSOC when diagnosed early through KRAS mutation markers. Although KRAS mutations are commonly associated with many types of cancer, their use in clinical practice is limited due to the lack of accurate methods to identify them. It is needed to further isolate the KRAS mu-

tation products and correlate the cancer-causing genes to make it a promising approach for cancer chemotherapy.

Keywords

KRAS Mutations, Non-Small Cell Lung Carcinoma (NSCLC), Colorectal Cancer, MAPK Pathway, Chemotherapy

1. Introduction

The high rate of death caused by cancer and the lack of progress in reducing mortality despite advancements in medical treatments are major concerns in cancer management [1]. Scientists are searching for novel strategies to effectively manage or at least diagnose at an early stage for a better prognosis. RAS signaling pathway is attributed to the development of many forms of solid tumors. Kirsten rat sarcoma viral oncogene (KRAS) is a proto-oncogene (family of RAS proteins) that serves as a signaling molecule and regulates various mutagenic pathways [2]. Located on chromosome 12p12.1, the KRAS gene produces a variety of proteins that contribute to cancer development through pathways such as Mitogen-activated protein kinase (MAPK), Protein Kinase, Phosphoinositide 3 kinase (PI3K), and mutL homolog1 [3]. Eighty-five mutations associated with the KRAS gene have been discovered, a starting basis for carcinogenesis. The presence of KRAS is directly associated with many cancers and thus serves as a prognostic indicator if it is detected early with suitable sensitive methods [4]. The mutagenic property of the KRAS gene makes it an ideal marker and spot for targeting anti-cancer drugs [5]. KRAS mutations are also used to create genetically engineered animal models that simulate human cancers and are used to develop anti-cancer drugs in the preclinical stages. Previous research identified KRAS as an oncogene yet developing drugs against it proved exceptionally challenging due to its complex signaling dynamics and reliance on upstream activators. Although indirect inhibitors (MEK/PI3K inhibitors) showed promise, KRAS mutation results remained limited. Emerging strategies encompass inhibiting downstream effectors, exploiting synthetic lethality, targeting feedback loops and employing combination therapies.

In this paper, a detailed study is carried out to explore the role of KRAS mutations in cancer etiology, the possible treatment niche around these mutations, and their potential to be used in animal models.

1.1. The Biological Role of the KRAS Gene

The KRAS gene on chromosome 12p12.1 is frequently mutated in various types of cancer, with the G12C mutation being the most prevalent in non-small cell lung cancer. This gene is mutated in a large percentage of pancreatic, colorectal, and lung adenocarcinomas. Additionally, KRAS mutations are more common in western populations and smokers. The KRAS protein, produced by the KRAS

gene, is involved in intracellular signaling and the growth of tumor cells by interacting with other proteins such as EGFR, MEK, MAPK, Raf, PI3K, and AKT [6]. The active form of KRAS is when it is bound to GTP, while the inactive form is when it is bound to GDP. The activity of the protein is affected by structural changes caused by GTP binding.

Switching inactive RAS, bound to GDP, to active RAS, bound to GTP, requires guanine nucleotide exchange factors (GEFs). RAS can convert back to its inactive form through its intrinsic hydrolytic activity. However, mutations in the KRAS gene can prevent this process by locking it in the active state, leading to the constant activation of downstream proteins and increasing cell proliferation and survival. Specifically, a mutation in codon 12, typically occupied by a glycine residue, blocks the binding of GAPs to KRAS and thus reduces the hydrolysis of GTP, resulting in high levels of active RAS [7].

1.2. KRAS Mutations in Different Types of Cancer

1.2.1. Solid Tumors

Many human cancers are linked to the RAS protein family, including HRAS, NRAS, KRAS4A, and KRAS4B. The most commonly mutated proteins within this family are KRAS, associated with the development of Non-Small Cell Lung Carcinoma (NSCLC), colorectal cancer, adenocarcinomas, ovarian cancer, ovarian cancer, and carcinoma, and endometrial tumors [8]. When bound to GTP, KRAS can activate a wide range of signaling pathways, including MAPK, PI3K, and RAF-MAPK ERK. This process can occur rapidly, activating various downstream molecules and cellular processes [9]. KRAS is a key player in regulating transcription factors such as ELK, JUN, and MYC, which play important roles in various cellular processes, including cell differentiation, proliferation, transformation, and survival. When KRAS is mutated, it can lead to a permanent binding to GTP and prolonged activation of signaling pathways, increasing cell proliferation and tumor formation [10]. Apart from cell signaling pathways, KRAS mutation is also involved in autocrine function, further aggravating the situation.

