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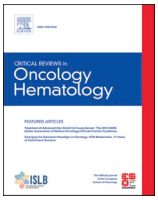

Detecting *BRAF* mutations in colorectal cancer in clinical practice: An Italian experts' position paper

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A R T I C L E I N F O

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A B S T R A C T

BRAF p.V600E exon 15 hotspot mutation can identify a molecular subgroup of metastatic colorectal cancer (mCRC) patients exhibiting poor prognosis under the conventional chemotherapy regimen. Recently, the chemotherapy-free combination of encorafenib and cetuximab has been approved as the standard of care for previously treated *BRAF* p.V600E mCRC patients, and genomic testing for *BRAF* mutations at the time of mCRC diagnosis is currently recommended. In clinical practice, *BRAF* mutation testing strategies are dramatically impacted by a lack of harmonization and standardization, both in the pre-analytical and analytical phases, which can result in *BRAF*-mutated patients not receiving the most appropriate therapy at recurrence. This paper proposes nine statements providing practical and concise advice on *BRAF* mutation testing in CRC, derived from collegial discussion and analysis of a multidisciplinary team of experts, including referral Italian oncologists and pathologists. The statements overview pivotal aspects implied in the detection, treatment and management of *BRAF*-mutated patients and have been drafted to represent a valuable tool for healthcare professionals committed to mCRC patient management. In addition, they represent a platform for implementing diagnostic-therapeutic workflows that can adapt to the variability of local resources while respecting the high-quality standards required by modern precision oncology.

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1. Introduction

Colorectal cancer (CRC) ranks as the third most prevalent cancer globally, with 1.1 million new cases reported annually, and stands as the second leading cause of cancer-related mortality (Cervantes et al., 2023). With over 50.000 new diagnoses and more than 24.000 deaths every year, CRC is the second most commonly diagnosed and deadliest cancer in Italy (I numeri del cancro, 2023).

Approximately 20% of patients with CRC have advanced disease at diagnosis, while approximately 35 % of those with resectable disease at diagnosis will develop advanced disease, mostly within 3 years from surgery (Associazione Italiana Oncologia Medica 2021). The estimated 5-year survival rate in CRC patients with metastatic disease is about 14% (Shin et al., 2023). The molecular profiling of metastatic CRC (mCRC) is fundamental for the use of treatments directly targeting the biological features of the tumour for specific patient subsets, significantly improving survival outcomes (Leowattana et al., 2023). In the past decade, the advent of precision medicine has revolutionized the clinical management of CRC patients. Particularly, *K/N RAS* exon 2–3–4 hotspot mutations clinically stratified CRC patients, electing wild type cases to target therapy based on the use of monoclonal antibodies against the *EGFR* extracellular domain (Giusti et al., 2007; Cohen et al. 2013).

Emerging evidence suggests that v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations, particularly *BRAF* p.V600E exon 15 hotspot mutation, can identify a novel molecular mCRC subgroup exhibiting poor prognosis under the conventional chemotherapy regimen (Shin et al., 2023).

BRAF encodes a protein that plays a pivotal role in the regulation of the Mitogen-Activated Protein (MAP) kinase/Extracellular signal-Regulated Kinases (ERK) signalling pathway, which affects cell growth, differentiation, and proliferation (Shin et al., 2023). Previous studies demonstrated that *BRAF* p.V600E mutations are some of the most common cancer-causing mutations in melanoma and other cancer types, including CRC (Shimada et al., 2018), and indeed, *BRAF* missense mutations may occur in up to 10% of CRC patients (Associazione Italiana Oncologia Medica, 2021; Clarke and Kopetz, 2015; Morkel et al., 2015). The most frequent *BRAF* mutations in CRC are detectable in codon 600, showing the highest prevalence rate for p.V600E hotspot mutation (Angerilli et al. 2022; Fanelli et al. 2020). Interestingly, *BRAF* clinically impacting mutations are mutually exclusive with *RAS* mutations (Morkel et al., 2015).

In 2020 the chemotherapy-free combination of the BRAF inhibitor encorafenib and the Epidermal Growth Factor Receptor (EGFR)-inhibitor cetuximab has been approved by European Medicines Agency for previously treated *BRAF* V600E mutated mCRC patients and then recognized as standard of care in this setting (Trullas et al., 2021; Tabernero et al., 2021; Cervantes et al., 2023; Morris et al., 2023; Associazione Italiana di Oncologia Medica, 2021). The key role of genomic testing for *BRAF* mutations at the time of mCRC diagnosis has been underlined in both national and international guidelines (Cervantes et al., 2023; Morris et al., 2023; Associazione Italiana di Oncologia Medica, 2021).

In this scenario, the lack of harmonized and standardized pre-analytical and analytical procedures when assessing *BRAF* mutation status in mCRC patients dramatically impacts molecular testing strategies in clinical practice. Consequently, BRAF p.V600E-mutated mCRC patients may not benefit from the most appropriate therapy as their molecular hallmark is not identified adequately or in a timely fashion.

To fill this gap, an Italian multidisciplinary expert panel on molecular testing and clinical management of CRC patients developed a set of expert recommendations. These guidelines aim at optimizing decision-making strategies for *BRAF* p.V600E mutated mCRC patients with the goal of improving the clinical outcome thorugh appropriate treatment selection. Upon reviewing the literature, the panel used a modified mini-Delphi algorithm (mm-Delphi) to score key points that arose from their collective discussions. The goal was to define statements that garnered the highest level of agreement among the experts.

This initiative aimed to reach an evidence- and experience-based consensus on the clinical and technical pitfalls for the clinical identification of *BRAF* mutated mCRC patients in diagnostic routine practice, The objectives were to:

- Raise awareness of the crucial role of *BRAF molecular* testing;
- Implement early testing strategies in the diagnostic series of CRC patients;
- Optimize molecular testing procedures (from the pre-analytical phase to the molecular reporting of clinically impacting variants);
- Sustain the integration of expertise supporting the identification of a multidisciplinary team for the clinical management of CRC patients.

2. Methods

A multidisciplinary panel of experts demonstrating proven expertise in clinical management and molecular profiling of CRC patients was grouped (Supplementary Figure 1).

During a first meeting (November 2023), an initial panel composed of 3 oncologists and 2 pathologists discussing clinical and molecular pitfalls related to *BRAF* testing in Italy, established 9 major discussion topics.

Subsequently, 3 of the experts reviewed all the available literature regarding each discussion topic and proposed a draft of 9 preliminary statements.

Each statement was then reviewed by the complete panel to reach an agreement using the mm-Delphi approach. This technique has been previously described (Gustafson et al., 1973; Gallego and Bueno, 2014); notably, in this project, the mm-Delphi method has been modified comparing the experimental model with the traditional method, allowing the initial collective drafting of the statements. Agreement on each statement was reached when $\geq$80 % of participants did not require further amendments.

Then, the panel selected 12 more experts (6 oncologists and 6 pathologists) and enrolled them in the project. Between December 2023 and January 2024, in the second round of the mm-Delphi process, the 9 previously selected experts' opinion statements circulated among the members of this extended panel. They independently reviewed all the statements and made further amendments, and rephrasing where needed (notably, only statements 4 and 5 required to be amended and revoted). Finally, all the statements reached an agreement. Details of this process are reported in Appendix 1. All the experts involved in the project contributed to the manuscript and are listed as authors.

3. Results

Table 1 shows the nine topics selected during the first mm-Delphi round and the final experts' opinion statements resulting from their discussion in the agreement process.

We present hereafter a summary of the evidence and of the experts' opinions that led to statements drafting and refinement in this project.

3.1. Clinico-pathological characteristics and molecular alterations

Overall, *BRAF* p.V600E mutations account for about 90 % of all *BRAF* mutations (Angerilli et al., 2022). Interestingly, a meta-analysis of 25 studies with a total of 11,955 CRC patients showed that these mutations were detected at diagnosis in 8.0 % and 11.6 % of stage I/II cases and stage III/IV, respectively, supporting a statistically significant association between BRAF p.V600E hotspot mutation and advanced TNM stage at diagnosis (Odd Ratio, OR, 1.59; 95 % IC 1.16–2.17) (Chen et al., 2014).

