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CLINICAL RELEVANCE OF KRAS MUTATION TESTING IN METASTATIC COLORECTAL USING DIFFERENT SEQUENCING PLATFORMS: A CASE REPORT

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Abstract

Introduction. Colorectal cancer (CRC) ranks as the second leading cause of cancer mortality and the third most common cancer worldwide. Recent statistics reveal a troubling rise in CRC incidence among adults under 50 in high-income countries, with rates increasing 1-4% annually. While screening advancements, including colonoscopy, have improved early detection and survival rates, metastatic cases with *KRAS* mutations remain challenging due to limited treatment options and mutation-driven resistance mechanisms.

Aim. The present study aims to analyze the clinical significance of multi-platform verification of *KRAS* gene mutation in a particular clinical case in order to optimize personalized treatment strategies for patients with *KRAS*-positive metastatic colorectal cancer.

Materials and methods. Our study reports the case of a 58-year-old female diagnosed with advanced-stage rectal adenocarcinoma with *KRAS* mutation and multi-organ metastasis. Following partial intestinal obstruction, the patient underwent resection surgery with cholecystectomy and metastasectomy, followed by FOLFOX chemotherapy and targeted therapy (TT). Despite initial stabilization, metastasis progressed to the lungs and liver. Multiplatform genetic analysis, including Next generation sequencing (NovaSeq 6000), Sanger sequencing, and droplet digital PCR (ddPCR), was employed to confirm *KRAS* variant status, highlighting minor platform-based discrepancies with clinical relevance.

Discussion. *KRAS* mutations profoundly affect CRC treatment strategies, particularly in relation to EGFR inhibitor eligibility. Multi-platform validation ensures accurate variant detection, critical for clinical decision-making. This case emphasizes the necessity of complementary assays, given the observed *KRAS* mutation's influence on therapeutic outcomes. Cross-platform diagnostic guidelines are advocated to standardize detection thresholds and improve precision in treatment planning.

Conclusion. This case underscores the clinical significance of multiplatform *KRAS* mutation validation in metastatic CRC, highlighting the potential for improved diagnostic protocols to optimize personalized treatment strategies for *KRAS*-mutant CRC patients.

Keywords: *colorectal cancer, metastasis, NGS, KRAS gene, mutations.*

Абстракт

КЛИНИЧЕСКАЯ ЗНАЧИМОСТЬ ТЕСТИРОВАНИЯ МУТАЦИЙ KRAS ПРИ МЕТАСТАТИЧЕСКОМ КОЛОРЕКТАЛЬНОМ РАКЕ С ИСПОЛЬЗОВАНИЕМ РАЗЛИЧНЫХ ПЛАТФОРМ СЕКВЕНИРОВАНИЯ: КЛИНИЧЕСКИЙ СЛУЧАЙ

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Актуальность. Колоректальный рак (КРР) является второй по значимости причиной смертности от рака и третьей по распространенности в мире. Последние статистические данные свидетельствуют о тревожном росте заболеваемости КРР среди взрослых в возрасте до 50 лет в странах с высоким уровнем дохода, причем показатели увеличиваются на 1-4% в год. В то время как достижения в области скрининга, включая колоноскопию, позволили улучшить раннее выявление и выживаемость, случаи метастазирования с мутациями *KRAS* остаются сложными из-за ограниченных возможностей лечения и механизмов резистентности, обусловленных мутациями.

Цель. Настоящее исследование направлено на анализ клинической значимости много платформенной проверки мутации гена *KRAS* при частном клиническом случае с целью оптимизации персонализированных стратегий лечения пациентов с *KRAS* -позитивным метастатическим колоректальным раком.

Материалы и методы. В данном исследовании сообщается о случае 58-летней женщины с диагнозом аденокарцинома прямой кишки на поздней стадии с мутацией *KRAS* и полиорганными метастазами. После частичной кишечной непроходимости пациентке была проведена резекция с холецистэктомией и удалением метастазов, а затем химиотерапия по методу FOLFOX и таргетная терапия (ТТ). Несмотря на первоначальную стабилизацию, метастазы прогрессировали в легких и печени. Для подтверждения статуса варианта *KRAS* был использован мультиплатформенный генетический анализ, включающий секвенирование следующего поколения (NovaSeq 6000), секвенирование по Сэнгеру и капельную цифровую ПЦР (ddPCR), что выявило незначительные несоответствия между платформами и клинической значимостью.