1.2.2. Non-Small Cell Lung Cancer (NSCLC)

Research has shown a strong link between KRAS mutations, particularly the G12C mutation, and non-small cell lung cancer (NSCLC), particularly in patients with a smoking history. Other KRAS mutations, such as G12V and G12D, have also been found to contribute to lung adenocarcinoma and have been found to occur in conjunction with mutations in other genes, such as p53, STK11, KEAP1, ATM, MET, ERBB2, and BRAF [11] [12]. Thus, KRAS mutation serves as a survival indicator for patients with NSCLC; the presence of KRAS mutation is a prominent marker in deciding the prognosis.

1.2.3. Pancreatic Cancer

KRAS mutation is one of the dominant pathological basis in the development of pancreatic cancer, with most of the mutations being G12D, G12V, G13D, G13C,

G13S, and G13R [10]. These mutations serve as early indicators in the development of tumors in the pancreas, such as pancreatic intraepithelial neoplasia and papillary mucinous neoplasm. Patients exhibiting KRAS mutation-derived pancreatic cancer have a poor prognosis and high mortality rate. KRAS mutation leads to increased metabolism in neoplasms, upregulation of granulocyte-macrophage colony-stimulating factor, Interleukins (IL-6), vascular endothelial growth factors, and chemokines resulting in heightened cell proliferation [13]. The cellular mechanism in pancreatic cancer is the activation of the ERK1-MAPK pathway, which alarmingly is resistant to most chemotherapy drugs leading to therapeutic failure. KRAS mutation also leads to increased turnover of other metastatic proteins, such as SARC, STAT3, and COX2, that causes further tumor assault in the systemic circulation.

1.2.4. Colorectal Cancer

KRAS mutations contribute to 4/5th of the total colorectal cancer and NRAS, and HRAS mutations cause the other 1/5th of the cancers. mutation variants involved in colorectal cancer are G12D, G12V, G12A, G12C, G13D, Q61H, Q61L, and Q61R. Thus, these mutations serve as an essential indicator in the development of colorectal tumors. The cellular mechanisms involved in colorectal cancer are MAPK pathway which resists the anti-epidermal growth factor antibodies [10]. Generally poor outcomes of the KRAS mutations in colorectal cancer can be attributed to faster progression into metastatic forms, a poor cellular demarcation that impedes the drug action, and simultaneous attack on hepatocytes leading to liver tumors.

1.2.5. Low-Grade Serous Ovarian Cancer (LGSOC)

Although LGSOC accounts for the low proportion of cancers, they are known to cause much devastating effects with poor prognosis and high fatality rate due to the tumor's drug resistance. The principal cellular mechanism behind the LGSOC is altered signaling in the MAPK pathway caused by somatic changes induced by KRAS mutation [14]. However, there is a light of hope in the management of LGSOC as patients treated with Selumetinib (a down regulator of RAS/RAF/MEK pathways) have responded well to the treatment with increased life expectancy [15]. Furthermore, a similar study with a combination of trametinib (antagonist of MEK1/2 pathway) and buparlisib (antagonist of PI3K) has shown a better response indicating that LGSOC is highly manageable when diagnosed early by screening KRAS mutation markers [16].

1.2.6. Endometrial Cancer

Endometrial cancer is highly prevalent among women in western countries. Type I endometrial cancer is known to have caused due to KRAS mutations. KRAS mutations stimulate estrogen receptors through the RAS/MAPK pathway [17]. Therefore, the current therapeutic modalities aim at KRAS mutant endometrial cancer for early diagnosis and treatment [18]. Current investigations with MEK/Akt inhibitors and Poly ADP ribose inhibitors have enlightened this

fact, although a thorough investigation has to be carried out to appreciate their use in endometrial cancer [19].

1.3. KRAS Gene and Its Role in Cancer Treatment

KRAS testing can identify patients with lung cancer who may benefit from targeted therapies that target the KRAS pathway. Several drugs, such as Selumetinib, a MEK inhibitor, have shown promise in treating advanced non-small cell lung cancer. Selumetinib is currently being studied in clinical trials for KRAS-mutant non-small cell lung cancer, with some studies showing an improvement in progression-free survival and overall response rate but no significant difference in overall survival. However, the trial size, the sensitivity of KRAS mutations to the Selumetinib, and other genetic changes may have affected the results [20]. Additionally, other MEK inhibitors and inhibitors of the PI3K/AKT/mTOR signaling cascade are also in development for KRAS-mutant non-small cell lung cancer. It is important to note that KRAS-mutant NSCLC may also be stratified by LKB1 status in future development.