Moreover, the clinical impact of detecting *BRAF* p.V600E mutation was also demonstrated. Several studies highlighted a statistically significant association between *BRAF* p.V600E mutation and CRC patient/

Table 1
Discussion topics and expert opinion statements.

Discussion topic	Expert opinion statement
1. Clinico-pathological characteristics and molecular alterations.	*BRAF*-mutated CRCs are characterized by distinct clinico-pathological characteristics and specific molecular alterations defining distinct subgroups.
2. Prognostic impact.	The *BRAF* p.V600E mutation is associated with poor prognosis, impacting not only recurrence rates but also the timing of recurrence.
3. Test availability in clinical practice.	The availability of molecular testing for *BRAF* p.V600E mutation is mandatory for the clinical management of patients with metastatic CRC at diagnosis.
4. Eligible patients.	Immediate testing for *BRAF* mutational status is mandatory for all patients with early CRC with loss of MLH1 as part of the Lynch syndrome diagnostic algorithm and should be considered in routine practice for patients with high-risk resected stage III CRC to add prognostic information and quickly inform for an appropriate treatment in case of disease recurrence.
5. Clinical evidence on targeted therapy.	In MSS *BRAF* p.V600E-mutated patients progressing during adjuvant or first-line chemotherapy-based regimens and MSI-H mCRC patients with *BRAF* p.V600E mutation progressing on immunotherapy, the combination of encorafenib-cetuximab should be considered as the first and preferred option.
6. Pre-analytical phase.	The performance of molecular testing relies not only on the quality of the method itself, but also on the quality of the analysed biospecimen.
7. Analytical phase.	Testing for the *BRAF* mutation should be carried out on FFPE tissues with quantitative real-time PCR or next-generation sequencing. Liquid biopsy could represent an alternative if no tissue is available.
8. Reporting of results.	Molecular pathology reports should present results in a clear and concise manner to guide clinicians in the selection of the best treatment options.
9. Multidisciplinary team.	A standardized, site-level specific, multidisciplinary clinical pathway and process is needed to allow for a timely and accurate diagnosis and adequate treatment.

BRAF: v-Raf murine sarcoma viral oncogene homolog B. CRC: colorectal cancer. mCRC: metastatic colorectal cancer. FFPE: formalin-fixed, paraffin-embedded. MSS: microsatellite stability. MSI-H: microsatellite instability-high. PCR: polymerase chain reaction.

disease characteristics, namely older age (≥60 years), female gender, proximal tumour location, peritoneal seeding, higher rates of peritoneal/lymph node metastases, and lower rates of lung metastases (Chen et al., 2014; Jang et al., 2017; Tran et al., 2011).

Particularly, *BRAF* V600E mutations demonstrated a robust association with specific CRC histopathological parameters, such as mucinous histology, poor differentiation, signet ring cells, serrated morphology, lymphovascular invasion, tumour budding, infiltrative tumour border and marked peritumoral lymphoid reaction (Chen et al., 2014; Jang et al., 2017).

The panel, however, underlined that the above-listed characteristics should not be considered selection factors for identifying *BRAF* mutations; they should not, therefore, be included among the criteria for selecting patients to be tested. As clearly stated by major clinical guidelines, all patients with mCRC should be tested for *BRAF*-activating mutations, regardless of their demographic, histological and clinical features (Cervantes et al., 2023; Morris et al., 2023; Associazione Italiana di Oncologia Medica, 2021).

Notably, a meta-analysis of 78 studies, including results from 7,000 CRCs, showed that *BRAF* p.V600E mutations are strongly associated with the "sporadic" somatic inactivation of the DNA Mismatch Repair (MMR) system. Remarkably, somatic *BRAF* mutation testing also plays a relevant role in Lynch Syndrome (LS) screening (Parsons et al., 2012; Fassan et al., 2020). *BRAF* p.V600E mutations are detected in both microsatellite stable (MSS) and CRCs patients with microsatellite instability (MSI-H) demonstrating a clinically relevant prognostic implication in this setting (Samowitz et al., 2005; Roth et al., 2010; Lochhead et al., 2013; Barras et al., 2017).

Further demonstrating a high heterogeneity among *BRAF* p.V600E mutated CRCs, a retrospective trial on *BRAF* p.V600E mutated CRC patients (n=218) revealed two distinct molecular subtypes distinguishing between BM1 and BM2. Particularly, the BM1 group included CRC patients with high KRAS/mTOR/AKT/4EBP1, EMT activation, and immune infiltration, whereas BM2 grouped patients are affected by cell-cycle checkpoint dysregulation. Taking into account clinical parameters, BM1 and BM2 subtypes showed a different clinical benefit from BRAF and MEK inhibitors (Barras et al., 2017).

A retrospective series of 155 *BRAF* p.V600E mCRC patients from eight Italian Oncology Units collected between January 2005 and December 2016 demonstrated how the identification of different promising biomarkers may optimize clinical management of real-world CRC patients. Among them, CRC patients with low CDX2 expression rate, high cytokeratin 7 (CK7) expression rate and low number of tumour-infiltrating lymphocytes (TILs) performed significantly worse under standard treatment regimen (Loupakis et al., 2019).

BRAF p.V600E mutations are heterogeneously distributed in the Consensus Molecular Subtypes (CMS) of CRC (70 %, 7 %, and 17 % found in CMS1, CMS2–3 and CMS4 subgroups respectively). For this reason, the prognostic role of CMS in *BRAF* V600E p.mCRC patients has been evaluated, showing that CMS1 cases had better clinical outcomes in terms of OS compared with CMS2–3/CMS4 groups (Loupakis et al., 2019).

Recent emerging data confirmed the role of synaptophysin expression in *BRAF*-mutated CRCs, as identified by immunohistochemistry. A relatively recent study showed that out of 159 mCRC *BRAF* p.V600E mutated patients (Fassan et al., 2021), synaptophysin expression identified 18 patients (11.3%) with drastically reduced PFS and OS.

Although *BRAF* p.V600E hotspot mutation represents the most common *BRAF* clinically relevant alteration in mCRC patients, a not negligible percentage of mutations are non-V600. Indeed, non-V600 *BRAF* mutations account for up to 22 % of all *BRAF* mutations tested by NGS and globally occur in about 2 % of mCRC patients (Jones et al., 2017). These mutations have been associated with a better prognosis: the median OS of *BRAF* non-V600E mCRC patients resulted in a significantly longer OS than those harboring both *BRAF* V600E mutations and wild-type *BRAF* (Jones et al., 2017).

However, since *BRAF* non-V600E mutations span over 19 different codons, different biological effects may depend on specific *BRAF* molecular alterations (Van Cutsem and Dekervel, 2017). *BRAF* mutations are grouped in three classes based on alteration type: Class 1, *BRAF* p.V600E mutations; Class 2, *BRAF* non-V600E mutations, harbouring codons 601 or 597 alterations; and Class 3, *BRAF* non-V600E mutations, harbouring codons 594 or 596 alterations. In a recent Italian trial that included 117 *BRAF*-mutated CRC patients, Class 2 patients presented lower median OS and PFS, similar to those associated with *BRAF* p.V600E mutations. Conversely, Class 3 patients showed median OS and PFS longer than wild-type *BRAF*. Moreover, Class 2 patients appeared to be not fully responsive to anti-EGRF therapies (Schirripa et al., 2019).

In conclusion, based on the evidence included in this section and on the panel members' opinion, the following statement reached the total agreement in the mm-Delphi process.

3.2. Prognostic impact

BRAF acts as a key actor in the MAPK/ERK signalling pathway

BRAF mutated CRCs are characterized by distinct clinico-pathological characteristics and specific molecular alterations defining distinct subgroups.

because activating mutations in the kinase domain promote uncontrolled cell cycle proliferation, driving CRC pathogenesis. Hence, *BRAF* p.V600E mutations have significant prognostic implications, as shown by several studies and meta-analyses.