Обсуждение. Мутации *KRAS* существенно влияют на стратегии лечения КРР, особенно в отношении приемлемости ингибиторов EGFR. Мультиплатформенная валидация обеспечивает точное выявление вариантов, что имеет решающее значение для принятия клинических решений. Данный случай подчеркивает необходимость дополнительных анализов, учитывая влияние мутации *KRAS* на результаты лечения. Рекомендуется использовать разные платформенные диагностические методы для стандартизации пороговых значений выявления и повышения точности планирования лечения.

Вывод. Этот случай подчеркивает клиническую значимость мультиплатформенной валидации мутации *KRAS* при метастатическом КРР, подчеркивая потенциал усовершенствованных диагностических протоколов для оптимизации персонализированных стратегий лечения пациентов с КРР с мутацией гена *KRAS*.

Ключевые слова: *колоректальный рак, метастазы, NGS, ген KRAS, мутации.*

Түйіндеме

ТҮРЛІ СИКВИНЕРЛЕУ ПЛАТФОРМАЛАРЫН ҚОЛДАНУ МЕН МЕТАСТАТАЛЫҚ КОЛОРЕКТАЛЬДЫ ҚАТЕРЛІ ІСІКТЕГІ KRAS МУТАЦИЯСЫН ТЕКСЕРУДІҢ КЛИНИКАЛЫҚ МӘНІ: КЛИНИКАЛЫҚ ЖАҒДАЙ

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Өзектілігі. Колоректальды қатерлі ісік (КҚІ) қатерлі ісіктен болатын өлім-жітімнің екінші себебі және әлемде үшінші орын алады. Соңғы статистика табысы жоғары елдерде 50 жасқа дейінгі ересектер арасында CRP ауруының алаңдатарлық өсуін көрсетеді, бұл көрсеткіштер жылына 1-4% - ға артады. Скринингтік жетістіктер, соның ішінде колоноскопия ерте анықтау мен өмір сүруді жақсартуға мүмкіндік бергенімен, kras мутациялары бар метастаз жағдайлары шектеулі емдеу мүмкіндіктері мен мутацияға байланысты төзімділік механизмдеріне байланысты күрделі болып қала береді.

Мақсаты. Бұл зерттеу *KRAS*-тың метастаздық колоректальды қатерлі ісігі бар науқастарды емдеудің жекелендірілген стратегияларын оңтайландыру үшін арнайы клиникалық жағдайда көп платформалы *KRAS* мутациясына тестілеудің клиникалық маңыздылығын талдауға бағытталған.

Материалдар мен әдістер. Бұл зерттеуде *KRAS* мутациясы және көп мүшелі метастаздары бар кеш сатыдағы тік ішектің аденокарциномасы диагнозы қойылған 58 жастағы әйелдің жағдайы туралы хабарланды. Ішінара ішек өтімсіздігінен кейін пациентке холецистэктомиямен және метастаздарды жоюмен резекция жасалды, содан кейін FOLFOX әдісімен химиотерапия және мақсатты терапия (ТТ) жүргізілді. Бастапқы тұрақтануға қарамастан, метастаздар өкпе мен бауырда дамыды. *KRAS* нұсқасының мәртебесін растау үшін келесі ұрпақ секвенциясы (NovaSeq 6000), Сангер сиквинерлеу және тамшылатып цифрлық ПТР (ddPCR) кіретін көп платформалы генетикалық талдау қолданылды, бұл платформалар мен клиникалық маңыздылық арасындағы шамалы сәйкессіздіктерді анықтады.

Талқылау. *KRAS* мутациялары КҚІ емдеу стратегияларына, әсіресе EGFR ингибиторларының қолайлылығына айтарлықтай әсер етеді. Көп платформалы валидация клиникалық шешімдер қабылдау үшін өте маңызды нұсқаларды дәл анықтауға мүмкіндік береді. Бұл жағдай байқалған kras мутациясының емдеу нәтижелеріне әсерін ескере отырып, қосымша сынақтардың қажеттілігін көрсетеді. Емдеуді жоспарлаудың дәлдігін анықтау және жақсарту шектерін стандарттау үшін әртүрлі платформалық диагностикалық әдістерді қолдану ұсынылады.

Қорытынды. Бұл жағдай метастатикалық КҚІ-де *KRAS* мутациясының мультиплатформалық валидациясының клиникалық маңыздылығын көрсетеді, *KRAS* мутациясы бар КҚІ науқастарын емдеудің жекелендірілген стратегияларын оңтайландыру үшін жетілдірілген диагностикалық хаттамалардың әлеуетін көрсетеді.

