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Therapeutic Impact of RAS Testing on Survival Outcomes in Moroccan Metastatic Colorectal Cancer Patients

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Abbreviations:
5-FU: 5-fluorouracil;
CRC: Colorectal cancer;
ECOG PS: Eastern Cooperative Oncology Group Performance Status;
EGFR: Epidermal growth factor receptor;
FFPE: Formalin-Fixed Paraffin-Embedded Tissue;
GLOBOCAN: Global Cancer Observatory;
mAbs: monoclonal antibodies;
mCRC: metastatic colorectal cancer;
mOS: median overall survival;
OS: Overall Survival;
VEGF: Vascular Endothelial Growth Factor;
WT: wild-type.

ABSTRACT

Introduction: RAS mutations, particularly KRAS and NRAS mutations, are frequent drivers of metastatic colorectal cancer (mCRC) and confer resistance to anti-EGFR targeted therapy. This study investigated the impact of RAS molecular testing on treatment patterns and survival outcomes in Moroccan mCRC patients.
Material and Methods: A retrospective study was conducted among patients diagnosed mCRC and treated at Ibn Rochd University Hospital, Casablanca, Morocco, between 2019 and 2023. All patients received first-line chemotherapy followed by KRAS and NRAS molecular testing to guide potential modifications to their second-line treatment regimens. Subsequently, we compared overall survival between patients whose second-line treatment regimens were adjusted based on their RAS mutation profile and patients who continued with their initial first-line treatment.
Results: A total of 55 patients with mCRC were included in this study. RAS testing revealed KRAS mutations as the most prevalent (56%), followed by NRAS (5%). Out of the total, 34 patients had their treatment regimens modified based on their RAS molecular profile, with 65% receiving anti-VEGF therapy and 35% receiving anti-EGFR therapy. The Kaplan-Meier analysis revealed that the RAS-guided treatment approach significantly improved overall survival compared to standard treatment, with a mOS of 24 months versus 18 months ($p=0.019$).
Conclusion: Our study confirms that systematic RAS testing enables the most personalized and effective treatments to be offered to mCRC patients, resulting in a remarkable improvement in mOS compared to traditional approaches.
Key words: mCRC, personalized medicine, KRAS, molecular testing, targeted therapy, chemotherapy

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INTRODUCTION

Despite significant efforts that have been made in Colorectal cancer (CRC) screening, diagnosis, and treatment, this cancer remains a significant global health challenge due to its high incidence, frequency and associated mortality. Based on the Global Cancer Observatory (GLOBOCAN) data, CRC ranks as the third most common cancer in Morocco, with more than 4558 new cases diagnosed and nearly 2374 deaths reported in 2020 (1). Upon diagnosis, approximately 25% of patients with CRC are found to have metastatic disease and up to 40% can develop metastases during the course of their illness (2). Those with localized metastases may undergo surgical resection. However, for most patients, the tumor is unresectable and chemotherapy remains the mainstay of treatment (3). Over the past decade, the treatment of metastatic colorectal cancer (mCRC) has undergone some improvements which have significantly enhanced the quality of life and survival rates of mCRC patients (4). These advancements included the use of different chemotherapy lines through the combination of cytotoxic agents such as irinotecan and oxaliplatin with 5-fluorouracil (5-FU) and folinic acid, which are referred to as FOLFIRI and FOLFOX, respectively (5). Recent advancements in molecular and cellular biology, along pharmacogenomics, have led to the emergence of new therapies known as "Targeted therapies". These drugs target specific molecules involved in the tumorigenesis of cancer cells in order to limit cytotoxic effects and improve the patient's quality of life. Various types of molecular Targeted therapies have been identified for cancer therapy; such as monoclonal antibodies (mAbs) or small-molecule tyrosine kinase inhibitors (6). Based on their origin and mechanism of action, we distinguish between anti-angiogenic agents, such as Bevacizumab (Avastin), a monoclonal antibody directed against Vascular Endothelial Growth Factor (VEGF), and epidermal growth factor receptor (EGFR) inhibitors, such as Cetuximab (Erbitux) or Panitumumab (Vectibix) (7,8). These molecules are most frequently used in combination with other conventional cancer treatments, such as chemotherapy, offering a more targeted and effective approach to mCRC (9). However, choosing the appropriate approach requires precision. In the case of mCRC, the decision to target VEGFR or EGFR often depends on the results of the RAS molecular testing (10). RAS protein plays a crucial role in signal transduction downstream of the EGFR and its activation by this receptor contributes to increased proliferation, survival, and production of angiogenic factors (11). Unfortunately, about 50% of patients with mCRC exhibit RAS mutations which result in the constitutive activation of RAS proteins independently of EGFR signals (12). Therefore, the indication for panitumumab and cetuximab is now limited to patients with RAS-WT (wild-type) mCRC, unlike bevacizumab, which can be used regardless of RAS status (10).