Overall, *BRAF* p.V600E CRCs often show a worse clinical outcome in comparison with *BRAF* wild-type CRC patients (Angerilli et al., 2022).

Moreover, *BRAF* p.*V600E* CRCs tend to have a lower response rate (RR) and OS rate compared with standard therapeutic approaches, regardless of stage at diagnosis (Fanelli et al., 2020). In addition, due to their aggressiveness, only 50 % of patients could receive second-line therapy in real-world clinical practice (Martinelli et al., 2022).

In a retrospective observational analysis of advanced CRC patients collected at Cork University Hospital histopathology database, median OS resulted significantly lower in the *BRAF* p.V600E group compared with the *BRAF* wild-type patients (17.3 vs. not reached; p=0.001). In the same study, the authors also demonstrated that *BRAF* p.V600E mutation was an independent marker increasing mortality risk in CRC patients (HR 12.76 (95 % CI 3.15–51.7; p<0.001)) (O'Riordan et al., 2022).

Remarkably, Passiglia et al., looking at a case series of 1,400 CRCs, observed a worse clinical outcome in mCRC patients eligible for surgical treatment for liver metastases when *BRAF* activating mutations are present. Indeed, the OS was significantly reduced in the *BRAF*-mutated group (HR 3.07 (95 % CI: 1.67–5.66)) compared with the wild-type group (Passiglia et al., 2016).

In a meta-analysis of seven randomized trials including more than 1,000 stage II/III CRC patients treated with curative surgical resection followed by adjuvant chemotherapy, *BRAF* activating mutations were significantly associated with shorter OS and disease-free survival (DFS) compared with *BRAF* wild-type CRC patients (HR 1.42 (95 % CI: 1.25–1.60, P <0.00001) and HR 1.26 (95 % CI: 1.07–1.48, P = 0.006), respectively) (Zhu et al., 2016).

Recently, a pooled analysis of two phase III studies enrolling more than 4,500 stage III CRC patients treated with standard oxaliplatin-based adjuvant chemotherapy showed shorter median time to retreatment (mTTR) in *BRAF* p.V600E CRC patients compared with *BRAF* wild-type CRC series (0.99 vs 1.54 years respectively; p < 0.0001). A low survival rate was also observed due to the early recurrence disease in the *BRAF* p.V600E CRC group (Rasola et al., 2023).

Several studies focused on the prognostic role of *BRAF* mutational status in guiding clinical management of MSI-H CRC patients.

CRCs with microsatellite instability (10–15 % of all CRCs) harbour defects in the highly preserved DNA mismatch repair (MMR) complex (Chang et al., 2020). *BRAF* pathogenetic mutations are often concomitant with MSI-H status as they derive from the high-level CpG island methylator phenotype (CIMP) which also leads to *MLH1* gene promoter methylation and MMR deficiency (Chen et al., 2014; Lochhead et al., 2013). A recent pooled analysis from the ACCENT/IDEA databases covering seven clinical trials and more than 8,400 stage III CRC patients after surgical resection showed that clinical parameters (older age, female sex, performance status > 0, N2 stage, poor differentiation, and proximal location) were more common in MSS *BRAF* p.V600E-patients compared with *BRAF/KRAS* exon 2 wild-type CRC patients. In this study, *BRAF* p.V600E mutations were significantly associated with shorter OS and survival after recurrence (SAR) compared with *BRAF/KRAS* exon 2 wild-type CRC patients both in MSS and MSI-H groups. However, *BRAF* p.V600E mutations highlighted a statistically significant lower time to recurrence only in the MSS group (HR=1.58, P<0.0001) and not in the MSI-H group (HR=0.98, P=0.91) (Taieb et al., 2023).

In conclusion, considering the evidence of this section, the expert panel reached a full agreement on the following statement adopting the mm-Delphi process.

3.3. Test availability in clinical practice

Given the clinical significance of *BRAF* activating mutations, international societies have established a set of decision-making guidelines that address the challenges and pitfalls of *BRAF* molecular testing in clinical practice.

According to the European Society of Medical Oncology (ESMO) clinical practice guidelines for mCRC patients, the National Comprehensive Cancer Network (NCCN) guidelines, and the guidelines of the Italian Association of Medical Oncology (AIOM), *BRAF* mutation testing is recommended in all mCRC patients at the time of diagnosis. Moreover, international societies consensually recommend testing *BRAF* p.V600E hotspot mutation as part of the LS diagnostic algorithm when IHC-based MMR protein analysis reveals absence of nuclear expression of MLH1 (Cervantes et al., 2023; Associazione Italiana di Oncologia Medica, 2021; National Comprehensive Cancer Network, 2024).

According to the panel of experts, it would be desirable for the oncologist to have information on the mutational status of *BRAF* routinely, without needing to request it, at the time of diagnosis of mCRC (reflex testing). Such information is crucial in directing first-line and subsequent-line therapeutic strategies and in the surgical evaluation of the patient.

On the other hand, the panel considered the role of reflex testing for *BRAF* mutations to be a controversial topic. Although *BRAF* molecular data are considered a clinically relevant tool for the management of CRC patients, supporting reflex *BRAF* testing at diagnosis, the economic and clinical benefits of this strategy still need to be thoroughly evaluated. The only published experience on this topic was presented during the 2023 ASCO Meeting. Here, reflex testing for *RAS/BRAF* clinically relevant mutations for mCRC patients was implemented starting from October 2019 (Cvetkovic et al., 2023). Results confirmed how this diagnostic testing algorithm drastically reduced turnaround time (TAT) of molecular profiling (Cvetkovic et al., 2023).

However, the panel of experts also discussed the low saving costs and logistically unfavorable nature of reflex testing for clinical management in routine practice. Considering these limitations, the reflex testing procedure cannot be adopted by our national institutions at this time. Moreover, if implemented, it could potentially create disparities in access to care across the country.

Overall, the panel confirmed the importance of *BRAF* p.V600E testing as key information in clinical management of mCRC patients, reaching the total agreement on the statement below.

3.4. Eligible patients

As stated in the previous section, the panel suggested investigating *BRAF* p.V600E molecular mutational status in all mCRC patients, regardless of their characteristics, because molecular analysis plays a crucial point in guiding treatment choice (Tabernero et al., 2021).

However, the eligibility of non-metastatic patients to *BRAF* p.V600E molecular testing is a debated topic.

The panel recommended assessing *BRAF* status in patients whose CRCs show loss of MLH1 expression by immunohistochemistry. As already mentioned, the presence of *BRAF* mutation implies that MLH1 expression is down-regulated by somatic methylation of the gene's

The *BRAF* p.V600E mutation is associated with poor prognosis, impacting not only the recurrence rate but also the time of recurrence.

The availability of molecular testing for *BRAF* p.V600E mutation is mandatory for the clinical management of patients with metastatic CRC at diagnosis.

promoter region and not by a germline mutation, essentially excluding LS (Hendriks et al., 2006). Indeed, LS is absent in the vast majority (about 99 %) of *BRAF* p.V600E CRC patients with MLH1 loss (National Comprehensive Cancer Network, 2024).

Overall, the panel considered *BRAF* mutational assessment as desirable (but not mandatory) in high-risk stage III CRC patients. In this context, it could be useful for the oncologist to be aware of the patient's poor expected OS/DFS, and increased risk of early recurrence (when *BRAF* p.V600E mutations occur in an MSS CRC patients) (Zhu et al., 2016; Taieb et al., 2023). If molecular data are available at diagnosis, the time required to set up a correct treatment program when relapse occurs can be reduced. It could also direct the physician toward enrolling the patient in studies (i.e. Unicorn Trial, ClinicalTrials.gov Identifier: NCT05845450) evaluating the preoperative use of targeted therapies in non-metastatic CRC patients.

The following statement summarizes the panel's discussion and final agreement on this topic; an initial version of this statement was amended since it reached a level of agreement below the required 80 % threshold during the mm-Delphi process (Appendix 1).