Түйінді сөздер: *колоректальды қатерлі ісік, метастаздар, NGS, KRAS гені, мутациялар.*

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Introduction

Colorectal cancer (CRC) is second in terms of mortality and third in the number of cases worldwide in the last decade. In recent years, the number of cases among young adults (younger than 50) has risen by 1%-4% in high-income countries, and with the given results, there is a prediction of an increase in cancer cases by 77% [1]. The reason for the rise among the younger generation is unknown, but there is a significant influence on the beginning of adulthood. The survival rate equals 65%, where the main survival predictor is stage diagnosis [2]. However, a healthy diet, physical activities, and uptake of screenings are considered protective. Colonoscopy screenings play a massive role in detecting early stages, increasing the survival rate [3]. However, selecting treatment regimens based on the tumor's genetic characteristics is essential. Single gene testing, such as KRAS, BRAF, and EGFR mutations, is commonly used in almost all cancer centres, primarily because of the National Comprehensive Cancer Network (NCCN) guidelines [4]. However, this approach only provides complete coverage of some relevant therapeutic targets since tumors' high biological heterogeneity likely affects therapeutic response. An example is the widespread KRAS mutation in human cancers, which, despite decades of research, remains a problematic therapeutic target; therapeutics directly targeting mutant KRAS still need to be developed. With the development of next-generation sequencing (NGS) technologies, assays have been expanded to panels of significant genes to identify tumor-specific driver mutations or those that can be targeted by FDA-approved or investigational new drugs [5]. However, the NGS method requires additional validation of the obtained results using various sequencing platforms.

Here, we present a case of multi-platform verification of *KRAS*gene mutation in multiorgan metastasis refractory to standard therapy.

Materials and methods

Archived Formalin-fixed, Paraffin-embedded (FFPE) tissue samples were obtained from a patient with multi-organ metastatic colorectal cancer for genomic sequencing. Representative FFPE blocks containing more than 30-70% tumor cell was identified with pathological examination. A series of histological tissue sections 5-8μm thick from the selected samples were prepared on a microtome for each glass. Glass slides were subsequently stained with hematoxylin and eosin solutions according to the standard protocol. Morphological examination of glass slides stained with hematoxylin and eosin was performed on an Olympus BX53 microscope (Japan) at X100 magnification. DNA from glass slides was isolated using the ReliaPrep™ FFPE gDNA Miniprep Kit (Promega, USA). Some DNA samples were pre-treated with the enzyme uracil DNA glycosylase (UDG), which reduces artefacts and sequencing errors for comparison. Next-generation sequencing was performed using the TruSight Oncology 500 HT 523-gene targeted oncopanel according to the standard manufacturer's protocol (Illumina, USA) on the NovaSeq 6000 platform. Droplet digital PCR (ddPCR) will be performed using the ddPCR *KRAS* Screening Multiplex Kit (Bio-Rad Laboratories, USA) on the QX200 Droplet Digital PCR platform (Bio-Rad Laboratories, USA) according to the manufacturer's protocol. Sanger sequencing was performed with a set of primers for mutations in exon 2 of the *KRAS* gene: forward pair 5'TATTTGATAGTGTATTAACCTTATGTGTG3' and the reverse pair - 5' GAAACCTTTATCTGTATCAAAGAATG 3' on the capillary electrophoresis platform 3730XL Genetic Analyzer according to the manufacturer's instructions (Applied Biosystems).

Case presentation

A 58-year-old female patient visited her local clinic in 2018 due to the presence of blood in her stool and a worsening of her symptoms. Following a series of examinations, she was referred to the Kazakh Institute of Oncology and Radiology (KazIOR) for further evaluation and treatment leading to hospitalization for specialized treatment. A colonoscopy revealed a narrowed and obstructed lumen 15 cm from the anus, with infiltrated, irregular, and ulcerated mucosa that fragmented easily during biopsy. The diagnosis was rectal cancer. Immunohistochemical analysis confirmed the presence of grade 2 adenocarcinoma of the rectum, St IV (T4N1M1). A pelvic MRI showed uneven thickening of

the walls and narrowing of the lumen in the rectosigmoid section of the colon, extending up to 4.5 cm and involving all layers of the bowel. There were isolated lymph nodes measuring up to 5 mm in the periphery. The MRI findings indicated a neoplasm in the rectosigmoid region of the colon, along with uterine fibroids. An abdominal ultrasound revealed diffuse changes in the liver consistent with hepatosis, metastatic lesions in both liver lobes, chronic calculous cholecystitis, and signs of chronic bilateral pyelonephritis. No focal changes were detected in the retroperitoneal space. A cardiology consultation diagnosed the patient with stage 1 arterial hypertension and a risk level of 2, with no contraindications for specialized treatment.

