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KRAS p.G12C mutation in metastatic colorectal cancer

Alfredo Colombo*; Concetta Maria Porretto

***Corresponding Author: Alfredo Colombo**
Oncology Unit C.D.C Macchiarella – Palermo, Italy.
Email: alfredocolombo63@gmail.com

Abstract

Tumors often have KRAS mutations. It occurs in around 30% of all cancer cases and almost 50% of metastatic colorectal cancer cases, a leading cause of cancer-related deaths globally. KRAS mutations plays a significant role in CRC progression, as recent advancements in CRC biology and genetics have revealed. KRAS gene encodes a GTPase that regulates growth factor and receptor signal pathways. KRAS mutations commonly occur in codons 12 and 13, possessing oncogenic characteristics. Abnormal KRAS activation boosts cancer-associated signals in CRC, including cell growth, movement, and new blood vessel formation. We examine the prognostic implications of KRAS mutations in this review. The KRAS p.G12C mutation is linked to a poorer prognosis in metastatic CRC. Mutations in amino acids at the same position in a protein can unexpectedly contribute to cancer due to the interplay of structure and conformation. Additionally, we provide a summary of the clinical evidence for the efficacy of novel inhibitors in targeting KRAS p.G12C as a potential treatment for metastatic CRC.

Keywords: Colon; Cancer; p.G12C; Mutations; KRAS.

Introduction

KRAS gene has frequent mutations in tumors [1]. About 30% of cancer cases and almost 50% of metastatic colorectal cancer (mCRC) cases have this mutation. CRC ranks third in global cancer-related mortality [2]. Around 50% of patients have or acquire metastatic disease, predominantly impacting the liver, lungs, lymph nodes, and peritoneum. Unfortunately, mCRC treatment advancements have not resulted in patient survival greater 30 months [3,4]. Growing knowledge of CRC biology and genetics highlights KRAS' importance. KRAS (Kirsten RAS viral oncogene homolog), encode, and N-RAS (Neuroblastoma RAS viral oncogene homolog), encode genes produce EGFR signaling GTPases [5]. The KRAS gene produces a 21.6 kD protein with 188 amino acids. KRAS recruits and activates crucial proteins for signal transduction of growth factors and receptors. KRAS becomes inactive after hydrolyzing GTP to GDP [6]. The overactive RAS proteins strongly enhance cancer-associated signals in CRC like growth, movement, and formation of

new blood vessels [7]. CRC tumors with KRAS mutations are resistant to anti-EGFR therapies (cetuximab or panitumumab) due to the EGFR pathway being constantly active and unaffected by ligands in these cases [5-7].

Prognostic differences in KRAS mutations

KRAS p.G12C and p.G12S mutations in mCRC have a significant negative prognostic impact [8]. The analysis of the prognosis of different types of mutations (KRAS p.G12D, p.G12V, p.G13D, p.G12A, p.G12C, p.G12S) was evaluated in 188 patients from the STORIA. The patients were divided according to the KRAS variant categories to enhance the focused evaluation of their prognostic capability.

There were no significant associations between KRAS mutational status and clinicopathological variables. In the multivariate analysis, after accounting for age, gender, and metastatic involvement, the prognostic effect was not observed in the p.G12V, p.G13D, p.G12A, and p.G12S variants, but it was still present in the p.G12D and p.G12C variants. The p.G12C variant had a significantly worse prognosis, with an hazard ratio of 13.6 (95% CI: 3.9-17.16) and a median overall survival of 4.3 months, compared to the wt form which had a median overall survival of 23.3 months ($p<0.001$)

This research shows that the KRAS p.G12C variant is clinically important in mCRC.

Multiple approaches for evaluating KRAS mutations have been employed, including PCR-based assays and NGS wide panel assays. PCR kits and pyrosequencing assays accurately detect KRAS mutations. They are relatively inexpensive and easy to perform. PCR kits detect known mutations but cannot identify unknown or uncommon ones beyond the targeted regions. Sanger sequencing is a proven method for detecting mutations, able to identify single-nucleotide variants and small insertions or deletions. However, it is both time-consuming, expensive, and unsuitable for high-throughput analysis. NGS revolutionized genetic research, enabling comprehensive assessment of KRAS mutations and other alterations by sequencing multiple genes simultaneously. NGS detects rare, novel mutations with high precision and sensitivity in the KRAS gene. The challenges of NGS include cost, data analysis complexity, and requirement for bioinformatics tools. The chosen methods and the variation in clinical samples can cause considerable differences in the occurrence of KRAS p.G12C.

