

2021

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

Cristina Orlov-Slavu

CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, FACULTY OF GENERAL MEDICINE,
DEPARTMENT OF ONCOLOGY, BUCHAREST, ROMANIA

Andreea Parosanu

CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, FACULTY OF GENERAL MEDICINE,
DEPARTMENT OF ONCOLOGY, BUCHAREST, ROMANIA, aparosanu@yahoo.com

Mihaela Olaru


CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, FACULTY OF GENERAL MEDICINE,
DEPARTMENT OF ONCOLOGY, BUCHAREST, ROMANIA

Dragos Serban

CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, EMERGENCY UNIVERSITY HOSPITAL
BUCHAREST, 4TH DEPARTMENT OF SURGERY, BUCHAREST, ROMANIA

Follow this and additional works at: <https://scholar.valpo.edu/jmms>

Ioana Paunica

 CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, FACULTY OF GENERAL MEDICINE,
Part of the Digestive System Diseases Commons, Gastroenterology Commons, Genetic Phenomena
BUCHAREST, ROMANIA, ioana.paunica@gmail.com
Commons, Genetic Processes Commons, Medical Genetics Commons, Neoplasms Commons, Oncology

Commons, and the Surgery Commons

See next page for additional authors

Recommended Citation

Orlov-Slavu, Cristina; Parosanu, Andreea; Olaru, Mihaela; Serban, Dragos; Paunica, Ioana; and Nitipir, Cornelia (2021) "How opportune is multigene testing in metastatic colorectal cancer? A review," *Journal of Mind and Medical Sciences*: Vol. 8 : Iss. 2 , Article 7.

DOI: 10.22543/7674.82.P215220

Available at: <https://scholar.valpo.edu/jmms/vol8/iss2/7>

This Review Article is brought to you for free and open access by ValpoScholar. It has been accepted for inclusion in *Journal of Mind and Medical Sciences* by an authorized administrator of ValpoScholar. For more information, please contact a ValpoScholar staff member at scholar@valpo.edu.

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

Authors

Cristina Orlov-Slavu, Andreea Parosanu, Mihaela Olaru, Dragos Serban, Ioana Paunica, and Cornelia Nitipir

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

Cristina Orlov-Slavu^{1,2}, Andreea Parosanu^{1,2*}, Mihaela Olaru^{1,2}, Dragos Serban³, Ioana Paunica⁴, Cornelia Nitipir^{1,2}

¹CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, FACULTY OF GENERAL MEDICINE, DEPARTMENT OF ONCOLOGY, BUCHAREST, ROMANIA

²ELIAS UNIVERSITY EMERGENCY HOSPITAL, DEPARTMENT OF MEDICAL ONCOLOGY, BUCHAREST, ROMANIA

³CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, EMERGENCY UNIVERSITY HOSPITAL BUCHAREST, 4TH DEPARTMENT OF SURGERY, BUCHAREST, ROMANIA

⁴CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, FACULTY OF GENERAL MEDICINE, BUCHAREST, ROMANIA

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

* **Corresponding author:**

Andreea Parosanu,

Carol Davila University of Medicine and Pharmacy, Faculty of General Medicine, Department of Oncology, Bucharest, Romania

E-mail: aparosanu@yahoo.com

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].

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, PIK3CA. 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.