The patient was discussed in a multidisciplinary team meeting on November 2018. Given the partial intestinal obstruction, the first step recommended was elective surgical intervention. In November 2018, the patient underwent laparotomy, abdominal cavity exploration, combined obstructive resection of the rectum with cholecystectomy, and removal of liver metastases, along with abdominal drainage.

The postoperative histological report indicated moderately differentiated adenocarcinoma of the rectum (GII) with ulceration, comedo necrosis, and inflammatory infiltration of the stroma. The tumor invaded all layers of the bowel wall and infiltrated the perirectal adipose tissue. No tumor cells were found at the resection margins. Metastases were identified in one of the two examined lymph nodes in the perirectal adipose tissue and in liver tissue. Chronic cholecystitis with xanthomatosis was also noted.

Molecular genetic testing for *KRAS* and *NRAS* mutations revealed the presence of a *KRAS* gene mutation in the sample analyzed.

DNA was isolated from the patient's FFPE tissue sample using the Wisard® Genomic DNA Purification Kit (Promega, USA). Quantitative evaluation of the isolated DNA performed by spectrophotometric method on the Nanodrop 2000 device, and by fluorometric method using the Qubit™ dsDNA HS kit (Invitrogen) on the Qubit 2.0 device. Genomic sequencing performed on the NovaSeq 6000 platform using the TruSight Oncology 500 High Throughput kit according to the manufacturer's standard protocol (Illumina, USA). Sanger sequencing performed using two pairs of commercial PCR primers for *KRAS* gene mutations in exon 2 and exon 3 (Sigma Aldrich, USA) according to standard protocol with or without UDG enzyme pretreatment. ddPCR performed on the QX200 (Bio-Rad, USA) device using cobas® *KRAS* Mutation Test (Roche, USA).

Sample materials conducted from FFPE blocks and were analyzed by pathomorphologist (Figure 1).

Patient had chemotherapy according to the scheme FOLFOX (oxaliplatin 130 mg, fluorouracil 600 m, fluorouracil 900 mg 22-hour infusion through a pump of disodium folinate 400 mg infusion through a pump of nafone premedication with sturgeon, dexamed). After a while target therapy (bevacizumab 400 mg) was recommended.

Patient received CT and TT for five years and were asked to participate in clinical trial for the Oxidative Drug Combination. And with the agreement of patient participated in 2 phases from 3,5g by increasing it by adding 3,5 g each time [6, 7].

The conclusion from the chest computed tomography (CT) scan, when compared to previous results, indicated metastatic lesions in both lungs, with a noted decrease in the size and number of these formations over time. Additionally, areas of pleuropulmonary fibrosis were observed in the lower and middle lobes of the right lung, suggesting stabilization of the condition with positive dynamics. Abdominal CT scans revealed metastatic lesions in the liver, with a formation in the right lobe that involved the right kidney, also showing a reduction in size over time. Furthermore, metastatic formations were found in the adipose tissue of the abdominal cavity, situated beneath the anterior abdominal wall and adjacent to loops of the colon, indicative of carcinomatosis, with a decrease in the size of these formations as well. When compared to the CT data from June 2023, the findings suggest stabilization of the process with positive dynamics. The pelvic MRI indicated that the rectal stump was thickened and in close proximity to the cervix, with signs of uterine fibroids present. The multidisciplinary team at the Oncology Center recommended adding two additional cycles of palliative chemotherapy (PCHT) and targeted therapy (TT) to the treatment plan, followed by maintenance therapy with TT.

Additionally, we utilized several sequencing platforms, including NovaSeq 6000, Sanger sequencing (both with and without Uracil DNA Glycosylase treatment), and droplet digital PCR (ddPCR), to ensure robust verification of *KRAS* mutations. Notably, while Sanger and ddPCR assays consistently identified the *KRAS* c.35G>A (p. Gly12Asp, G12D) mutation (Table 1), NovaSeq 6000 platform results unambiguously identified the *KRAS* c.35G>T (p.Gly12Val) variant (Table 2).