Significantly, in the STORIA study they have found a median overall survival of 4.3 months for patients harboring KRAS p.G12C mutations. These findings are worse than the poorest survival outcome reported in a study for KRAS p.G12C mutated patients (15.2 months) [14]. This difference in prognoses may be attributed to clinical factors associated with the inherent heterogeneity of patient populations. In the study (STORIA trial), were excluded oligometastatic patients, who made up 10% of the metastatic colorectal cancer cases. Additionally, the survival (23.3 months) is significantly lower than the higher survival of KRAS wt patients (60.0 months), indicating a potential contributing factor. Therefore, excluding oligometastatic patients and including a higher proportion of poly-metastatic high tumor burden disease might have impacted the survival outcomes for KRAS p.G12C patients in our study. Considering these factors when interpreting and comparing this findings with prior studies is crucial. Nevertheless, studies consistently

show that patients with non-p.G12C mutations have a median survival decrease of 2–7.7 months compared to those with KRAS p.G12C mutations [9,10,11]. Additionally, studies consistently show that the p.G12C mutation in KRAS has a significant detrimental effect, resulting in median survival differences of less than 10 months compared to wild-type KRAS [12,13,14]. The adverse impact of KRAS p.G12C on metastatic CRC prognosis is well-established.

KRAS Mutations and Prognosis

KRAS Mutations: Connections between structure and prognosis

KRAS p.G12C displays intricate interactions with other molecules, resulting in a complex impact beyond its own modification.

Mainly, KRAS hotspot mutations mainly happen in codons 12 and 13. Codons 61 and 146 mutations, despite being oncogenic, make less than 5% of all KRAS mutations [5,6]. Mutations in codons 12 and 13 cause allosteric distortion of the GDP/GTP binding pocket, which decreases GTPase activity in KRAS when bound to guanine nucleotide activating protein (GAP). This change keeps the molecule active, bound to GTP. Codons 12 and 13 in the KRAS wild type encode glycine residues. Bulky side chains of different amino acids hinder steric hindrance in GTP hydrolysis by protruding into the GDP/GTP binding pocket of KRAS when substituted at positions such as codons 12 (aspartate and valine) and 13 (aspartate) [15]. However, the functional implications differ greatly, especially in terms of how they interact with effector proteins. This disparity exists both between wt and mutant variants and among different mutant forms. Surprisingly, the p.G12D mutation in KRAS, which is the most common in cancer, closely resembles the wild type in its dynamics [16]. KRAS dynamics change via an allosteric mechanism due to mutations, leading to various modifications in distal protein regions. Mutated forms of KRAS have structural variations due to allosteric effects, causing spatial distortions in regions responsible for binding effectors and GAPs. These GAPs regulate GTP hydrolysis, aiding in the transition of KRAS into its inactive GDP-bound state [17]. These changes in KRAS three-dimensional structure impact its interactions with effector and regulatory molecules.

This conformational change can disrupt the binding of other molecules, particularly those involved in KRAS deactivation. The clinical and prognostic implications of KRAS mutations, hinge on these essential considerations.

Recent improvement in understanding individual mutations have further complicated the study of KRAS mutations. KRAS p.G12C binds to GTP, which causes constant activation and renders it insensitive to p120RAS and neurofibromin, important GTPase activators. AMG510 (sotorasib) selectively identifies the inactive GDP-bound variant of mutant KRAS p.G12C, contradicting the common assumption that these proteins are unaffected by GAPs. RGS3 was found to be an unexpected KRAS p.G12C GAP, resolving this paradox [18].

GEFs further complicate the activation of inactive KRAS-GDP. GEF proteins induce GTP binding, activating KRAS including the specific variant KRAS p.G12C. RASGEF SOS1 stimulates KRAS p.G12C activation

via receptor tyrosine kinase. Thus, SOS1 inhibitors may have therapeutic potential against continuously active KRAS p.G12C, when combined with direct inhibitors [19].

The functional impact of the KRAS p.G12C mutation differs across various cancer types. The observed variation may be due to tissue-specific expression and the influence of regulatory protein SOS1 on the response to inhibiting KRAS p.G12C. Sotorasib achieves an objective response in around 30% of metastatic non-small cell lung cancer cases, while in colorectal cancer, the objective response rate is approximately 7% [20,21]. Furthermore, lung cancer with the KRAS p.G12C mutation has a strong correlation with high Tumour Mutational Burden (TMB) and high PD-L1 expression, indicating a possible response to immunotherapy [22]. The reasons for the different effects of KRAS mutations are unknown, but it has been shown that having two inactive copies of the MUTYH gene is linked to colorectal cancer with KRAS p.G12C and PIK3CA p.Q546K mutations [23]. MUTYH repairs DNA errors caused by guanine oxidation due to oxidative stress. It acts as an adenine DNA repair enzyme, specifically eliminating misplaced adenine within 7,8-dihydro-8-oxoguanine (8-oxoG) pairs. This process causes G:C to T:A mutations. MUTYH and OGG1 collaborate to remove 8-oxodG. MUTYH gene mutations cause MAP, an autosomal recessive disorder with colorectal adenoma or polyp formation. MAP individuals have a higher lifetime risk of colorectal cancer [24].

Studying KRAS mutations and their impact on different tumors is a fascinating challenge that can improve our approach to cancer therapies, such as inhibiting KRAS p.G12C.