References

- Mosele F, Remon J, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2020;31(11):1491-1505. doi: 10.1016/j.annonc.2020.07.014
- Lynch JP, Hoops TC. The genetic pathogenesis of colorectal cancer. *Hematol Oncol Clin North Am*. 2002;16(4):775-810. doi: 10.1016/s0889-8588(02)00029-1
- Moon BS, Jeong WJ, Park J, Kim TI, Min do S, Choi KY. Role of oncogenic K-Ras in cancer stem cell activation by aberrant Wnt/ β -catenin signaling. *J Natl Cancer Inst*. 2014 Feb;106(2):djt373. doi: 10.1093/jnci/djt373
- Frattini M, Balestra D, Suardi S, Oggionni M, Alberici P, Radice P, Costa A, Daidone MG, Leo E, Pilotti S, Bertario L, Pierotti MA. Different genetic features associated with colon and rectal carcinogenesis. *Clin Cancer Res*. 2004 Jun 15;10(12 Pt 1):4015-21. doi: 10.1158/1078-0432.CCR-04-0031
- Harada K, Hiraoka S, Kato J, Horii J, Fujita H, Sakaguchi K, Shiratori Y. Genetic and epigenetic alterations of Ras signalling pathway in colorectal neoplasia: analysis based on tumour clinicopathological features. *Br J Cancer*. 2007 Nov 19;97(10):1425-31. doi: 10.1038/sj.bjc.6604014
- Giehl K. Oncogenic Ras in tumour progression and metastasis. *Biol Chem*. 2005 Mar;386(3):193-205. doi: 10.1515/BC.2005.025
- Miranda E, Destro A, Malesci A, Ballardore E, Bianchi P, Baryshnikova E, Franchi G, Morengi E, Laghi L, Gennari L, Roncalli M. Genetic and epigenetic changes in primary metastatic and nonmetastatic colorectal cancer. *Br J Cancer*. 2006 Oct 23;95(8):1101-7. doi: 10.1038/sj.bjc.6603337
- Müller MF, Ibrahim AE, Arends MJ. Molecular pathological classification of colorectal cancer. *Virchows Arch*. 2016 Aug;469(2):125-34. doi: 10.1007/s00428-016-1956-3
- Porcu G, Parsons AB, Di Giandomenico D, Lucisano G, Mosca MG, Boone C, Ragnini-Wilson A. Combined p21-activated kinase and farnesyltransferase inhibitor treatment exhibits enhanced anti-proliferative activity on melanoma, colon and lung cancer cell lines. *Mol Cancer*. 2013;12(1):88. doi: 10.1186/1476-4598-12-88
- Kirov KG. A scientometric approach of dynamic science institutionalization in the field of laparoscopic proctocolectomy. *J Clin Invest Surg*. 2019;4(2):88-95. doi: 10.25083/2559.5555/4.2/88.95
- Van Cutsem E, Lenz HJ, Köhne CH, Heinemann V, Tejpar S, Melezínek I, Beier F, Stroh C, Rougier P, van Krieken JH, Ciardiello F. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol*. 2015 Mar 1;33(7):692-700. doi: 10.1200/JCO.2014.59.4812
- Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med*. 2013 Sep 12;369(11):1023-34. doi: 10.1056/NEJMoa1305275
- Barras D, Missiaglia E, Wirapati P, Sieber OM, Jorissen RN, Love C, Molloy PL, Jones IT, McLaughlin S, Gibbs P, Guinney J, Simon IM, Roth