To emphasize the significance of RAS molecular profile and its therapeutic implications, we conducted this study on Moroccan patients with mCRC who initially received standard chemotherapy as their first-line treatment and later underwent RAS molecular testing to guide subsequent treatment decisions. To our knowledge, this is the first study of its kind conducted among Moroccan patients with mCRC.

MATERIALS AND METHODS

Study Design

This was a retrospective cohort study that included 55 patients diagnosed with mCRC between 2019 and 2023 at University Hospital Center Ibn Rochd, Casablanca, Morocco. Histopathological and molecular data were obtained from the Anatomopathological laboratory and the clinical data were retrospectively collected from the Mohammed VI Cancer Treatment Center data base of the same hospital.

Inclusion criteria

Patients were eligible for this study if they met the following criteria: 18 years or older, histologically confirmed adenocarcinoma of the colon or rectum and had received first-line chemotherapy treatment with FOLFOX, XELOX, or FOLFORINOX.

Exclusion criteria

Were excluded from this study patients who had undergone fewer than two cycles of chemotherapy as first line treatment or had a previous history of any other malignancy within the past 5 years.

RAS Molecular Testing

DNA extraction

Genomic DNA was extracted from 5-6 mm sections Formalin-Fixed Paraffin-Embedded Tissue (FFPE) using the cobas® DNA sample preparation kit (Roche Molecular Systems, Inc) according to the manufacturer's instructions. DNA was then purified and quantified using NanoDrop 2000c Spectrophotometer (ThermoFisher Scientific, USA).

PCR amplification

Molecular analysis of the RAS genes (KRAS, NRAS) was conducted using the cobas® KRAS Mutation Test and the cobas® BRAF/NRAS Mutation Test (Roche Molecular Systems, Inc, Pleasanton, CA, USA) which are a real-time PCR assays designed to detect the presence of mutations involving codons 12, 13 and 61 of the KRAS gene and exons 2 and 3 of the NRAS gene. Mutations were then achieved by melting curve analysis using the cobas z 480 analyzer.

Statistical analysis

The main evaluation criterion was the Overall Survival (OS), defined as the time from initiation of first line treatment to death or last follow-up. Survival was estimated using the Kaplan-Meier method and compared using the log-rank test. All statistical analyses were performed using SPSS version 20.0 software (IBM Corporation, Armonk, NY, United States). p-values < 0.05 were considered to be statistically significant.

RESULTS

Patient Characteristics

Among the 55 participants, 56% were male and 44% were female, resulting in a sex ratio of 1.3. The median age of our patients was 60 years, ranging from 34 to 81 years. The Eastern Cooperative Oncology Group Performance Status (ECOG PS) was 1 or higher in 44% of cases at the time of diagnosis. CRC tumors were primarily located in the colon in 69% of cases while 31% were identified in the rectum. Further analysis of the tumor sidedness revealed that the majority of cases, accounting for 78%, were situated on the left side of the colon. In contrast, the right side of the colon exhibited a lower incidence, with only 21% of cases. Regarding metastatic spread, liver metastases were the most common among the included cases accounting for 84%, followed by pulmonary metastases in 36% of cases. Histologically, the most common histological type in our study was the Lieberkühnien adenocarcinoma, which was observed in 94% of cases followed by Mucinous carcinoma with a proportion of 5.45%.

All 55 patients included in this study received first-line chemotherapy, and the chemotherapeutic regimens administered are detailed in *table 1*. Specifically, 42% of patients received a monotherapy regimen as their first-line treatment and 58% were treated with first-line doublet chemotherapy (FOLFOX or FOLFIRI). Following an average of 4 chemotherapy cycles, patients underwent RAS molecular testing to guide their second-line therapy based on their mutation status. Among those tested, 62% (34/55) had their treatment regimens adjusted according to their molecular profile. The remaining 38% (21/55) patients continued on their initial regimen. Subsequently, all patients were closely monitored for a period ranging from 6 to 37 months, during which 17 patients passed away.