Overcoming resistances

The inhibition of oncogenic KRAS is hard because it lacks druggable pockets. Moreover, the high affinity of GTP and GDP to KRAS poses challenges for the development of direct KRAS inhibitors. There has been a renewed interest in studying KRAS due to the belief that it can be targeted using small organic molecules that have a strong binding to the protein. The idea arose from finding two pockets on KRAS, specifically the SII-pocket on top of the Switch II loop in GDP-KRAS p.G12C, placed between the α3-helix and Switch II loop [25-27]. These findings have transformed KRAS-targeting pharmacology. Subsequently, a development in KRAS inhibition for mCRC was achieved with the identification of covalent inhibitors specifically designed to target the p.G12C mutation in KRAS, namely Sotorasib and Adagrasib.

These compounds enable a covalent bond between their electrophilic acryloyl components and the nucleophilic thiol group of the cysteine (Cys) residue at position 12. The recent clinical trials have shown positive results for KRAS p.G12C inhibitors, but it should be emphasized that their focus is exclusively on the inactive form of KRAS.

Sotorasib and Adagrasib in clinical practice

The clinical trials yielded positive outcomes for both drugs. Sotorasib was given to 42 pretreated mCRC patients in a phase I trial. The escalation cohorts were planned with dose levels of 180,360,720, and 960 mg once daily. The response rate was 7.1% and the disease control rate was 73.8%. No toxic effects

led to dose limitations, and no adverse events caused death. Diarrhea occurred in 38 patients (29.5%), fatigue in 30(23.3%), and nausea in 27(20.9%). The established phase II dosage is 960 mg per day [21]. 62 patients with the same disease were studied in phase II. Results showed a 9.7% response rate and 82.3% disease control rate. Sotorasib was administere at 960 mg daily until disease progression, intolerable side effects, withdrawal of consent, or death. Grade 3 treatment-related adverse events were observed in 10% of patients, mainly manifested as diarrhea. No fatal events were reported [28]. Two heavily pretreated patients with KRAS p.G12C-mutant CRC achieved a partial response in a first-in-human phase I study of adagrasib. Nausea, diarrhea, vomiting, and fatigue were the most frequent treatment-related adverse events. 15.0% of patients experienced fatigue, which was the most common grade 3-4 treatment-related adverse event. The determined recommended phase II dose is 600 mg twice daily, considering safety, tolerability, and observed pharmacokinetics properties [29]. Pretreated individuals with the KRAS p.G12C mutation in mCRC were enrolled in a non-randomized phase II clinical trial. Patients were given either adagrasib alone (600 mg orally twice daily) or a combination of adagrasib (same dose) and intravenous cetuximab once weekly (standard doses). Adagrasib monotherapy resulted in a 19% response rate among 44 patients. The response rate in the combination therapy group (with 32 patients) rose to 46%. Grade 3 or 4 treatment-related adverse events were observed in 34% of the monotherapy group and 16% of the combination therapy group. There were zero grade 5 adverse events reported. Thus, adagrasib and sotorasib demonstrated similar safety and encouraging efficacy in heavily pretreated mCRC patients with the KRAS G12C mutation.

Anti-EGFR therapy in combination may enhance clinical outcomes [30]. The US FDA granted orphan drug status to sotorasib in June 2019 for KRAS p.G12C-positive NSCLC and colorectal cancer. Sotorasib and adagrasib currently only have FDA approval for NSCLC at the time of writing. Sotorasib and adagrasib are being tested in phase I/II clinical trials across various countries for assessing their effectiveness in treating KRAS p.G12C-mutated mCRC. The trials include combinations using immunotherapy, SHP-2 inhibitors, ULK 1/2 kinase inhibitors, anti-EGFR therapies, and SOS-2 inhibitors.

Conclusion

Oncologists get a molecular report on a particular KRAS mutation in clinical practice. Guidelines widely recommend excluding anti-EGFR-based treatments when patients are RAS mutated [31]. Some KRAS mutations have different prognostic implications. The p.G12C mutation suggests a worse prognosis, while the p.G12D mutation implies intermediate outcomes between the wt form and the p.G12C mutation. However, the p.G12V variant may have a similar prognosis as wild-type KRAS in patients. In addition, limited knowledge exists regarding the predictive and prognostic consequences of other rare mutations (p.A146T, 2 p.A146V, 2 p.G13R, 2 p.K117N, 2 p.G13C, 1 p.G12_G13insG, 1 p.G12F). We must adopt caution and promote participation in clinical studies or multicenter research to collect sufficient data. Prognostic evaluations in mCRC are complex due to multiple clinical factors such as age, tumor burden, chemotherapy response, CEA levels, tumor site, lymph node involvement, grading, histology, and more. More research is needed to better comprehend the predicted outcomes and treatment implications related to various KRAS mutations in mCRC patients. These investigations will enhance our understanding and improve treatment

approaches for this intricate disease. The KRAS p.G12C mutation is clinically significant in metastatic colorectal cancer patients. Exploring and fully utilizing it as a treatment focus is a key priority in oncology soon.

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Authors Information: Alfredo Colombo*; Concetta Maria Porretto
Oncology Unit C.D.C Macchiarella – Palermo, Italy.

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