3.5. Clinical evidence on targeted therapies

Recently, *BRAF* mutational status has emerged as a promising predictive biomarker, identifying mCRC patients for target therapy. The panel particularly discussed the optimal placement of targeted therapies involving BRAF inhibitor (encorafenib) combined with anti-epidermal growth factor receptor (EGFR) therapy (cetuximab) within the treatment algorithm for metastatic colorectal cancer (mCRC).

This approach has been approved as the standard of care for the treatment of *BRAF* p.V600E-mutated mCRC patients who have previously received systemic therapy (BEACON trial). In this trial, encorafenib plus cetuximab with or without binimetinib improved mOS, ORR, and mPFS compared with standard chemotherapy plus cetuximab. Moreover, by adding binimetinib to the experimental arm, the overall efficacy was not modified, while higher rates of adverse events were observed, supporting the use of encorafenib plus cetuximab schedule, which was subsequently approved by regulatory agencies worldwide (Trullas et al., 2021; Tabernero et al., 2021).

An Italian, multicenter retrospective observational study confirmed clinical outcomes from the BEACON trial in a real-life setting. Overall, n=133 *BRAF* V600E mCRC patients were enrolled and treated with encorafenib plus cetuximab with or without binimetinib in 21 participating centers. Among them, 97 patients only received encorafenib plus cetuximab. As shown by the authors, encorafenib plus cetuximab treatment showed consistent clinical outcomes in line with the BEACON trial in terms of safety and efficacy (Boccaccino et al., 2022).

Italian national guidelines approved ICIs (first line pembrolizumab as monotherapy and nivolumab plus ipilimumab in the second line) (Keytruda® SmPC; Opdivo® SmPC; Yervoy® SmPC) in patients with CRCs showing microsatellite instability (d-MMR/MSI-H).

Of note, all Italian and international guidelines recommend first-line immunotherapy, regardless of *BRAF* mutational status in MSI-H mCRC patients (Cervantes et al., 2023; Morris et al., 2023; National Comprehensive Cancer Network, 2024; Associazione Italiana di Oncologia Medica, 2021).

The BEACON trial lacks clinical data on target therapies after the first-line with ICIs regimen, reducing clinical evidence for this recommendation. However, as pointed out by the panel, CRC patients previously treated with ICIs were assessed in the BEACON trial, and targeted therapy is a key weapon for second-line treatment in clinical practice. In accordance with ESMO guidelines, the clinical rationale suggests that *BRAF*-mutated /MSI-H positive CRC patients receiving first-line immunotherapy could also benefit from encorafenib plus cetuximab upon progression (Cervantes et al., 2023).

The role of encorafenib plus cetuximab for the first-line treatment in both MSS and MSI-H mCRC patients is currently under investigation in randomized clinical trials (BREAKWATER study, ClinicalTrials.gov Identifier: NCT04607421; SEAMARK study, ClinicalTrials.gov Identifier: NCT05217446).

The following statement summarizes the panel's discussion and final agreement on this topic; an initial version of this statement was amended since it reached a level of agreement below the common required threshold of 80 % adopting mm-Delphi algorithm (Appendix 1).

3.6. Pre-analytical phase

BRAF molecular testing has become a crucial step for the clinical management of CRC patients however the pre-analytical phase still requires some standardization (Supplementary Figure 2). The term "pre-analytical phase" refers to several sequential working procedures necessary for the preservation of the diagnostic specimen, such as fixation in formalin, processing of tissue and short cold ischemia time (Malapelle et al., 2023).

Following this assessment, the expert panel considered both primary and metastatic specimens feasible for successful molecular analysis. In this scenario, it is essential to receive specimens as soon as possible to immediately activate preserving procedures able to improve morphological and molecular assessment of diagnostic samples (Malapelle et al., 2023). Therefore, "cold ischemia time" (the time ranging between surgical resection and fixation procedure) should be minimal (reaching a maximum of one hour) to decrease nucleic acid fragmentation rate. Overall, pre-analytical optimized procedures are fundamental both for correct morphological and molecular evaluation and for predictive/prognostic-pivotal parameters (Hammond et al., 2010; Wolff

Immediate testing for *BRAF* mutational status is mandatory for all patients with early CRC with loss of MLH1 as part of the Lynch syndrome diagnostic algorithm and should be considered in routine practice for patients with high-risk resected stage III CRC to add prognostic information and quickly inform for an appropriate therapy in case of disease recurrence.

In MSS *BRAF* p.V600E-mutated patients progressing during adjuvant or first-line chemotherapy-based regimens and MSI-H mCRC patients with *BRAF* p.V600E mutation progressing on immunotherapy, the combination of encorafenib-cetuximab should be considered as the first and preferred option.

et al., 2013).

Each specimen should be fixed in 10 % neutral buffered formalin (4 % formaldehyde) for 6–48 hours (Malapelle et al., 2023), depending on sample type (surgical resection or small biopsy). Formalin fixation is considered the most critical step in the pre-analytical phase since over-fixation can drastically impact on nucleic acid integrity (Cappello et al., 2022). Pathologists are key actors in molecular diagnostic procedures: the selection of micro- or macro-dissecting neoplastic areas to evaluate neoplastic cell percentage dramatically impacts on the identification of the most suitable technical approach for molecular testing (Cappello et al., 2022).

Indeed, to obtain adequate tumor cell content (>20 %) the available specimens should be reviewed by a pathologist and enriched by microdissection before DNA extraction (Malapelle et al., 2023). This step removes from the samples large portions of non-neoplastic cells which would otherwise dilute neoplastic cellularity (Hunt and Finkelstein, 2004; Fassan, 2018; Parente et al. 2023). Specimens resected after adjuvant chemotherapy/radiation therapy or affected by a high mucin content and necrotic debris (two potential PCR inhibitors) should be rejected (Hunt and Finkelstein, 2004; Fassan, 2018). Moreover, neoadjuvant treatments may decrease tumor cell percentage generating a false negative result. Therefore, in patients undergoing neoadjuvant therapy, it is recommended to use the pretreatment biopsy samples for molecular analysis rather than the surgical specimen (Boissière-Michot et al., 2012).

In addition, Formalin-Fixed Paraffin-Embedded (FFPE) biospecimens should be appropriately stored while waiting for the technical availability of molecular testing. Three years of storage at room temperature may impact FFPE block adequacy for NGS-based molecular tests (Hedegaard et al., 2014).

Before DNA extraction, FFPE specimens should also be deparaffinized. It has been shown that improved DNA yield and integrity can be obtained by adopting deparaffinized slides in comparison with tissue sections in tubes (Kofanova et al., 2020). Although xylene drastically impacts on nucleic acid integrity, alternative deparaffinization reagents may be useful (namely mineral oil, hexadecane, pentadecane or tetradecane), yielding higher nucleic acids concentration compared with standardized protocols (Malapelle et al., 2023).

Nucleic acid extraction procedures and heat treatment conditions applied for reverting crosslinks should be set to optimize the preanalytical management of diagnostic samples. Currently, a plethora of different technical procedures are available to successfully carry out nucleic acid purification from diagnostic routine samples: membrane-based kits, silica column-based or magnetic bead-based methods, dual DNA/RNA extraction kits, and automatized FFPE extraction methods (Malapelle et al., 2023). Several clinical trials have compared the technical performance of these diagnostic procedures to harmonize pitfalls in preanalytical managing procedures of tissue specimens.

After reviewing and discussing all the above-listed evidence, the panel reached an agreement using the mm-Delphi approach on the following statement. Of note, panelists considered as routinely available specimens both tissue from primary tumor and from metastatic sites.

3.7. Analytical phase

At the present time, two distinct technical approaches are routinely available for *BRAF* molecular analysis: the Real-Time Polymerase Chain Reaction (RT-PCR) and the Next Generation Sequencing (NGS) based systems, both methods present pros and cons which should be discussed (Cappello et al., 2022).

RT-PCR is a highly sensitive, cheaper, and easy-to-use technology, showing lower turnaround than NGS. Thus, RT-PCR-based platforms are the most diffuse technical approach for detecting *BRAF* clinically relevant alterations in the diagnostic routine practice of small and medium institutions. Unfortunately, RT-PCR-based approaches are affected by a scant reference range able to identify selected hotspot mutations (Cappello et al., 2022; Angerilli et al., 2021; Russo et al., 2021).