Table 1 - Clinicopathological features of the 55 colorectal patients included in this study

Characteristics	Total (N=55)	%
Age		
Median (range)	60 (34-81)	
< 60	25	45.4%
≥ 60	30	54.5%
Gender		
Male	31	56.4%
Female	24	43.6%
ECOG PS		
0	31	56.3%
≥ 1	24	43.6%
Primary tumor site		
Colon	38	69%
Rectum	17	30.9%
Sideness		
Left	43	78.1%
Right	12	21.8%
Metastatic sites		
Liver		
Yes	46	83.6%
No	9	16.36%
Lung		
Yes	16	29%
No	39	70.9%
Peritoneal		
Yes	6	10.9%
No	49	89%
Bone		
Yes	4	7.2%
No	51	92.7%
Histological type		
Lieberkühnien adenocarcinoma	52	94.5%
Mucinous adenocarcinoma	3	5.4%
First-line regimen		
Mono*	23	41.8%
Doublet**	32	58.2%

* Mono (single-agent therapy): involve using only one chemotherapy drug such as capecitabine

** Doublet (two-drug combination): combine two chemotherapy drugs which can be oxaliplatin-based (FOLFOX, XELOX) or irinotecan-based (FOLFIRI, FOLFORINOX)

Abbreviations: ECOG PS: Eastern Cooperative Oncology Group performance status

Molecular Profile

Molecular testing revealed that 62% (34/55) of patients exhibited RAS mutations, with the KRAS

mutation being the most prevalent (56%, 31/55). More precisely, 49% of these KRAS mutations were detected at codons 12 and 13 of the KRAS gene. As for the NRAS mutation, it was detected in 5% of cases (3/55). Additionally, 4 (7%) patients had invalid results, mainly due to insufficient DNA (*fig. 1*).

Treatment patterns

After conducting RAS molecular testing, 62% (34/55) of patients had their treatment regimens modified to align with their molecular findings. Specifically, 65% (22/34) received anti-VEGF therapy, while the remaining 35% (12/34) received anti-EGFR treatment. These targeted therapies were frequently combined with chemotherapy regimens. The most common combination for anti-VEGF therapy was with XELOX (capecitabine + oxaliplatin) in 29% of cases. Similarly, anti-EGFR therapy was frequently paired with XELOX, in 18% of cases (*fig. 2*).

Conversely, 38% (21/55) remained on the same regimen either due to their general health not being suitable for targeted therapies or because their molecular testing results were invalid. The treatment regimen of these patients primarily consisted of doublet and triplet chemotherapy combinations. Specifically, 17 patients (81%) received doublet chemotherapy, which typically included a combination of two chemotherapeutic agents such as XELOX (capecitabine + oxaliplatin) or FOLFIRI (5-Fluorouracil + Leucovorin + Irinotecan), or XELIRI (capecitabine + irinotecan). The remaining 4 patients (19%) were administered triplet chemotherapy, which consisted of

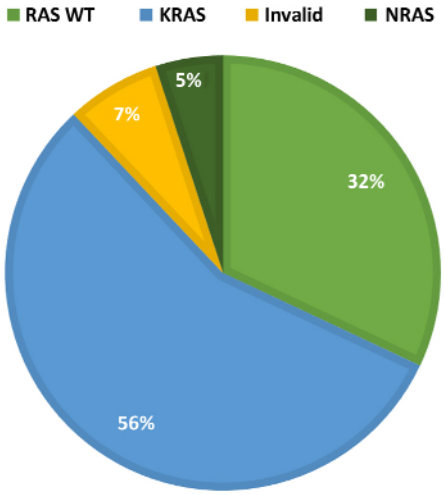


Figure 1 - Distribution of RAS molecular testing

FOLFIRINOX (5-Fluorouracil + Leucovorin + Irinotecan + Oxaliplatin). The choice between doublet or triplet chemotherapy was influenced by several factors, including the patient's performance status, comorbidities, and prior treatment response.