- AD, Bosman FT, Tejpar S, Delorenzi M. BRAF V600E Mutant Colorectal Cancer Subtypes Based on Gene Expression. *Clin Cancer Res.* 2017 Jan 1;23(1):104-115. doi: 10.1158/1078-0432.CCR-16-0140
14. Strickler JH, Wu C, Bekaii-Saab T. Targeting BRAF in metastatic colorectal cancer: Maximizing molecular approaches. *Cancer Treat Rev.* 2017 Nov;60:109-119. doi: 10.1016/j.ctrv.2017.08.006
15. Cremolini C, Loupakis F, Antoniotti C, Lupi C, Sensi E, Lonardi S, Mezi S, Tomasello G, Ronzoni M, Zaniboni A, Tonini G, Carlomagno C, Allegrini G, Chiara S, D'Amico M, Granetto C, Cazzaniga M, Boni L, Fontanini G, Falcone A. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. *Lancet Oncol.* 2015 Oct;16(13):1306-15. doi: 10.1016/S1470-2045(15)00122-9
16. Van Cutsem E, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zube A, Celik I, Rougier P, Ciardiello F. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol.* 2011 May 20;29(15):2011-9. doi: 10.1200/JCO.2010.33.5091
17. Frunza TC, Lunca S, Baciuc I, Axinia I, Mocanu CV, Crudu A, Bujor N, Livenschi LF, Nicolaiev CA, Hulubencu A, Diaconu A, Valasciu E, Jalobceastai I, Tibirna M, Dimofte MG. LARS-like symptoms in the general population may suggest the significance of postoperative functional problems and emotional implications of rectal surgery. *J Mind Med Sci.* 2019; 6(2): 278-285. DOI: 10.22543/7674.62.P278285
18. Corcoran RB. New therapeutic strategies for BRAF mutant colorectal cancers. *J Gastrointest Oncol.* 2015; 6(6):650-9. doi: 10.3978/j.issn.2078-6891.2015.076
19. Kopetz S, Desai J, Chan E, Hecht JR, O'Dwyer PJ, Maru D, Morris V, Janku F, Dasari A, Chung W, Issa JP, Gibbs P, James B, Powis G, Nolop KB, Bhattacharya S, Saltz L. Phase II Pilot Study of Vemurafenib in Patients With Metastatic BRAF-Mutated Colorectal Cancer. *J Clin Oncol.* 2015 Dec 1;33(34):4032-8. doi: 10.1200/JCO.2015.63.2497
20. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature.* 2012 Jan 26;483(7387):100-3. doi: 10.1038/nature10868
21. Yaeger R, Cercek A, O'Reilly EM, Reidy DL, Kemeny N, Wolinsky T, Capanu M, Gollub MJ, Rosen N, Berger MF, Lacouture ME, Vakiani E, Saltz LB. Pilot trial of combined BRAF and EGFR inhibition in BRAF-mutant metastatic colorectal cancer patients. *Clin Cancer Res.* 2015 Mar 15;21(6):1313-20. doi: 10.1158/1078-0432.CCR-14-2779
22. Corcoran RB, André T, Atreya CE, Schellens JHM, Yoshino T, Bendell JC, Hollebecque A, McRee AJ, Siena S, Middleton G, Muro K, Gordon MS, Tabernero J, Yaeger R, O'Dwyer PJ, Humblet Y, De Vos F, Jung AS, Brase JC, Jaeger S, Bettinger S, Mookerjee B, Rangwala F, Van Cutsem E. Combined BRAF, EGFR, and MEK Inhibition in Patients with BRAFV600E-Mutant Colorectal Cancer. *Cancer Discov.* 2018 Apr; 8(4):428-443. doi: 10.1158/2159-8290.CD-17-1226
23. Pietrantonio F. Encorafenib, Binimetinib, and Cetuximab in BRAF V600E-Mutated Colorectal Cancer. *N Engl J Med.* 2020 Feb 27;382(9):876-877. doi: 10.1056/NEJMc1915676
24. Kopetz S, Grothey A, Yaeger R, et. Encorafenib, Binimetinib, and Cetuximab in BRAF V600E-Mutated Colorectal Cancer. *N Engl J Med.* 2019 Oct 24;381(17):1632-1643. doi: 10.1056/NEJMoa1908075
25. Luchini C, Bibeau F, Ligtenberg MJL, Singh N, Nottegar A, Bosse T, Miller R, Riaz N, Douillard JY, Andre F, Scarpa A. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach. *Ann Oncol.* 2019 Aug 1;30(8): 1232-1243. doi: 10.1093/annonc/mdz116
26. Benson AB 3rd, Venook AP, Cederquist L, et al. Colon Cancer, Version 1.2017, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw.* 2017;15(3):370-398. doi: 10.6004/jnccn.2017.0036
27. Alecu L, Tulin A, Enciu O, Bărbulescu M, Ursuț B, Obrocea F. Gastrointestinal Stromal Tumors - Diagnosis and Surgical Treatment. *Chirurgia (Bucur).* 2015 Nov-Dec;110(6):525-9.
28. Alecu L, Tulin A, Ursuț B, Ursuț B, Oproiu A, Obrocea F, Ionescu M. Gastrointestinal stromal tumor with primary hepatic unique location--clinical case. *Chirurgia (Bucur).* 2011 Sep-Oct;106(5):677-81.
29. Middha S, Zhang L, Nafa K, Jayakumaran G, Wong D, Kim HR, Sadowska J, Berger MF, Delair DF, Shia J, Stadler Z, Klimstra DS, Ladanyi M, Zehir A, Hechtman JF. Reliable Pan-Cancer Microsatellite Instability Assessment by Using Targeted Next-Generation Sequencing Data. *JCO Precis Oncol.* 2017; 2017:PO.17.00084. doi: 10.1200/PO.17.00084
30. Cocco E, Benhamida J, Middha S, et al. Colorectal Carcinomas Containing Hypermethylated MLH1 Promoter and Wild-Type BRAF/KRAS Are Enriched for Targetable Kinase Fusions. *Cancer Res.* 2019 Mar;