The potential benefits of using dual inhibition of the PI3K/AKT and MEK/ERK signaling pathways to block oncogenic RAS signaling in KRAS-mutant NSCLC have been shown in several studies using genetically engineered mouse models. Preclinical studies have also confirmed that dual inhibition is necessary to achieve maximum efficacy and prevent resistance to these inhibitors. Several clinical trials testing the combination of MEK inhibitors and PI3K inhibitors, as well as inhibitors of RAS signaling, c-met, and more in patients with advanced solid tumors and NSCLC, are currently underway to determine the effectiveness of these treatments in blocking oncogenic RAS signaling [21] [22].

The evidence from laboratory studies and early clinical trials suggests that treatment with heat-shock protein-90 (HSP-90) inhibitors may have potential as a therapy for KRAS-mutant non-small cell lung cancer (NSCLC). However, results from Phase II clinical trials investigating HSP-90 inhibitors Ganetespib and Etaspimycin in patients with advanced NSCLC have not shown significant therapeutic activity. Further research is ongoing, including studies examining the use of HSP-90 inhibitors in combination with Chemotherapy and KRAS testing in developing new treatments such as EGFR TKIs [23]. In addition, two Phase III trials are currently underway comparing the pan-human epidermal growth factor receptor inhibitor dacomitinib with either placebo or erlotinib in patients with stage IIIB/IV NSCLC who have progressed after standard therapy. They prospectively collect tumor tissue for KRAS testing [24].

2. KRAS Mutation and Its Resistance to Cancer Therapy

2.1. KRAS and Epidermal Growth Factor Receptor (EGFR)

The KRAS gene mutation in colorectal cancer is linked to a lower response rate to specific chemotherapy treatments and a worse overall prognosis. Studies have shown that patients with the wild-type KRAS gene have better outcomes than

those with the mutant KRAS gene. The KRAS protein is important in mediating the EGFR-induced signaling pathways, and mutations in other mediators of the EGFR pathway, such as BRAF, PTEN, and PIK3CA, may also affect the response to anti-EGFR therapies [25] [26]. Adding anti-EGFR treatments to Chemotherapy has been shown not to improve response in patients with mutant KRAS and may even be harmful. However, not all KRAS point mutations lead to resistance to anti-EGFR monoclonal antibodies. Some studies have shown that patients with the KRAS G13D mutation have better survival and progression-free survival after treatment with cetuximab compared to other KRAS mutant tumors, but this mutation has also been linked to worse overall survival compared to patients with other KRAS mutations or wild-type KRAS. Additionally, a pooled analysis of 533 metastatic colorectal cancer patients from the CRYSTAL and OPUS trials suggests that adding cetuximab to first-line Chemotherapy may benefit patients with KRAS G13D mutation [26].

2.2. KRAS and v-raf Murine Sarcoma Viral Oncogene Homolog B1 (BRAF)

Research suggests that specific mutations in the KRAS and BRAF genes may predict resistance to anti-EGFR therapies in patients with metastatic colorectal cancer. Studies have found that KRAS G13D mutation may have a better progression-free survival after treatment with cetuximab, but it is also associated with worse overall survival compared to patients with other KRAS mutations or wild-type KRAS [27]. Similarly, BRAF mutations are also linked to resistance to anti-EGFR treatment in colorectal cancer and have been associated with poor prognosis and worse outcomes when compared to tumors with wild-type BRAF. However, more studies are needed to understand the specific mechanisms underlying these associations and to confirm these findings. It has been observed that a small percentage of colorectal cancer samples carry a BRAF mutation, which is known to activate the ERK/MAPK signaling pathway and lead to changes in gene expression. Studies such as OPUS and CRYSTAL have also shown that BRAF mutations are mainly found in the absence of KRAS mutations, but due to the small sample size, definitive conclusions about the impact of BRAF mutations on prognosis cannot be made [28]. The presence of a BRAF mutation has been linked to poor outcomes in patients with colorectal cancer

2.3. KRAS and miRNA

Despite the high prevalence of KRAS mutations in various types of cancer, researchers have yet to develop effective therapies that target KRAS. Recent studies have proposed using microRNAs (miRNAs) to inhibit KRAS mutations in cancer treatment. miRNAs are small RNA molecules that regulate gene expression by binding to the 3'-untranslated regions of target messenger RNAs [29]. They can control transcription or translation, depending on their level of complementarity to the target mRNA. miRNAs have been studied for their potential use in treating colon cancer due to their role in controlling gene expression and their

tendency to be lost in cancer. Studies have identified specific miRNAs, such as let-7 and miR-143, linked to cell proliferation in colorectal cancer and are known to interact with KRAS [30].