Treatment outcomes

To further explore the impact of RAS testing on treatment outcomes, we compared the overall survival rates of patients whose regimens were adjusted based on the RAS molecular results to those who maintained their initial treatment (*fig. 3*). Our analysis revealed a median overall survival (mOS) of 18 months for patients who maintained their initial treatment regimen (blue

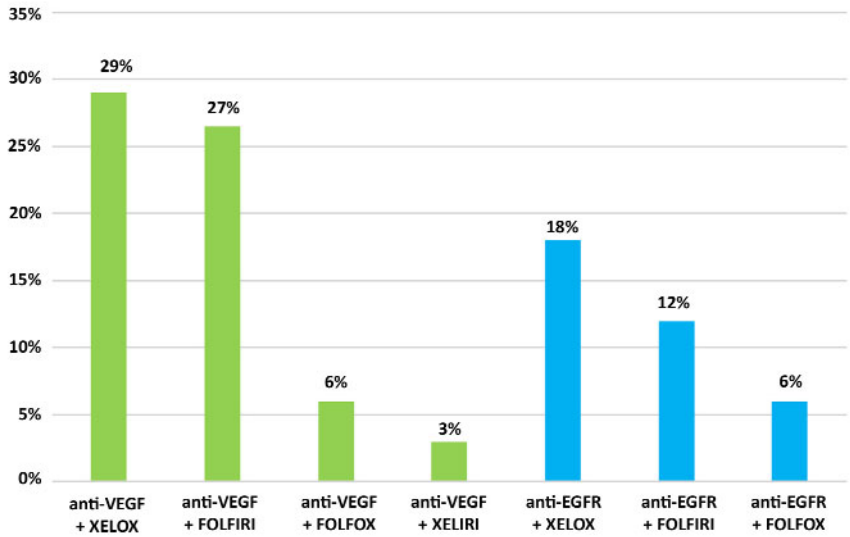
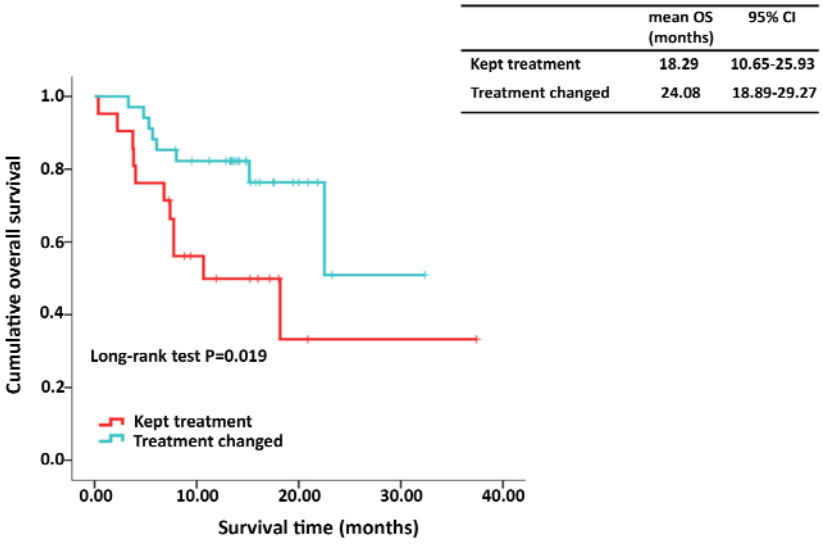


Figure 2 - Targeted therapy combinations with chemotherapy in second-line treatment

Figure 3 - Kaplan–Meier survival curve for OS according to the treatment regimen used in second-line treatment



line), whereas those who underwent treatment modifications guided by RAS testing (green line) achieved a significantly improved mOS of 24 months (p=0.019). Hence, this suggest that RAS testing may play a crucial role in optimizing treatment strategies for mCRC patients in Morocco.

DISCUSSION

Recent advancements have significantly enhanced our understanding of the molecular mechanisms involved in CRC carcinogenesis, leading to the development of a personalized and effective therapeutic strategy. Notably, RAS mutational analysis has become a crucial step in the management of patients with mCRC, through the identification of specific subpopulations of patients who may substantially benefit from targeted therapies such as anti-EGFR mABs. As demonstrated by the CRYSTAL study, RAS status has enabled the identification of patients likely to benefit from cetuximab (anti-EGFR mAB) combined with chemotherapy, which significantly improved the survival of these patients compared to those receiving chemotherapy alone (mOS = 23,5 vs. 20 months; p=0.0093) (13). While anti-EGFR targeted therapies may not be effective for mCRC patients with RAS mutations, anti-VEGF therapies such as bevacizumab, have emerged as promising alternatives and showed positive outcomes regardless of RAS status. However, despite the established benefits of RAS testing, its use in Morocco faces challenges and lacks systematic integration into clinical practice. This constraint underscores a significant barrier to providing optimal and personalized care for individuals with mCRC in developing countries. To address this gap and potentially pave the way for wider adoption, we conducted this study in order to investigate the impact of RAS molecular testing on treatment outcomes and survival rates in Moroccan mCRC patients.

