Journal of Mind and Medical Sciences

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https://proscholar.org/jmms/

ISSN: 2392-7674

How opportune is multigene testing in metastatic colorectal cancer? A review

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ABSTRACT

Personalized treatment in oncology is the most innovative method of care. The best method to establish personalized treatment is by genetic characterization of the malignant cell.

Theoretically, the more detailed the characterization, the more effective the choice of treatment becomes. Currently, there are fast and relatively low-cost options that allow such genetic characterization. However, test results sometimes do not detect targetable alterations and, even if they do detect, the use of the treatment-alteration combination does not always generate a satisfactory oncological response.

The present paper aims to answer two questions. First, how targetable can the most common gene alterations in colorectal cancer be. Second, whether it makes sense to use broad molecular testing as a standard in all metastatic patients.

Category: Review

Received: July 08, 2021
Accepted: September 06, 2021
Published: October 10, 2021

Keywords:

multigene assay, metastatic, colorectal cancer, targeted therapy

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Introduction

In order to personalize oncological systemic therapy, it is essential to characterize the tumor cell as good as possible. In order to do so, broad molecular testing is often required, including next-generation sequencing for a large panel of gene alterations. Following the information obtained, it is possible to decide upon the optimal treatment to target the mutations found. Although it sounds ideal, this process is also full of obstacles, such as the accessibility of the tests, the availability of the drugs that correspond to these alterations, the costs, the lack of expertise of the doctors who should interpret the results. Last but not least, the level of evidence for alteration-treatment associations is different for each cancer site.

The present review aims at answering two questions: how targetable can the most common gene alterations in colorectal cancer be and whether it makes sense to use broad molecular testing as a standard procedure in all metastatic patients.

In order to get the answers, an inquiry was made in the PubMed and Scopus databases using keywords such as: genomic alteration, next-generation sequencing and colorectal cancer.

Only the articles published between 2015 and 2020 were included. In addition, some landmark trials that had been published before this time were also taken into consideration.

A number of 218 articles were found (both original articles and systematic literature reviews). Out of these, 36 have been selected for this paper. Their selection was made using the PICO criteria.

The most noteworthy alterations for targeting metastatic colorectal cancer are KRAS/ NRAS, BRAF, NTRK1, ERBB2, PIK3CA, ATM, MET, AKT1, RET and ALK.

The case of the patients with high tumor mutational burden (TMB) and microsatellite instability should also be considered, as they have been shown to be of particular importance in colorectal cancer [1].

To cite this article: Cristina Orlov-Slavu, Andreea Parosanu, Mihaela Olaru, Dragos Serban, Ioana Paunica, Cornelia Nitipir. How opportune is multigene testing in metastatic colorectal cancer? A review. *J Mind Med Sci.* 2021; 8(2): 215-220. DOI: 10.22543/7674.82.P215220

Several data found in the literature will be detailed in this review, which will conclude with discussions and interpretations related to the level of evidence for targeting each of the factors presented.

Discussion

RAS

The genetic evolution of colorectal cancer involves tumor suppressor genes, mismatch repair genes, or epigenetic processes such as the hypomethylation or the hypermethylation of the DNA. They participate in tumorigenesis through the involvement in the stages of the cell cycle, but also through the direct clinical consequences of mutations [2].

The most important oncogene involved in the onset and development of colorectal cancer is RAS (Rat Sarcoma).

The RAS oncogene has three sub-variants with relevance in colorectal cancer: HRAS, KRAS and NRAS. All of them can lead to normal cell transformation, but KRAS is the most often involved in carcinogenesis. RAS oncogenes encode proteins involved in the transmission of multiple extracellular growth signals to the nucleus. They ensure the transition from the inactive form related to GDP (guanosine diphosphate) to the active form that implies GTP (guanosine triphosphate). In the case of the above-mentioned mutations, the GTP- bound active form is maintained in this continuous stage, stimulating cell division and growth [3-5].

These mutations are also involved in later processes involving tumor invasion and metastasis. RAS mutations are more common in proximal colon cancers [6,7].

The detection of RAS mutations in colorectal cancer is clinically important from at least two points of view: therapeutically and as a method of excluding patients for targeted therapy [8].

The involvement of the farnesyl transferase enzyme in the continuous activation of growth and division pathways by RAS is a scientific certainty. By inhibiting this enzyme, it is assumed that the effect of the KRAS mutation would be slowed down or stopped, thus this is currently the most interesting therapeutic target in these patients [9,10].