2.4. KRAS and let-7 miRNA

Studies have suggested that let-7 miRNA plays a key role in regulating KRAS expression. The KRAS gene has binding sites for let-7 miRNA, and lower levels of let-7 in cancer tissues have been linked to higher levels of KRAS mRNA. Research has also shown that treatment with let-7 miRNA can decrease KRAS protein levels by up to 70%. The decreased let-7 expression has also been associated with poor patient outcomes in lung cancer and may affect the sensitivity of cells to radiation therapy. In colon cancer, let-7 miRNA may have a suppressive effect on cell growth and proliferation. Additionally, a specific genetic mutation in the KRAS gene that affects let-7 binding has been linked to increased KRAS expression and poor patient survival [31]. More research is needed to understand how let-7 is regulated and if its expression or its repressors can be used to target KRAS-driven cancers.

2.5. KRAS and miR-143

miR-143 is a miRNA found in lower levels in colorectal cancer samples thanin healthy tissue. It has been linked to KRAS-driven cancer development and has been shown to bind to the KRAS gene. Studies have shown that miR-143 acts as a tumor suppressor by decreasing KRAS protein levels and blocking cell proliferation. Low levels of miR-143 have been linked to shorter cancer-specific survival and progression-free survival in colorectal cancer patients [10]. These findings suggest that miRNA-based therapies may be promising for targeting KRAS-driven colorectal cancers. Various KRAS mutations associated with different types of cancers and the underlying cellular/molecular pathway are shown in **Table 1**.

Table 1. KRAS mutations-associated cancers and cellular signaling pathway involved.

KRAS mutations	Associated cancer(s)	Cellular/Molecular Pathway
KRAS G12V	Lung adenocarcinoma, Colorectal cancer, Pancreatic cancer	MAP kinase pathway [32]
KRAS G13D	Lung adenocarcinoma, Colorectal cancer	MAP kinase pathway [33]
KRAS Q61H	Lung adenocarcinoma, Colorectal cancer, Pancreatic cancer	MAP kinase pathway [34]
KRAS G12C	Lung adenocarcinoma	MAP kinase pathway [35]
KRAS G12R	Lung adenocarcinoma, Colorectal cancer	MAP kinase pathway [36]
KRAS A146T	Colorectal cancer	MAP kinase pathway [37]
KRAS G38A	Colorectal cancer	MAP kinase pathway [6]
KRAS G12S	Lung adenocarcinoma, Colorectal cancer	MAP kinase pathway [7]

2.6. Prognostic and Predictive Value of KRAS Mutation

KRAS is a protein that plays a significant role in the development of colorectal cancer. The Kirsten Ras In-Colorectal-Cancer Collaborative Group (RASCAL) study aimed to determine the prognostic significance of KRAS mutations in colorectal cancer [38]. The study included data from 2721 patients from 22 research groups in 13 countries and found that KRAS mutation was associated with poorer prognosis. Specific mutations, such as the glycine to valine substitution on codon 12 (C12V), had the greatest adverse effect on overall survival. Additionally, any G to T transition on codon 12 was linked to lower disease-free survival and overall survival. Studies such as Zlobec *et al.* and Andreyev *et al.* [39] [40] also found that KRAS mutations have a significant impact on the prognosis of colorectal cancer patients and that KRAS mutations are linked to more aggressive and rapid metastasis, particularly in the liver [39] [40].

2.7. KRAS Mutation as a Biomarker

KRAS is a protein that functions as a signaling transducer for various receptors, including the EGFR found on colon and rectal cells. Mutations in KRAS can lead to resistance to anti-EGFR chemotherapy drugs in colorectal cancer. Research suggests that KRAS plays a significant role in the early stages of endometrial cancer development, with a frequency of 10% - 30% in Type I oestrogen-related EC and 6% - 16% in endometrial hyperplasia specimens. It also has a role in predicting the invasive potential of certain tumors. KRAS mutations occur before TP53 gene inactivation, which marks the transition from low-grade to high-grade Type I EC. And it is used as a predictive biomarker to determine a patient's response to anti-EGFR Chemotherapy [18].