NGS platforms, on the other hand, are routinely adopted in high-volume molecular testing institutions. NGS systems detect any variant from a large number of covered genes (from 2 to 500 genes) in the same run. In addition, NGS approaches are scalable depending on the reference range of the selected panel. Considering this aspect, small medium NGS panels (2–50 genes) and comprehensive genomic profiling panels (>200 genes) should be used in clinical practice and research trials, respectively (Cappello et al., 2022; Havel et al., 2019; Russo et al., 2021). Moreover, NGS panels allow simultaneous *RAS* and *BRAF* analysis, which is currently recommended in routine practice, with testing for additional emerging biomarkers (Havel et al., 2019; Angerilli et al., 2021).

Despite these technical advantages, NGS systems have not yet replaced RT-PCR as for *BRAF* testing in CRC patients, pinpointing a possible integrated role for these technical approaches. ESMO guidelines recommend NGS implementation when the costs are comparable to those of RT-PCR (Mosele et al., 2024).

Although NCCN guidelines consider IHC as a valid technical option for *BRAF* p.V600E detection in CRC patients, the panel did not recommend this assay due to the low-analytical performance of dedicated antibodies requiring internal validation, and intra-tumoral and inter-operator variability impacting on the technical results (Grillo et al., 2024, National Comprehensive Cancer Network, 2024; Galuppini et al. 2017).

Molecular testing for *BRAF* mutations can also be performed on liquid biopsy (LB) (Russo et al. 2021). LB overcomes some of the technical limitations of tissue-based molecular approaches, such as spatial and temporal heterogeneity (Ou et al., 2018). Moreover, LB consists of a noninvasive, dynamic, simple molecular analysis approach with low TAT (Russo et al., 2021). The prospective Poseidon study compared LB and standard tissue biopsy (STB) for *RAS/BRAF* mutation detection rate on a real-world mCRC series. In this trial the overall agreement between LB and STB reached 83 %; LB sensitivity and specificity compared with STB were 90 % and 80 %, respectively. However, about 13 % of the results obtained with LB were false negatives (Procaccio et al., 2021). In this scenario, an expert opinion of the AIOM-SIAPEC-IAP-SIBIOC-SIF Italian Scientific Societies recommended LB to be used for *BRAF* mutation only when an inadequate surgical sample or biopsy is available for molecular test (Russo et al., 2021).

The performance of molecular testing relies not only on the quality of the method itself, but also on the quality of the analysed biospecimen.

Panelists reviewed this statement on the *BRAF* mutational testing strategy in mCRC patients reaching the agreement in the mm-Delphi procedure, with only 2 panelists preferring NGS over real-time PCR.

3.8. Reporting of the molecular profiling

According to the panel, a clearly interpretable molecular and clinical report is still an open challenge in diagnostic routine practice. Particularly, NGS-derived molecular records require easy-to-understand and clinically informative report for clinicians. Reports from external laboratories can also be challenging if explanatory comments, which are increasingly needed as diagnostic technology evolves, are not included.

In addition, the panel highlighted that molecular reports lack uniformity, with heterogeneous technical procedures (platforms, infrastructure, biosources) available in each testing institution. Therefore, standardized guidelines are mandatory to build easily interpretable clinical reports.

In this scenario, a molecular analysis report should clearly and concisely include (Schmid et al., 2022):

- General clinical data (name, surname, date of birth, sex), the ordering physician, the laboratory that performed the analysis, and the specimen (ID number, date of collection).
- Type of specimen used for molecular testing (FFPE – biopsy or resection specimen, frozen tissue, LB). In the case of a tissue sample, histological diagnosis, tumor cell content, whether microdissection was performed, and the identification of the physician supervising the diagnostic procedure should be included. If a liquid biopsy sample is available, collecting time and pre-analytical managing procedures should be summarized.
- Details regarding methodology/procedure, i.e. type of assay, limit of detection, target description (genes and exons/codons tested - if applicable).
- Molecular results (list of molecular alterations using standard nomenclature system and including variant allele frequencies – if applicable; additional analytic and clinical interpretative comments).

Finally, *BRAF* testing should be completed and reported within a TAT of $\leq$10 working days for at least 90 % of the test requests (Cervantes et al., 2023; Malapelle et al., 2023).

As a summary of this discussion on clinical reporting of *BRAF* mutation tests, the panel proposed the following statement, which reached an agreement in the mm-Delphi process.

3.9. Multidisciplinary team

According to the panel, it is important to establish a standardized mCRC patient journey, designed according to each centre's peculiarities and aimed at making information on *BRAF* mutational status readily (and with low TAT times) available to the healthcare professionals who need it (Fig. 1).

The panel underlined the pivotal role of the pathologist in the diagnostic scenario of *BRAF*-mutated CRC patients and, generally, in personalized diagnostics, which is the pillar of precision oncology (Angerilli et al., 2021).

Pathologists should be aware of the challenges associated with the molecular assessment of *BRAF* in mCRC and act flexibly, tailoring their practice to each individual case.

In particular, they should choose the most appropriate diagnostic strategy based on the characteristics of the available sample and select the most technically adequate platform taking into account the limit of detection.

Finally, they should provide other health professionals who manage mCRC patients with comprehensive information on the mutations detected since different types of *BRAF* mutations have different prognostic and therapeutic significance.

This discussion was summarized in the following and last statement of this expert's opinion paper; the statement reached the agreement in the mm-Delphi process.

4. Conclusions

Considering the clinical benefit of targeted drugs in *BRAF* p.V600E mCRC patients, molecular analysis has become an essential part of clinical management.

This experts' opinion paper proposes nine statements that provide practical and concise advice on *BRAF* mutation testing in CRC patients derived from collegial discussion and analysis of a multidisciplinary team of experts, including referral Italian oncologists and pathologists.

The cited statements represent a key weapon for healthcare professionals committed to managing mCRC patients overviewing the pivotal aspects implied in the detection, treatment, and management of *BRAF*-mutated CRC patients. Indeed, it is of paramount importance to raise awareness regarding the importance of *BRAF* mutation testing in mCRC patients. Despite the relatively low incidence of BRAF mutations in mCRC, the availability of a specific targeted treatment has added a predictive value giving the opportunity to improve clinical outcomes for CRC patients.

Where needed, optimization of the test journey should be pursued. This paper should therefore be considered a starting point in the implementation of diagnostic-therapeutic workflow able to adapt to the variability of local resources while respecting the high-quality standards required by modern precision oncology.

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CRediT authorship contribution statement

Conceptualization: MF, SL. Data curation: VA, RI, FB. Methodology: MF, SL, VA, RI, FB. Supervision: MF, SL, UM. Validation: all the panelists. Visualization: all authors. Roles/Writing original draft: VA, RI, FB. Writing review & editing: MF, SL, UM. Approval to submit: All Authors

Declaration of Competing Interest

FB received personal honoraria as invited speaker from Eli-Lilly, MSD, EISAI, Bristol Myers Squibb, AstraZeneca, Pierre Fabre; participation in advisory board for Servier, AAA Novartis. **CC** reported the following: advisory board or consultant role with Astra Zeneca, Merck Serono, MSD, Nordic Pharma, Roche, Pierre Fabre, Takeda, Tempus; invited speaker with compensation for Amgen, Bayer, Merck Serono, MSD, Pierre Fabre Servier, Takeda; Research grants by Amgen, Merck,

Testing for the *BRAF* mutation should be carried out on FFPE tissues with quantitative real-time PCR or next-generation sequencing. Liquid biopsy could represent an alternative if no tissue is available.

Molecular pathology reports should present results in a clear and concise manner to guide clinicians in the selection of the best treatment options.