Initially, we collected data from patients whose cancer had been histopathologically confirmed at the Anatomical Pathology Laboratory of CHU Ibn Rochd, Casablanca and who subsequently received treatment at the Mohammed VI Center for Cancer Treatment. After screening, we included 55 patients diagnosed with mCRC who had received first-line chemotherapy. These patients received a range of initial treatment regimens, highlighting the importance of personalized approaches in mCRC management. Specifically, 58% received first-line doublet chemotherapy, while the remaining 42% underwent monotherapy. Following an average of 4 cycles of first-line chemotherapy, all patients underwent RAS molecular testing targeting codons 12, 13, and 61 of the KRAS gene, as well as exons 2 and 3 of the NRAS gene, to guide potential modifications to their second-line treatment regimens. RAS analysis identified mutations in 62% (34/55) of patients. Remarkably, 56% of these patients exhibited KRAS mutations (31/55). These results align with those of a previous study by Musselwhite et al., which also reported that 56% of mCRC patients had KRAS mutations, with the G12D variant being the most common (47%) (14). NRAS mutations were less common, detected in only 5% (3/55) of cases. Additionally, 7% (4/55) of tests yielded invalid results due to

insufficient DNA. Finally, the remaining 38% (21/55) of patients tested negative for RAS mutations.

To highlight the impact of RAS testing on mCRC treatment outcomes, we compared OS rates between two groups: patients whose second-line regimens were modified based on their KRAS/NRAS mutation profile and patients who continued with their initial first-line treatment. The Kaplan-Meier analysis revealed that changing treatment based on RAS testing offered a significant survival advantage compared to keeping the same treatment (OS = 24 months vs. 18 months; p=0.019). This finding underscores the potential of personalized therapeutic approaches driven by RAS status results in the management of mCRC.

One limitation of our study was the relatively small sample size analyzed. Inclusion challenges were due to difficulties in tracking patients who adhered to their treatment schedules and attended follow-up appointments. This is partly attributable to the fact that a significant proportion of patients treated at CHU Ibn Rochd, Casablanca reside in neighboring towns. Another limitation relates to the timing of molecular testing. Due to delays in requesting these tests, all patients received systemic therapy before the results were available. This approach could have potentially delayed the initiation of targeted therapy for some patients who might have benefited from it sooner.

Although our study sheds light on the molecular testing influencing the treatment management of advanced CRC, it is essential to better understand the patients' personal experiences. Future research could investigate the factors influencing patients' decisions regarding targeted therapies, especially among those eligible based on molecular testing but who did not undergo these treatments. Additionally, exploring new strategies could help establish more equitable and patient-centered approaches in low- and middle-income countries.

CONCLUSION

The positive impact of RAS testing on treatment outcomes in patients with mCRC, in this first-of-its-kind study conducted in Morocco, highlights the potential of personalized medicine in their care. By tailoring treatment decisions to the RAS molecular status, we can significantly improve clinical outcomes and quality of life for these patients. Consequently, the incorporation of RAS testing into routine clinical practice in Morocco proves to be crucial. We urge healthcare providers to prioritize this critical step through collaborative efforts aimed at increasing awareness and improving access to such promising therapeutic strategy.

Author's Contributions

SF developed the study design, and took the lead in writing the manuscript. OA, SG, NT, and SS contributed to revising the manuscript. FG provided critical comments that shaped the analysis and manuscript. MK directed the research, analysis and successive drafts. All authors carefully read and approved the final manuscript.

Conflict of Interest

The authors declare that the work described in this document has not been influenced by any known competing financial interests or personal relationships.

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Ethics Committee Approval

All participants provided written consent in accordance with the principles of the Declaration of Helsinki. Ethical clearance was granted by the Ethics Committee of the University Hospital Center Ibn Rochd, Casablanca (13/09/2022, No. 2/2022).

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