Table 1.

Summary of *KRAS* Mutation results by Sanger sequencings' and ddPCR.

Sanger sequencing without UDG Treatment	Sanger sequencing with UDG Treatment	ddPCR_*KRAS*_c.12,c13 (G12A, G12C, G12D, G12S, G12V,G13D)_57bp	Clinical *KRAS* status
c.35G>A p.G12D	c.35G>A p.G12D	Mutated 14.6% MAF	*KRAS* mutant

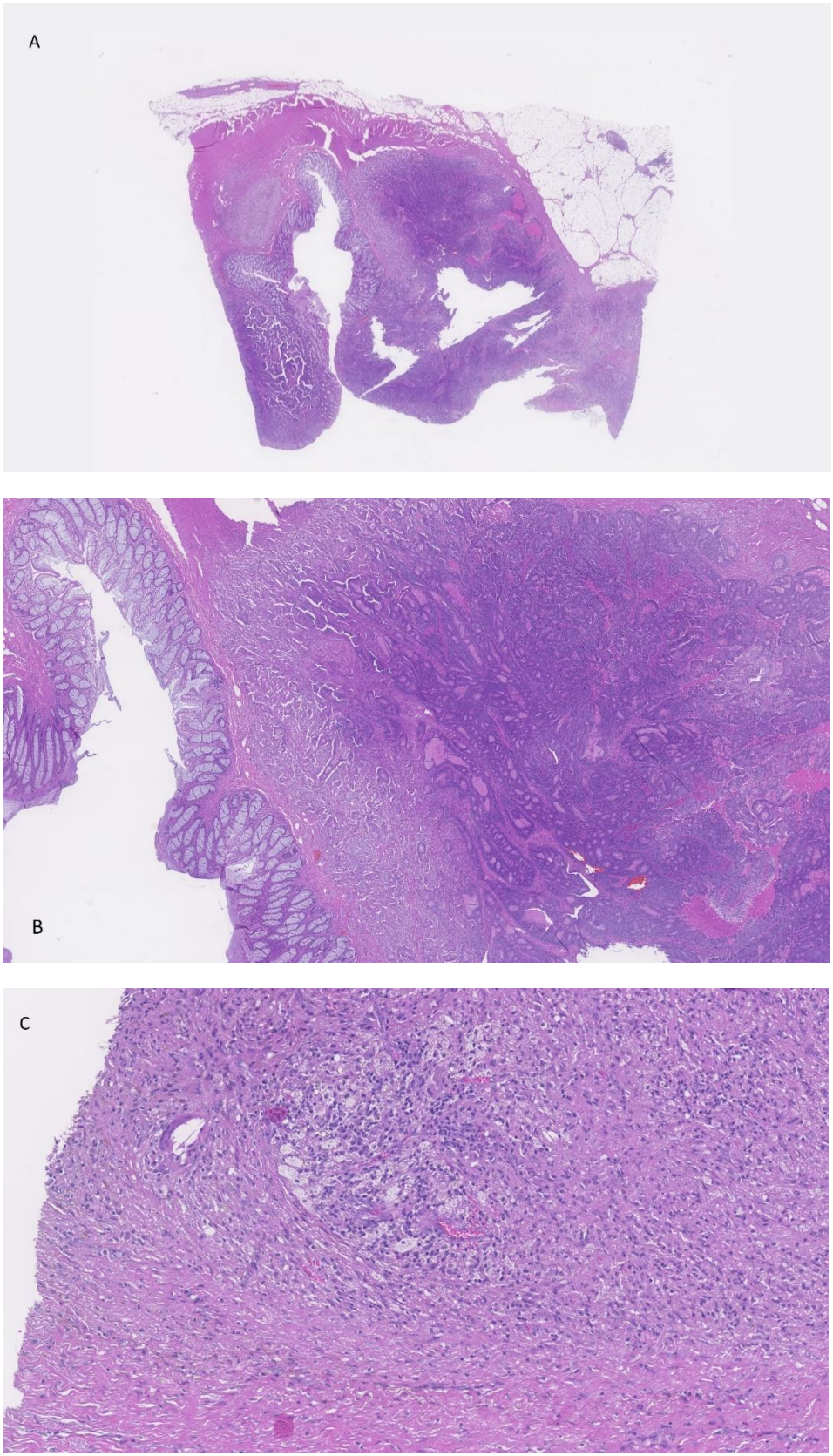

Figure 1. Histopathological and imrnunohistochemical images of a core biopsy of colon.