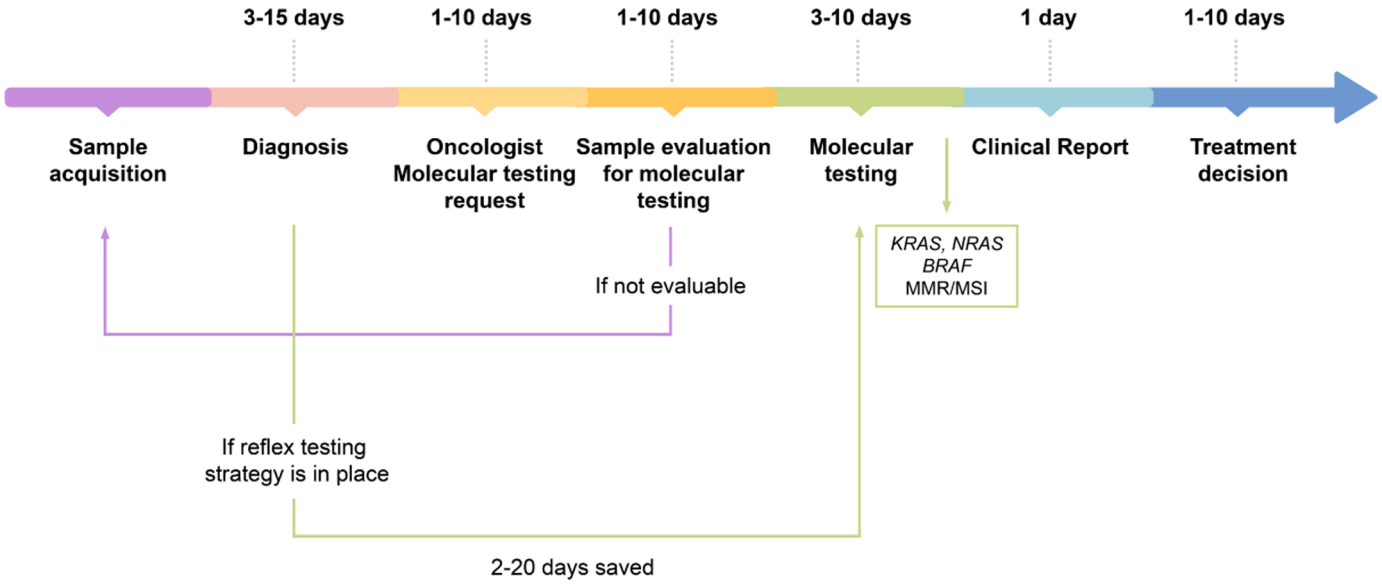

Fig. 1. Overview of the mCRC patient's journey in the context of *BRAF* testing*. *Based on experts' opinion.

A standardized, site-level specific, multidisciplinary clinical pathway and process is needed to allow for a timely and accurate diagnosis and adequate treatment.

Pierre Fabre, Roche, Seagen (Pfizer), Servier, Tempus. **MF** reported the following: advisory board or consultant role with Amgen, Astellas, Astra Zeneca, BMS, Sanofi, Diapath, Eli Lilly, GSK, Incyte, IQvia, Janssen Pharma, MSD, Novartis, Pierre Fabre, Roche; Research Funding (To Institution): Astellas, Diaceutics, Roche. **FG** reported the following: ADVISORY BOARDS: - MSD; GSK; Beigene; LECTURE FEES: Pierre Fabre; MSD; GSK; AAA; Novartis; Servier; Astellas; Incyte; BMS; AstraZeneca; BeiGene; Daiichi-Sankyo. **EGR**, outside the submitted work, has received advisory fees, honoraria, travel accommodation/expenses, grants and/or non-financial support from AstraZeneca, Exact Sciences, GSK, Illumina, MSD, Novartis, Roche, Sophia Genetics and Thermo Fisher Scientific; receipt of honoraria or consultation fees for speaker, consultancy or advisory roles: Amgen, Bayer, Eisai, Merck Serono, Pierre Fabre, Roche, Servier, Incyte, ESMO, MSD, Takeda; travel grant: AstraZeneca, Pierre Fabre,Bayer. **RI** reports consulting or advisory role for Pierre-Fabre and Bayer. **TPL** state no conflict of interest and have received no payment in the preparation of this manuscript. TPL reports receipt of honoraria or consultation fees for speaker, consultancy or advisory roles: Bayer, Pierre Fabre, Servier, Takeda. **SL** reports: consulting or Advisory Role from Amgen, Astellas, Astra Zeneca, Bayer Bristol-Myers Squibb, Daiichi-Sankyo, GSK, Incyte, Lilly, Merck Serono, MSD, Servier, Takeda, Rottapharm, Beigene; speakers' Bureau: Amgen, Astra Zeneca, Bristol-Myers Squibb, Incyte, GSK, Lilly, Merck Serono, MSD, Pierre-Fabre, Roche, Servier; Research Funding (To Institution): Amgen, Astellas, Astra Zeneca, Bayer, Bristol-Myers Squibb, Daichii Sankyo, Hutchinson, Incyte, Merck Serono, Mirati, MSD, Pfizer, Roche, Servier. **UM** has served as consultant and/or speaker bureau for Boehringer Ingelheim, Roche, MSD, Amgen, Thermo Fisher Scientifics, Eli Lilly, Diaceutics, Diatech, GSK, Merck, AstraZeneca, Menarini Steamline, Novartis, Hedera outside the current work. **EM** reported receiving honoraria or consultation fees for speaker, consultancy or advisory roles from Amgen, Bayer, Eisai, Merck Serono, Pierre Fabre, Roche, Servier, Incyte, ESMO, MSD, Takeda; travel grant: AstraZeneca, Pierre Fabre, Bayer. **NN** reports: Personal financial interests (speaker's fee and/or advisory boards): MSD, Bayer, Biocartis, Illumina, Incyte, Roche, BMS, MERCK, Thermofisher, Astrazeneca, Eli Lilly, Novartis, Servier; Institutional financial interests (financial support to research projects): MERCK, Thermofisher, QIAGEN, Roche, Astrazeneca, Biocartis, Illumina; Non-financial interests: President, International Quality Network for Pathology (IQN Path); Past President, Italian Cancer Society (SIC); Scientific Director, IRST "Dino Amadori", Forlì. **PP** received personal fees from MSD, Pierre-Fabre, Servier, Astellas, Incyte, AstraZeneca, GSK, Pharmacogenetics, BeiGene, Bristol-Myers Squibb, Lilly. **AP** reported consulting or advisory role for Daiichi, Beigene, Pierre-Fabre, Merck, Amgen, Bayer, Servier. **FP** reported receiving institutional research grants from BMS, Incyte, Agenus, Amgen, Lilly and AstraZeneca, and personal fees from BMS, MSD, Amgen, Merck-Serono, Pierre-Fabre, Servier, Bayer, Takeda, Astellas, Johnson&Johnson, Rottapharm, Ipsen, AstraZeneca, GSK, Daiichi-Sankyo, Seagen/Pfizer, Beigene. **LS** is currently supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC) under My First Grant (MFAG) No. MFAG27367. LS reports consulting or advisory role for Pierre-Fabre, AstraZeneca, Bayer, SERVIER, Merck, Amgen, GSK, Incyte, Leopharma, MSD, Takeda. **VA**, **FP** reports no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.critrevonc.2024.104574.

References

Angerilli, V., Galuppini, F., Pagni, F., Fusco, N., Malapelle, U., Fassan, M., 2021. The role of the pathologist in the next-generation era of tumor molecular characterization. Published 2021 Feb 18. Diagnostics 11 (2), 339. https://doi.org/10.3390/diagnostics11020339.

Angerilli, V., Sabella, G., Centonze, G., et al., 2022. BRAF-mutated colorectal adenocarcinomas: pathological heterogeneity and clinical implications. Crit. Rev. Oncol. Hematol. 172, 103647. https://doi.org/10.1016/j.critrevonc.2022.103647.

Associazione Italiana di Oncologia Medica (AIOM). Linee guida tumori del colon. October 2021.

Barras, D., Missiaglia, E., Wirapati, P., et al., 2017. BRAF V600E mutant colorectal cancer subtypes based on gene expression. Clin. Cancer Res 23 (1), 104–115. https://doi.org/10.1158/1078-0432.CCR-16-0140.