KRAS mutations are also frequently linked to a condition known as microsatellite instability (MSI) endometrial cancer. Additionally, KRAS mutations have been found to occur early in the development of endometrial cancer and are also associated with a subtype of cancer known as MSI-positive endometrial cancer. This is thought to be due to the fact that KRAS promoter can be affected by a process known as hypermethylation, which can lead to reduced expression of DNA repair proteins and contribute to the development of cancer [41].

3. Therapies Targeting KRAS Mutations

3.1. Non-Small Cell Lung Cancer

Previous efforts to block KRAS downstream signaling pathways using inhibitors such as selumetinib and trametinib have had limited success due to the presence of alternative feedback mechanisms. However, inhibitors targeting the KRAS G12C mutation, such as adagrasib, have shown promise in early studies. Sotorasib (AMG 510) is a small molecule that specifically targets the KRAS G12C mutation, and preclinical studies have shown it to be effective in causing tumor regression and enhancing the effectiveness of Chemotherapy and other targeted therapies. The results of a phase II trial called CodeBreak 100, which evaluated

the safety and efficacy of sotorasib in advanced non-small cell lung cancer patients with the KRAS G12C mutation, were recently published. The results showed that sotorasib had a notable anti-tumor effect, with a 37% response rate and a median progression-free survival of 6.8 months. The most common side effects were diarrhea, fatigue, nausea, vomiting, and elevations of aminotransferase levels. A phase III trial called CodeBreak 200 is currently recruiting patients to compare sotorasib and docetaxel in advanced NSCLC patients with KRAS G12C mutations who have progressed after prior Chemotherapy and checkpoint inhibitor treatment [42].

3.2. Pancreatic Cancer

Previous attempts to inhibit KRAS in pancreatic ductal adenocarcinoma (PDAC) have had limited success since the majority of KRAS mutations found in PDAC are G12D and G12V, which are difficult to target. Additionally, the molecular structure of mutated KRAS in PDAC has a stronger attraction to GTP and a blocked active site, making it harder to target therapeutically. There have been several attempts to inhibit KRAS in PDAC, including using small interfering RNAs (siRNA) delivered through a vector such as Local Drug Eluter (LODER), inhibitory exosomes (iExosomes), and nanoliposomal delivery platforms. These approaches have shown promising results in preclinical studies and some small clinical trials, but further research is needed to determine their effectiveness in treating PDAC. Other potential strategies for directly inhibiting KRAS, such as targeting the RAS-binding pocket and anti-RAS vaccination, have thus far not yielded positive results in patients with PDAC [8]

Attempts to target KRAS as a therapeutic option in pancreatic ductal adenocarcinoma (PDAC) have had limited success, as G12D and G12V mutations are more common in PDAC than G12C mutations. Indirect targeting of KRAS through downstream signaling pathways, such as MEK, EGFR, PI3K, AKT, and mTOR, has also been ineffective. Previous attempts to inhibit KRAS downstream signaling pathways, such as RAF/MEK/ERK, have had limited success in pancreatic ductal adenocarcinoma (PDAC) due to most KRAS in PDAC being G12D and G12V, which are harder to target than G12C mutations. Additionally, the molecular structure of mutated KRAS has a stronger affinity for GTP and a blocked active site, making it more challenging to target. Studies have also shown that adaptive reactivation of MAPK pathways and multiple parallel signaling redundancy may limit the efficacy of MEK inhibitors. Despite promising results with a combined inhibition of MEK and EGFR or dual inhibition of the EGFR pathway, these have not translated into significant therapeutic changes. Research is currently being conducted on other targets, such as RALA, RALB, JAK1 and 2, NF-Kb, cell cycle regulators, and molecules involved in autophagy mechanisms, hoping to find a more effective treatment for PDAC patients [8].

3.3. Colorectal Cancer

Various therapeutic strategies have been investigated for treating colorectal can-

cer (CRC), such as directly targeting the mutant KRAS protein, parallel inhibiting downstream pathways, and targeting KRAS-membrane association. Sotorasib, a specific and irreversible KRAS G12C inhibitor, has shown promise in a phase I trial among heavily pretreated patients with KRAS-mutated CRC. However, Selumetinib, another KRAS inhibitor, did not show an improvement in disease progression or survival. The combination of downstream MEK and PI3K/mTOR inhibition has shown promising activity in blocking tumor cell proliferation in CRC xenografts. Additionally, targeting KRAS-membrane structure association with EMICORON, a synthetic compound binding to G4 structures, has shown positive preclinical results in downregulating KRAS mRNA and protein expression and decreasing tumor volume in patient-derived xenografts bearing KRAS mutations [43].