(A) Overview (hematoxylin and eosin (H&E) stain, 5x magnification).
(B) Adenocarcinoma (H&E stain, 10x magnification).
(C) Desmoplastic reaction in tumors with single scyrrhous sites. (H&E stain, 30x magnification).

Table 2.

Genetic mutations detected on NovaSeq 6000.

Gene Name	Chr	Position	ClinVar Significance	Variant	Protein Change	CDSChange	Consequence
PIK3CA	chr3	1,79E+08	likely pathogenic	Somatic	NP_006209.2: p.(Lys111Glu)	NM_006218.3: c.331A>G	missense variant
APC	chr5	1,12E+08	likely pathogenic; pathogenic	Somatic	NP_000029.2: p.(Arg1450Ter)	NM_000038.5: c.4348C>T	stop gained
BMPR1A	chr10	88683229	NA	Somatic	NP_004320.2: p.(Arg480Gln)	NM_004329.2: c.1439G>A	missense variant
KRAS	**chr12**	**25398284**	**likely pathogenic; pathogenic**	**Somatic**	**NP_203524.1: p.(Gly12Val)**	**NM_033360.3: c.35G>T**	**missense variant**
AMER1	chrX	63411291	NA	Somatic	NP_689637.3: p.(Arg626Ter)	NM_152424.3: c.1876C>T	stop gained

Discussion

This case highlights the complexities and importance of multi-platform validation in detecting clinically actionable mutations, specifically *KRAS* variants, in metastatic colorectal cancer (CRC). The clinical and therapeutic implications of *KRAS* mutations in CRC are profound, given their influence on treatment decisions, particularly in determining eligibility for targeted therapies like EGFR inhibitors [8-10]. Accurate mutation detection is therefore critical, as inconsistencies could directly affect patient outcomes. Our results showed different *KRAS* mutations when using different sequencing and driver mutation detection approaches in the same patient. Such discrepancies have been reported in previous studies, with platform-specific biases attributed to differences in sequencing chemistry, coverage depth, and bioinformatics pipelines. For instance, a study by Zehir et al. observed that distinct sequencing platforms could yield variability in detecting low-frequency mutations, potentially leading to conflicting clinical interpretations [11]. These discrepancies underscore the importance of complementary assays in clinical diagnostics, especially when single-platform findings diverge, as observed here with TruSight Oncology 500 panel on the NovaSeq 6000. Validation through multi-platform approaches aligns with best practices in precision oncology, as it can mitigate risks associated with platform-specific limitations and improve the accuracy of mutation reporting [12-16].

This case also illustrates the potential *KRAS* variants, like G12V, to uniquely influence therapeutic outcomes compared to more frequently observed variants such as G12D. Research has shown that *KRAS* mutation subtypes may differentially affect tumor response to specific therapies, including targeted treatments and immunotherapy [17]. For example, patients harboring *KRAS* G12C or G12V mutations have demonstrated varied responses to emerging targeted inhibitors, suggesting that accurate characterization of these mutations is vital for personalized treatment planning [18].

The implications of these findings suggest that future clinical protocols should advocate for standardized cross-platform guidelines, particularly in cases where actionable mutations directly affect treatment selection. Standardizing thresholds for variant calling and sequencing depth across platforms, as advocated by Frampton et al., could further enhance diagnostic reliability, ensuring that discrepancies are minimized and that all clinically relevant variants are consistently detected [19]. These practices are increasingly important as clinical genomics integrates diverse NGS technologies, each with unique operational and interpretative characteristics [20]. Future studies are encouraged to explore these discrepancies further and develop optimized protocols that could be universally applied in clinical laboratories, ultimately improving the reliability of molecular diagnostics in oncology.

Conclusion

Sequencing of the cancer genome has fundamentally advanced our understanding of the biology of the disease and, more recently, has expanded approaches for monitoring tumors in the clinic and guiding treatment. Although cancer research increasingly relies on whole genome characterization, the clinical application of genomics has primarily been limited to targeted sequencing approaches designed to identify specific clinically relevant biomarkers. In some tumor types, such as colon cancer, lung cancer, and melanoma, it has become standard practice to profile tumors for recurrent targetable mutations. The results of this study highlight the clinical relevance of multi-platform validation of driver tumor mutations. Given the diversity of tumor types that may harbor potentially actionable mutations, genetic testing is needed to identify novel biomarkers that predict resistance response to therapy.

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