Boccaccino, A., Borelli, B., Intini, R., et al., 2022. Encorafenib plus cetuximab with or without binimetinib in patients with BRAF V600E-mutated metastatic colorectal cancer: real-life data from an Italian multicenter experience. ESMO Open 7 (3), 100506. https://doi.org/10.1016/j.esmoop.2022.100506.

Boissière-Michot, F., Lopez-Crapez, E., Frugier, H., et al., 2012. KRAS genotyping in rectal adenocarcinoma specimens with low tumor cellularity after neoadjuvant treatment. Mod. Pathol. 25 (5), 731–739. https://doi.org/10.1038/modpathol.2011.210.

Cappello, F., Angerilli, V., Munari, G., et al., 2022. FFPE-based NGS approaches into clinical practice: the limits of glory from a pathologist viewpoint. Published 2022 May 5. J. Pers. Med 12 (5), 750. https://doi.org/10.3390/jpm12050750.

Cervantes, A., Adam, R., Roselló, S., et al., 2023. Metastatic colorectal cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. Ann. Oncol. 34 (1), 10–32. https://doi.org/10.1016/j.annonc.2022.10.003.

Chang, S.C., Li, A.F., Lin, P.C., et al., 2020. Clinicopathological and molecular profiles of sporadic microsatellite unstable colorectal cancer with or without the CpG island methylator phenotype (CIMP). Published 2020 Nov 23. Cancers (Basel) 12 (11), 3487. https://doi.org/10.3390/cancers12113487.

Chen, D., Huang, J.F., Liu, K., et al., 2014. BRAFV600E mutation and its association with clinicopathological features of colorectal cancer: a systematic review and meta-analysis. Published 2014 Mar 3. PLoS One 9 (3), e90607. https://doi.org/10.1371/journal.pone.0090607.

Clarke, C.N., Kopetz, E.S., 2015. BRAF mutant colorectal cancer as a distinct subset of colorectal cancer: clinical characteristics, clinical behavior, and response to targeted therapies. J. Gastrointest. Oncol. 6 (6), 660–667. https://doi.org/10.3978/j.issn.2078-6891.2015.077.

Cohen, M.H., Chen, H., Fuchs, C., et al., 2013. Approval summary: cetuximab in combination with cisplatin or carboplatin and 5-fluorouracil for the first-line treatment of patients with recurrent locoregional or metastatic squamous cell head and neck cancer. Oncologist 18, 460–466. https://doi.org/10.1634/theoncologist.2012-0458.

Cvetkovic, L., Said, L., Ayoub, J.P.M., et al., 2023. Reflex testing for RAS and BRAF mutations in metastatic colorectal cancer: a single Canadian academic oncology center experience (abstr). J. Clin. Oncol. 41 (4), 86. https://doi.org/10.1200/JCO.2023.41.4_suppl.86.

Fanelli, G.N., Dal Pozzo, C.A., Depetris, I., et al., 2020. The heterogeneous clinical and pathological landscapes of metastatic Braf-mutated colorectal cancer. Published 2020 Jan 29. Cancer Cell Int 20, 30. https://doi.org/10.1186/s12935-020-1117-2.

Fassan, M., 2018. Molecular diagnostics in pathology: time for a next-generation pathologist? Arch. Pathol. Lab Med. 142 (3), 313–320. https://doi.org/10.5858/arpa.2017-0269-RA.

Fassan, M., Milione, M., Maddalena, G., et al., 2021. Synaptophysin expression in V600EBRAF-mutated advanced colorectal cancers identifies a new subgroup of tumours with worse prognosis. Eur. J. Cancer 146, 145–154. https://doi.org/10.1016/j.ejca.2021.01.016.

Fassan, M., Scarpa, A., Remo, A., et al., 2020. Current prognostic and predictive biomarkers for gastrointestinal tumors in clinical practice. Pathologica 112 (3), 248–259. https://doi.org/10.32074/1591-951X-158.

Gallego, D., Bueno, S., 2014. Exploring the application of the Delphi method as a forecasting tool in Information Systems and Technologies research. Technol. Anal. Strateg Manag 26, 987–999. https://doi.org/10.1080/09537325.2014.941348.

Galuppini, F., Pennelli, G., Loupakis, F., Lanza, C., et al., 2017. BRAF p.V600E-specific immunohistochemical assessment in colorectal cancer endoscopy biopsies is consistent with the mutational profiling. Histopathology 71 (6), 1008–1011. https://doi.org/10.1111/his.13315.

Giusti, R.M., Shastri, K.A., Cohen, M.H., et al., 2007. FDA drug approval summary: panitumumab (Vectibix™). Oncologist 12, 577–583. https://doi.org/10.1634/theoncologist.12-5-577.

Grillo, F., Paudice, M., Pigozzi, S., et al., 2024. BRAF^{V600E} immunohistochemistry can reliably substitute BRAF molecular testing in the Lynch syndrome screening algorithm in colorectal cancer. Histopathology 84, 877–887. https://doi.org/10.1111/his.15133.

Gustafson, D.H., Shukla, R.K., Delbecq, A., et al., 1973. A comparative study of differences in subjective likelihood estimates made by individuals, interacting groups, Delphi groups, and nominal groups. Organ Behav. Hum. Perform. 9, 280–291. https://doi.org/10.1016/0030-5073(73)90052-4.

Hammond M.E., Hayes D.F., Dowsett M., et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer [published correction appears in J Clin Oncol. 2010 Jul 20;28(21):3543]. J Clin Oncol. 2010;28(16):2784-2795. doi:10.1200/JCO.2009.25.6529.

Havel, J.J., Chowell, D., Chan, T.A., 2019. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat. Rev. Cancer 19 (3), 133–150. https://doi.org/10.1038/s41568-019-0116-x.

Hedegaard, J., Thorsen, K., Lund, M.K., et al., 2014. Next-generation sequencing of RNA and DNA isolated from paired fresh-frozen and formalin-fixed paraffin-embedded samples of human cancer and normal tissue. Published 2014 May 30. PLoS One 9 (5), e98187. https://doi.org/10.1371/journal.pone.0098187.

Hendriks, Y.M., de Jong, A.E., Morreau, H., et al., 2006. Diagnostic approach and management of Lynch syndrome (hereditary nonpolyposis colorectal carcinoma): a guide for clinicians. CA Cancer J. Clin. 56 (4), 213–225. https://doi.org/10.3322/canjclin.56.4.213.

Hunt, J.L., Finkelstein, S.D., 2004. Microdissection techniques for molecular testing in surgical pathology. Arch. Pathol. Lab Med. 128 (12), 1372–1378. https://doi.org/10.5858/2004-128-1372-MTFMTI.

I numeri del cancro in Italia, 2023. ⟨https://www.aiom.it/wp-content/uploads/2024/02/2023_AIOM_NDC-web_def.pdf⟩.

Jang, M.H., Kim, S., Hwang, D.Y., et al., 2017. BRAF-mutated colorectal cancer exhibits distinct clinicopathological features from wild-type BRAF-expressing cancer independent of the microsatellite instability status. J. Korean Med Sci. 32 (1), 38–46. https://doi.org/10.3346/jkms.2017.32.1.38.

Jones, J.C., Renfro, L.A., Al-Shamsi, H.O., et al., 2017. Non-V600 BRAF mutations define a clinically distinct molecular subtype of metastatic colorectal cancer. J. Clin. Oncol. 35 (23), 2624–2630. https://doi.org/10.1200/JCO.2016.71.4394.

Kofanova, O., Bellora, C., Garcia Frasquilho, S., et al., 2020. Standardization of the preanalytical phase of DNA extraction from fixed tissue for next-generation sequencing analyses. N. Biotechnol. 54, 52–61. https://doi.org/10.1016/j.nbt.2019.07.005.

Leowattana, W., Leowattana, P., Leowattana, T., 2023. Systemic treatment for metastatic colorectal cancer. World J. Gastroenterol. 29 (10), 1569–1588. https://doi.org/10.3748/wjg.v29.i10.1569.