Other potential therapies for KRAS-mutated CRC being studied include targeting the association between KRAS and the cell membrane, altering metabolic pathways important for KRAS-mutated cells, utilizing immunotherapy, and utilizing synthetic lethality by combining MEK inhibitors with other targeted therapies such as BCL2, AKT, and SHP2. Inhibition of KRAS-membrane association has shown promise in laboratory studies, but the chemicals used in these studies have been unstable in vivo. Modulating metabolic pathways, such as using glutaminase inhibitors and GAPDH, aims to kill KRAS-mutated cells by blocking the pathways that sustain their metabolic needs. Immunotherapy, such as immune checkpoint inhibitors, has shown promise in treating KRAS-mutated NSCLC. Finally, synthetic lethality approaches are evaluated in clinical trials [44].

3.4. Low-Grade Serous Ovarian Carcinoma

In LGSOC (Low-Grade Serous Ovarian Cancer), studies have been conducted on using inhibitors that target the RAS/RAF/MEK pathway. One trial found that selumetinib provided an 80% response in disease control, with an average time of 11 months before the disease progressed and a 2-year survival rate of 55% in 52 patients with LGSOC, 14 of whom had KRAS mutations [45]. Additionally, there have been instances where patients with KRAS or NRAS mutations in LGSOC responded positively to MEK inhibitors. Another trial that combined a MEK1/2 inhibitor (trametinib) and a PI3K inhibitor (buparlisib) showed a 28.6% response rate and 76.2% disease control rate in a group of LGSOC patients [45].

3.5. Endometrial Cancer

Endometrial cancer (EC) caused by KRAS mutations is known as Type I EC and occurs in 10% - 30% of cases. These mutations are often associated with a specific subtype of EC called microsatellite instability (MSI) EC. Investigations have discovered that epithelial cells (ECs) with KRAS mutations exhibit heightened activation of estrogen signaling. This suggests that anti-estrogen therapy may be

crucial in treating these types of cancer. However, a phase II trial of selumetinib did not show significant results in treating recurrent EC. Other combination therapies, such as combining MEK inhibitors with Akt inhibitors and PARP inhibitors with Akt or PI3K/mTOR inhibitors, have also been investigated but have not produced significant clinical results [46].

4. Methods for Targeting KRAS Mutation

4.1. Indirect KRAS Inhibition

The oncogenic properties of RAS require farnesylation, and by disrupting this process, the membrane localization and overall transformation activity of KRAS can be reduced. Studies have shown that targeting the post-translational modification of KRAS through farnesyltransferase inhibitors (FTIs) can inhibit KRAS farnesylation and potentially be used as a therapeutic strategy. However, these FTIs have not shown clinical activity in NSCLC trials and have been found only effective in HRAS-mutant cancer cells [47]. Although Salirasib and other methods can inhibit all isoforms of Ras, they have also been tested in clinical trials but have not shown significant anti-tumor activity. Recently, vaccination using mRNA-5671 has been explored as a treatment option in clinical trials, both as a standalone therapy and in combination with pembrolizumab [48].

4.2. Direct KRAS Inhibition

Researchers have looked into ways to target KRAS mutations in lung cancer, including indirect methods such as inhibiting the farnesylation of KRAS using farnesyltransferase inhibitors (FTIs) and targeting pathways that are affected by KRAS mutations like PI3K/AKT/mTOR and RAF/MEK/ERK. While FTIs have not yet been proven effective in non-small cell lung cancer clinical trials, other methods, such as inhibiting fatty acid synthase (FASN), have shown promise in early trials. A Phase II trial is underway to evaluate the effectiveness and safety of the FASN inhibitor TVB-2640 in KRAS-mutant non-small cell lung cancer patients [11].

A phase II clinical trial, Selected-1, tested the effectiveness of Selumetinib, a MEK pathway inhibitor, on patients with KRAS-mutated non-small cell lung cancer who had previously undergone treatment [49]. The trial results showed that adding selumetinib to Chemotherapy did not improve progression-free survival compared to treatment with docetaxel alone. Additionally, the overall survival rates between the two treatment groups were not significantly different. A separate preclinical study using animal models discovered that other mutations, such as those in TP53 or Lkb1, can influence the response of KRAS-mutant cancers to treatment with selumetinib and docetaxel [50].