- 79(6):1047-1053. doi: 10.1158/0008-5472.CAN-18-3126
31. Marchiò C, Scaltriti M, Ladanyi M, Iafrate AJ, Bibeau F, Dietel M, Hechtman JF, Troiani T, López-Rios F, Douillard JY, André F, Reis-Filho JS. ESMO recommendations on the standard methods to detect NTRK fusions in daily practice and clinical research. *Ann Oncol*. 2019 Sep 1;30(9):1417-1427. doi: 10.1093/annonc/mdz204
32. Motofei IG. Biology of Cancer; From Cellular Cancerogenesis to Supracellular Evolution of Malignant Phenotype. *Cancer Invest*. 2018;36(5):309-317. doi: 10.1080/07357907.2018.1477955
33. de Moor JS, Gray SW, Mitchell SA, Klabunde CN, Freedman AN. Oncologist Confidence in Genomic Testing and Implications for Using Multimarker Tumor Panel Tests in Practice. *JCO Precis Oncol*. 2020 Jun 11;4:PO.19.00338. doi: 10.1200/PO.19.00338
34. Tannock IF, Hickman JA. Molecular screening to select therapy for advanced cancer? *Ann Oncol*. 2019 May 1;30(5):661-663. doi: 10.1093/annonc/mdz088
35. Costea DO, Enache FD, Baz R, Suceveanu AP, Suceveanu AI, Ardeleanu V, Mazilu L, Costea AC, Botea F, Voinea F. Confirmed child patient with Covid-19 infection, operated for associated surgical pathology – first pediatric case in Romania. *Rom Biotechnol Lett*. 2020;25(6):2107-2110. doi: 10.25083/rbl/25.6/2107.2110
36. Meric-Bernstam F, Hurwitz H, Raghav KPS, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol*. 2019 Apr;20(4):518-530. doi: 10.1016/S1470-2045(18)30904-5
37. Sartore-Bianchi A, Trusolino L, Martino C, et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2016 Jun;17(6):738-746. doi: 10.1016/S1470-2045(16)00150-9
38. Yakirevich E, Resnick MB, Mangray S, et al. Oncogenic ALK Fusion in Rare and Aggressive Subtype of Colorectal Adenocarcinoma as a Potential Therapeutic Target. *Clin Cancer Res*. 2016 Aug 1;22(15):3831-40. doi: 10.1158/1078-0432.CCR-15-3000
39. Fabrizio DA, George TJ Jr, Dunne RF, et al. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. *J Gastrointest Oncol*. 2018 Aug;9(4):610-617. doi: 10.21037/jgo.2018.05.06
40. Juric D, Rodon J, Tabernero J, Janku F, et al. Phosphatidylinositol 3-Kinase α -Selective Inhibition With Alpelisib (BYL719) in PIK3CA-Altered Solid Tumors: Results From the First-in-Human Study. *J Clin Oncol*. 2018 May 1;36(13):1291-1299. doi: 10.1200/JCO.2017.72.7107
41. Li AY, McCusker MG, Russo A, Scilla KA, Gittens A, Arensmeyer K, Mehra R, Adamo V, Rolfo C. RET fusions in solid tumors. *Cancer Treat Rev*. 2019 Dec; 81:101911. doi: 10.1016/j.ctrv.2019.101911
42. Wang C, Jette N, Moussienko D, Bebb DG, Lees-Miller SP. ATM-Deficient Colorectal Cancer Cells Are Sensitive to the PARP Inhibitor Olaparib. *Transl Oncol*. 2017 Apr;10(2):190-196. doi: 10.1016/j.tranon.2017.01.007