Lochhead, P., Kuchiba, A., Imamura, Y., et al., 2013. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. J. Natl. Cancer Inst. 105 (15), 1151–1156. https://doi.org/10.1093/jnci/djt173.

Loupakis, F., Biason, P., Prete, A.A., et al., 2019. CK7 and consensus molecular subtypes as major prognosticators in V600EBRAF mutated metastatic colorectal cancer. Br. J. Cancer 121 (7), 593–599. https://doi.org/10.1038/s41416-019-0560-0.

Malapelle, U., Angerilli, V., Pepe, F., et al., 2023. The ideal reporting of RAS testing in colorectal adenocarcinoma: a pathologists' perspective. Pathologica. https://doi.org/10.32074/1591-951X-895. Published online June 14,.

Martinelli, E., Cremolini, C., Mazard, T., et al., 2022. Real-world first-line treatment of patients with BRAFV600E-mutant metastatic colorectal cancer: the CAPSTAN CRC study. ESMO Open 7 (6), 100603. https://doi.org/10.1016/j.esmoop.2022.100603.

Morkel, M., Riemer, P., Bläker, H., Sers, C., 2015. Similar but different: distinct roles for KRAS and BRAF oncogenes in colorectal cancer development and therapy resistance. Oncotarget 6 (25), 20785–20800. https://doi.org/10.18632/oncotarget.4750.

Morris, V.K., Kennedy, E.B., Baxter, N.N., et al., 2023. Treatment of metastatic colorectal cancer: ASCO guideline. J. Clin. Oncol. 41 (3), 678–700. https://doi.org/10.1200/JCO.22.01690.

Mosele, M.F., Westphalen, C.B., Stenzinger, A., et al., 2024. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann. Oncol. 35 (7), 588–606. https://doi.org/10.1016/j.annonc.2024.04.005.

National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Colon Cancer. Version 1.2024 - January 29, 2024. ⟨https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf⟩.

O'Riordan, E., Bennett, M.W., Daly, L., Power, D.G., 2022. The implication of BRAF mutation in advanced colorectal cancer. Ir. J. Med Sci. 191 (6), 2467–2474. https://doi.org/10.1007/s11845-021-02689-x.

Ou, S.I., Nagasaka, M., Zhu, V., 2018. Liquid biopsy to identify actionable genomic alterations. Am. Soc. Clin. Oncol. Educ. Book 978–997. https://doi.org/10.1200/EDBK_199765.

Parente, P., Grillo, F., Vanoli, A., Mastracci, L., Fassan, M., 2023. A charming molecular profile in the wrong tissue: the pathologist in the era of molecular diagnostics in the gastrointestinal tract. Expert Rev. Mol. Diagn. 23 (12), 1045–1047. https://doi.org/10.1080/14737159.2023.2288267.

Parsons, M.T., Buchanan, D.D., Thompson, B., Young, J.P., Spurdle, A.B., 2012. Correlation of tumour BRAF mutations and MLH1 methylation with germline mismatch repair (MMR) gene mutation status: a literature review assessing utility of tumour features for MMR variant classification. J. Med Genet. 49 (3), 151–157. https://doi.org/10.1136/jmedgenet-2011-100714.

Passiglia, F., Bronte, G., Bazan, V., Galvano, A., Vincenzi, B., Russo, A., 2016. Can KRAS and BRAF mutations limit the benefit of liver resection in metastatic colorectal

cancer patients? A systematic review and meta-analysis. Crit. Rev. Oncol. Hematol. 99, 150–157. https://doi.org/10.1016/j.critrevonc.2015.12.015.
Procaccio, L., Bergamo, F., Daniel, F., et al., 2021. A real-world application of liquid biopsy in metastatic colorectal cancer: the poseidon study. Published 2021 Oct 13. Cancers 13 (20), 5128. https://doi.org/10.3390/cancers13205128.
Rasola, C., Laurent-Puig, P., André, T., et al., 2023. Time to recurrence and its relation to survival after recurrence in patients resected for stage III colon cancer. Eur. J. Cancer 194, 113321. https://doi.org/10.1016/j.ejca.2023.113321.
Roth, A.D., Tejpar, S., Delorenzi, M., et al., 2010. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J. Clin. Oncol. 28 (3), 466–474. https://doi.org/10.1200/JCO.2009.23.3452.
Russo, A., Incorvaia, L., Del Re, M., et al., 2021. The molecular profiling of solid tumors by liquid biopsy: a position paper of the AIOM-SIAPEC-IAP-SIBioC-SIC-SIF Italian Scientific Societies. ESMO Open 6 (3), 100164. https://doi.org/10.1016/j.esmoop.2021.100164.
Samowitz, W.S., Sweeney, C., Herrick, J., et al., 2005. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res 65 (14), 6063–6069. https://doi.org/10.1158/0008-5472.CAN-05-0404.
Schirripa, M., Biason, P., Lonardi, S., et al., 2019. Class 1, 2, and 3 BRAF-mutated metastatic colorectal cancer: a detailed clinical, pathologic, and molecular characterization. Clin. Cancer Res 25 (13), 3954–3961. https://doi.org/10.1158/1078-0432.CCR-19-0311.
Schmid, S., Jochum, W., Padberg, B., et al., 2022. How to read a next-generation sequencing report-what oncologists need to know. ESMO Open 7 (5), 100570. https://doi.org/10.1016/j.esmoop.2022.100570.
Shimada, Y., Tajima, Y., Nagahashi, M., et al., 2018. Clinical significance of BRAF non-V600E mutations in colorectal cancer: a retrospective study of two institutions. J. Surg. Res 232, 72–81. https://doi.org/10.1016/j.jss.2018.06.020.
Shin, A.E., Giancotti, F.G., Rustgi, A.K., 2023. Metastatic colorectal cancer: mechanisms and emerging therapeutics. Trends Pharmacol. Sci. 44 (4), 222–236. https://doi.org/10.1016/j.tips.2023.01.003.
Tabernero, J., Grothey, A., Van Cutsem, E., et al., 2021. Encorafenib plus cetuximab as a new standard of care for previously treated BRAF V600E-mutant metastatic colorectal cancer: updated survival results and subgroup analyses from the BEACON study. J. Clin. Oncol. 39 (4), 273–284. https://doi.org/10.1200/JCO.20.02088.
Taieb, J., Sinicrope, F.A., Pederson, L., et al., 2023. Different prognostic values of KRAS exon 2 submutations and BRAF V600E mutation in microsatellite stable (MSS) and unstable (MSI) stage III colon cancer: an ACCENT/IDEA pooled analysis of seven trials. Ann. Oncol. 34 (11), 1025–1034. https://doi.org/10.1016/j.annonc.2023.08.006.
Tran, B., Kopetz, S., Tie, J., et al., 2011. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer 117 (20), 4623–4632. https://doi.org/10.1002/cncr.26086.
Trullas, A., Delgado, J., Koenig, J., Fuerstenau, U., Dedorath, J., Hausmann, S., Stock, T., Enzmann, H., Pignatti, F., 2021 Feb. The EMA assessment of encorafenib in combination with cetuximab for the treatment of adult patients with metastatic colorectal carcinoma harbouring the BRAFV600E mutation who have received prior therapy. ESMO Open 6 (1), 100031. https://doi.org/10.1016/j.esmoop.2020.100031.
Van Cutsem, E., Dekervel, J., 2017. Not all BRAF-mutant metastatic colorectal cancers are identical: distinct clinical consequences of non-V600 BRAF mutations. J. Clin. Oncol. 35 (23), 2598–2599. https://doi.org/10.1200/JCO.2017.72.7057.
Wolff, A.C., Hammond, M.E., Hicks, D.G., et al., 2013. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J. Clin. Oncol. 31 (31), 3997–4013. https://doi.org/10.1200/JCO.2013.50.9984.
Zhu, L., Dong, C., Cao, Y., et al., 2016. Prognostic role of BRAF mutation in stage II/III colorectal cancer receiving curative resection and adjuvant chemotherapy: a meta-analysis based on randomized clinical trials. Published 2016 May 3. PLoS One 11 (5), e0154795. https://doi.org/10.1371/journal.pone.0154795.