The presence of RAS mutations in colorectal cancer patients is associated with a lack of response to epidermal growth factor receptor (EGFR) inhibitors such as cetuximab or panitumumab. Therefore, testing metastatic patients for these mutations is essential for the decision on the treatment, which is currently standard [11,12].

B-RAF

There are two subtypes of B-RAF mutations, depending on the different gene expression and molecular outcome: BM1 and BM2. In the case of the first, there is an activation of the KRAS / AKT pathway that translates into mesenchymal-epithelial transition and immune response, while the second involves abnormalities in the cell cycle and the immune checkpoint [13,14].

It is known that patients with B-RAF mutations have a worse prognosis than the wild-type ones (the wild-type ones have a double or even triple survival rate) [15,16].

However, the BM1 subtype of the B-RAF mutation is linked to the worst outcome. These remarks also explain the different responses to the treatment of these subtypes of patients [14].

The B-RAF mutation is less targeted in colorectal cancer than previously expected. The use of B-RAF inhibitors alone in metastatic colorectal cancer resulted in a 5% response rate [17-19].

From a molecular point of view, this reduced response is explained through the activation of the pathway involving EGFR with B-RAF blockade in this localization. In melanoma, for example, this does not happen because these cells have poorly expressed EGFR [20].

For this reason, the most widely used therapeutic option in patients with metastatic colorectal cancer is the combination of an EGFR inhibitor (cetuximab or panitumumab) and a B-RAF inhibitor (vemurafenib / dabrafenib / encorafenib) [21-24].

MSI

Microsatellite instability (MSI) is a genetic predisposition to mutations that results from impaired DNA mismatch repair (MMR). Tumors with high microsatellite instability and DNA mismatch repair (MSI-H/dMMR) are expressing higher numbers of neo-antigens which increase T-cell activation. Therefore, testing for mismatch repair deficiency (MMR-D)/MSI provides a predictive response to immune checkpoint inhibitors, but it is also a prognostic marker for fluorouracil chemotherapy response.

Even though MSI detection was initially performed for the screening of Lynch syndrome, this molecular signature is found across a broad range of tumor types, and screening for microsatellite instability must become standard practice.

Consequently, the international guidelines (ESMO-European Society for Medical Oncology and NCCN-The National Comprehensive Cancer Network) recommend MSI/ MMR testing for all the patients with colorectal cancer and uterine endometrioid carcinoma [25,26].

Traditionally, there are 2 methods of assessment of MSI /MMR deficient status: MSI analysis can be directly performed using the five microsatellite loci through polymerase chain reaction (PCR), and immunohistochemistry (IHC) is indirectly used to determine the loss of MMR gene expression (MLH1, MSH2, PMS2 or MSH6).

Nowadays, emerging techniques have improved the detection of MSI. Next-Generation Sequencing (NGS) is a novel genetic diagnostic approach for tumor profiling for

microsatellite instability. NGS can scan countless microsatellites, or other targetable alterations suitable for the treatment, which allows a more thorough assessment. Other cancer sites can be tested as well [27,28].

Middha et al., developed a computational software program that combines NGS with biostatistics to address MSI in tumor tissue sampling, without the need for additional biological testing. MSI sensor is a computational tool that reports the percentage of unstable microsatellites as a score. An MSI high tumor is defined by an MSI sensor score higher than 10. This study predominantly included colorectal and endometrial cancers and found a high concordance between the traditional methods (PCR, IHC) and NGS (99.4%). However, NGS seemed slightly more sensitive than PCR and it had the potential of identifying the MSI missed through current laborious and time-consuming methods [29].

As mentioned above, KRAS/ NRAS mutation status or NGS tests should be performed in patients with MSS (microsatellite stable) colorectal tumors. Patients with MSI-H and MLH1 deficient colorectal cancers are at a high risk of developing Lynch syndrome and should undergo testing for the hypermethylation status of MLH1 (MLH1ph).

What is the relationship between MSI-H, MLH1 deficient, BRAF /RAS wild type colorectal cancers and the presence of kinase fusions?