In a phase II trial, another MEK inhibitor, trametinib, was tested on patients with previously treated KRAS-mutant non-small cell lung cancer, usually results showed that the progression-free survival and response rates were similar to those of patients treated with the chemotherapy drug docetaxel [46]. Researchers

have found that targeting the BRAF gene in KRAS-mutated cancers is ineffective and are instead exploring other targets. For example, a phase III trial of the RAF inhibitor sorafenib called MISSION was conducted on patients with relapsed or refractory non-squamous non-small cell lung cancer who had failed previous treatment and results showed a significant improvement in progression-free survival in the KRAS-mutant subpopulation compared to the KRAS wild-type group but no significant improvement in overall survival. Studies on the PI3K/AKT/mTOR pathway have also shown that inhibiting these alterations with monotherapy inhibitors may not be sufficient. A phase II trial of the drug ridaforolimus, an oral inhibitor of mTOR, was given to 79 patients, but it only had a 1% objective response rate, and no significant benefit in overall survival was seen [51]. Post-translational modification of KRAS promotes the membrane localization of this protein and allows KRAS signaling to occur.

5. KRAS Mutations in Animal Models

Animal models serve as a simulation medium to recreate the tumor pathology and test the efficacy of the anti-cancer drugs in the preclinical stages. The major drawback of the animal models is their too-simple nature which does not correlate with the complex human cellular pathways that lead to false conclusions on the drugs of interest [52]. Scientists are exploring new pit stops for fine-tuning the animal models that precisely recreate the human cellular mechanisms and provide a better understanding of the drug actions. One such breakthrough is the usage of genetically engineered mouse models. The mouse models with KRAS allele modification can serve as a true model that experimentally recreates the human tumor cell signals and can greatly help screen anti-cancer drugs [27]. The KRAS LSL-G12D mutants, p53 frt/frt mice models are widely used for modeling Non-small-cell lung cancer, adenocarcinomas, and pancreatic ductal adenocarcinoma [53].

6. Applications

The RAS families of proteins are highly notorious in cancer pathogenesis and are found to be ineffective targets for Chemotherapy due to the lack of proper binding sites [10]. However, KRAS proteins need a special mention in this family as they provide a sufficient niche for drug binding and can function as an effective target molecule for anti-cancer drugs [54]. This property of KRAS proteins and their active role in tumorigenesis provides a new opportunity for developing drug molecules that can actively or passively target KRAS mutations [55]. Recent advances and applications around KRAS mutations are shown in **Table 2**.

7. Challenges and Limitations

The main challenge in using KRAS mutations as clinical markers for early cancer diagnosis is that some of the KRAS mutations are dormant and can be a false alarm [8].

Table 2. Recent Advances and applications around KRAS mutation.

KRAS Mutation/biomarker	Recent advances/diagnostic applications
KRAS G12V [18]	Trametinib + Docetaxel (NSCLC), Cetuximab (CRC), Panitumumab (CRC) approved. Novel MEK, PI3K and AKT inhibitors in trials (e.g. cobimetinib, alpelisib, ipatasertib). KRAS G12V peptide vaccine in Phase 1 for NSCLC **Diagnostic applications:** Liquid biopsies detecting circulating tumor DNA (ctDNA) for monitoring response. ctDNA analysis provides early signal of clinical benefit or resistance. Biomarkers such as ctDNA, tMAF, mutation specific PD-L1 serve as hallmarks for cancer detection
KRAS G13D [31]	Similar targeted drug approvals and trials as KRAS G12V based on MAP kinase pathway. Tumor mutant allele frequency (tMAF) tracks treatment effects.
KRAS Q61H [34]	Rare mutation with MEK and AKT inhibition testing in preclinical models. Liquid biopsies offer insights into selective kinase inhibitor sensitivity. KRAS Q61H vaccine got 14% response rate leading to larger studies.
KRAS G12C [18]	Chemosensitive mutation, standard treatment is chemotherapy. New MEK inhibitors (e.g. refametinib, trametinib) allow dual use with chemotherapy in trials. Co-blocking MEK and PI3K/AKT pathways under investigation. Mutation specific PD-L1 expression determines immunotherapy eligibility
KRAS G12R [56]	Standard treatment based on limited data, newer agents needed. Increased genomic sequencing required to stratify patients for clinical trials. Advances include targeted approvals, molecular markers for selection/monitoring, liquid biopsies and clinical trials integrating genomics.

Limitations in mutation detection lack sensitivity to detect minor mutant clones enabling resistance or recurrence. Newer targeted sequencing misses covert mutations. Liquid biopsies facilitate earlier detection but are limited by tumor heterogeneity and mutation evolution.