Approximately 15% of advanced MSI-H/ MMR-D, BRAF /RAS wild-type colorectal cancers harbor kinase fusions. These kinase fusions are strongly associated with sporadic MLH1ph than with Lynch syndrome. Therefore, this subset of advanced colorectal tumors may benefit from targeting kinase fusions [30].

NTRK

It is of interest to determine tumors that harbor NTRK fusions in colorectal cancer. NTRK fusion is frequently identified in rare cancer types and there are many strategies to target these oncogenic drivers through targeted kinase inhibitors.

The European Society for Medical Oncology Translational Research and Precision Medicine Working Group reviewed the testing methods currently available for NTRK1/2/3 gene fusions.

The methods of choice for the NTRK1/2/3 fusion gene include real-time PCR (RT-PCR), FISH (fluorescence in situ hybridization), IHC (immunohistochemistry), and NGS technology for DNA and RNA sequencing.

There are limitations and strengths of each method. IHC is a time and tissue-efficient screening with lower costs for NTRK fusions especially in a population with a lower prevalence of molecular alterations. Some advantages of NGS sequencers are the high sensitivity and specificity in detecting a large number of mutations in a single assay, the RNA-based NGS methods being preferred for the detection of NTRK fusions [31,32].

Oncologists have a different attitude toward genomic testing, which may vary according to the types of assessment and their own experience.

Recent data from the National Survey of Precision Medicine in Cancer Treatment revealed how confident oncologists are in using genomic medicine in clinical practice. To guide patient care, doctors are highly (60.1%) confident using next-generation sequencing (NGS) or gene expression (GE) tests. They are more confident when using a single gene testing approach than an entire genome sequencing.

The oncologists' confidence is mostly influenced by the number of patients, the testing platform available and practice infrastructure. Moreover, doctors' training and instruction are very important; continuous education can help keep their interest in medical advances [33].

Is genomic testing routine care?

A French study tried to answer this question. In the ProfiLER trial, 2,579 metastatic and previously treated subjects (both adult and pediatric ones) underwent Next Generation Sequencing molecular profiling of 59 or 69 cancer-related genes and whole-genome comparative genomic hybridization. The goals of this study were to explain the nature and prevalence of specific alterations in the genetic material, and to assess the molecular profiling for precision cancer therapies.

The results showed that the most common mutations were in the genes KRAS, CCND1, CDKN2A, PIKC3A. A molecular tumor board recommended targeted treatments in 27% of the patients, but unfortunately, only 6% received the recommended therapies.

As a consequence, this study failed to prove its hypotheses, and genomic testing should not be used as routine screening care to select appropriate targeted therapies [34].

How can we stratify multigene testing in metastatic colorectal cancer?

For the understanding of the targetability of gene alterations in colorectal cancer, we must first detail the ESCAT ((ESMO) European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets) classification. ESCAT I level means that the combination of gene alteration-drug has been validated in a clinical trial important enough for this to be the standard of care. ESCAT level II assumes that this combination is effective in phase II or I trial or in retrospective analyses. The alterations with ESCAT III evidence level are those with proven efficiency for other locations, but not for the one of interest. The data supporting alterations with ESCAT IV come from preclinical studies. This classification was validated by a panel of oncology experts with experience

in genetics and then validated by two other experts from outside the working group [1,35].

Thus, the gene alterations described above are classified as follows: KRAS has a prevalence of 44% and in its case, ESCAT classification is not possible, B-RAF with a prevalence of 8.5% and ESCAT I, MSI-H 4-5% and ESCAT I, NTRK fusion 0.5% and ESCAT I, ERBB2 amplification 2% and ESCAT II, PIK3CA mutation 17% and ESCAT III, ATM 5% and ESCAT III, MET 1.7% and ESCAT III, RET and ALK fusions with a prevalence of 0.3% and 0.2% and ESCAT III [36-42].

All in all, after stratifying the data, the ESMO recommendations are that multigene NGS tumor should remain an alternative to PCR (polymerase chain reaction) with the purpose of determining the above alterations if it does not involve additional costs [1].

Conclusions

The use of NGS multigene makes sense in colorectal cancer as long as cost-effectiveness is maintained. Excluding the financial effect, these tests are a particularly important step in refining cancer research and accelerating the development of oncology drugs. To choose a large panel of genes is an option in non-targetable cancer sites with the hope that we will find those rare responders. If the treating oncologist decides to proceed this way, he must always inform them about the limited possibility of finding a corresponding treatment.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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