Challenges target KRAS mutations [36]:

- KRAS is hard to target directly given dependence on upstream activators and complex signaling. Indirect MEK/PI3K inhibitors have limited efficacy.
- No drugs directly target frequent KRAS cancer drivers though most target other mutants (e.g. EGFR/BRAF inhibitors).
- Mutant KRAS constantly binds GTP causing chronic downstream signaling hard to inhibit selectively. Inhibitors target wild-type KRAS causing toxicity.
- KRAS mutations become quickly resistant through feedback reactivation, bypass activation and clonal evolution. Combination therapies are needed but hard to develop and implement.
- Patients lack effective targeted options due to lack of actionable mutations or limited mutation-specific advances. Immunotherapy shows limited KRAS-driven cancer benefit.

Areas for progress include [37]:

- Improved minor mutant detection through ultra-deep sequencing of liquid/tissue biopsies and single-cell analysis enabling early treatment and resistance/response monitoring.
- Novel tools directly targeting KRAS by inhibiting mutant interactions, pre-

venting membrane localization or disturbing effector binding. Proximal RAS activators also hold promise.

- Synthetic lethality exploits KRAS-mutant vulnerabilities killing them sparing wild-type cells. Polo-like kinase 1 (PLK1) inhibition is an example.
- KRAS-targeted vaccines and immunotherapies harnessing immune system against mutant KRAS expressing tumor cells.
- Genomics integrates into trials defining mutation-specific mechanisms of response/resistance, enabling customized combination therapies.
- Collaborations across academia, pharma, funding agencies and patient groups prioritize and accelerate hard-to-treat KRAS-driven cancer progress.

The number of mutations involved in the RAS family makes screening and selecting specific markers hard. The analytical methods should be rugged enough with ample sensitivity and specificity to detect the various alleles and should be able to differentiate between normal and pathogenic variants [55]. The volatile mutations in the KRAS gene make it a notorious molecule to be chosen as a drug target site [57]. Limited knowledge about the KRAS mutations makes it highly challenging to make clinical decisions for diagnosis. Once the limitations around KRAS mutations are overcome, it can be an excellent area where cancer chemotherapy can be exploited with fruitful results.

8. Future Directions

New methods for detecting KRAS mutations [35] precisely include:

- Applying nanoparticle-based techniques to improve detection of rare mutations. Nanoparticles concentrate DNA for more sensitive analyses. Some nanoparticles enhance fluorescence for accurate detection.
- Conducting single-cell genome analysis to recognize mutations in a subset of cells. This could identify mino or KRAS clones driving resistance or recurrence.
- Using multi-omic approaches combining genetics, epigenetics, transcripts and proteins. This provides comprehensive insights into KRAS mutations and downstream signaling pathways.

Developing therapies targeting KRAS or associated pathways [6] includes:

- Designing small molecules blocking KRAS-GTP binding and downstream activation. However, challenges inhibit this approach.
- Inhibiting pathway effectors like RAF, MEK, PI3K or AKT kinases. Approved MEK and AKT inhibitors show limited effectiveness against KRAS mutations.
- Blocking feedback loops and crosstalk preventing resistance. For example, inhibiting BRAF prevents paradoxical MEK signaling.
- Exploiting synthetic lethality and gene-dosage analysis to pinpoint genes selectively lethal when mutated. This facilitates mutation-specific targets though still explored.
- Applying combination therapies inhibiting multiple pathways or combining

targeted drugs with immunotherapies. This addresses resistance via comprehensive approaches.

- Developing precise medicine by analyzing KRAS mutation subtypes to generate mutation-specific personalized therapies.

Understanding KRAS functions thoroughly, improving detection methods precisely, and innovating therapeutic strategies efficiently targeting oncogenic KRAS signaling challenge studies. Future progress in these areas advances management of cancers with KRAS mutations.

9. Conclusion

Although KRAS mutations are associated with many types of cancers, their use in clinical practice is currently limited due to the lack of rugged and robust methods that can identify this marker accurately. Some of the KRAS mutations are also known to be associated with non-malignant outcomes or dormant conditions which may trigger a false positive reaction. Thus, there is a need to further isolate the KRAS mutation products and clinically correlate the cancer-causing genes in order to make it a promising approach for cancer chemotherapy. However, these mutations can be used to create genetically engineered animal models that give a more clinical picture while testing anti-cancer drugs in the preclinical stages.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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