This is a peer-reviewed, accepted author manuscript of the following research article: Mauri, D., Kamposioras, K., Matthaios, D., Tolia, M., Nixon, I., Dambrosio, M., Zarkavelis, G., Papadimitriou, K., Petricevic, B., Kountourakis, P., Kopecky, J., Kuhar, C. G., Popovic, L., Chilingirova, N. P., De Mello, R. A., Plavetić, N. D., Katsanos, K., Mostert, B., Alongi, F., ... Valachis, A. (2022). Next-generation sequencing of circulating tumor DNA can optimize second-line treatment in RAS wild-type metastatic colorectal cancer after progression on anti-EGFR therapy: time to rethink our approach. *Oncology Research and Treatment*, *45*(4), 216-221. https://doi.org/10.1159/000521845

Next generation sequencing of circulating tumor DNA can optimize second line treatment in RAS wild type metastatic colorectal cancer after progression on anti-EGFR therapy: time to rethink our approach…

Davide Mauri[1*], Georgios Zarkavelis[1],Konstantinos Kamposioras[2], MariaTolia[3], Joanna Nixon[4], MarioDambrosio[5], Konstantinos Papadimitriou[6], Branka Petricevic[7], Pantelis Kountourakis[8], Jindrich Kopecky[9], Cvetka Grašič Kuhar[10], Lazar Popovic[11], Nataliya P Chilingirova[12], Ramon Andrade De Mello[13,14], Natalija Dedić Plavetić[15], Konstantinos Katsanos[16], Bianca Mostert[17], Filippo Alongi[18,19], Berardino de Bari[20], Stefanie Corradini[21], Eleytherios Kampletsas[1], Ioanna Gazouli[1], Stefania Gkoura[1], Anna-Lea Amylidi[1], Antonios Valachis[22].

1) Department ofMedical Oncology, University of Ioannina, Greece.
2) Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, UK.
3) Department of Radiotherapy/Radiation Oncology, Faculty of Medicine, University of Crete, Heraklion, Greece.
4) Scottish Sarcoma Network (SSN)UK Chair NCRI HN Epidemiology and Survivorship subgroupThe Beatson West of Scotland Cancer Centre, Glasgow, UK.
5) Department of Medical Oncology, Clinica San Carlo, Paderno Dugnano, Milan, Italy.
6) University Hospital of Antwerp, Antwerp, Belgium.
7) Department of Hematology and Oncology, KlinikOttakring, Vienna, Austria.
8) Medical Oncology Department,Bank of Cyprus Oncology Centre,Nicosia, Cyprus.
9) Department of clinical oncology and radiotherapy, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic.
10) Department of Medical Oncology, Institute of Oncology Ljubljana, Slovenia.
11) Medical Oncology Department Oncology Institute of VojvodinaUniversity of Novi Sad Serbia.
12) Medical Oncology Clinic at Specialized Hospital for Active Treatment in Oncology, University Pleven, Sofia, Bulgaria.
13) Department of Biomedical Sciences & Medicine, University of Algarve, Faro, Portugal.
14) Precision Oncology Group, Federal University of São Paulo (UNIFESP), & Nine of July University (UNINOVE), São Paulo, Brazil.

15) University Hospital Centre Zagreb, Department of Oncology, University of Zagreb, School of Medicine, Zagreb, Croatia.
16) Departmentof Gastroenterology, University Hospital of Ioannina, Greece.
17) Department of Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, The Netherlands.
18) Advanced Radiation Oncology Department, IRCCSSacroCuore Don Calabria Hospital, Cancer Care Center, Negrar, Italy.
19) University of Brescia, Brescia, Italy.
20) Hospitalier Neuchâtelois, La Chaux-de-Fonds, Switzerland.
21) Department of Radiation Oncology, University Hospital, LMU Munich, Munich, Germany.
22) Department of Oncology, Faculty of Medicine and Health, Örebro University, Örebro, Sweden.

*** Corresponding Author**

Davide Mauri

Contact information:

Email address: dvd.mauri@gmail.com

Address: Department of Medical Oncology, School of Medicine, University of Ioannina, Ioannina, Greece.

Abstract

Management of *Ras* wild-type colorectal cancer (CRC) patients upon disease progression after the successful use of targeted treatment with anti-EGFR monoclonal antibodies and backbone chemotherapy remains a clinical challenge. Development of treatment resistance with prevalence of pre-existing *RAS* mutated clones, *RAS* mutation conversion, truncation of extracellular receptor domains as well as *HER2* and *MET* amplification are molecular events that can be difficult to follow without the use of sophisticated laboratory techniques. The clinical hurdle of re-biopsy and tumor heterogeneity can be overcome by the implementation Next Generation Sequencing (NGS) to analyze circulating tumor DNA (ctDNA) and identify drugable mutations or recovery of *RAS*-wildness. In this opinion paper we summarize with critical thinking the clinical approach to be followed after the failure of first line treatment in *Ras* wild-type CRC tumors with the use of NGS. Rechallenge with anti-EGFR inhibitors, in case of persistent or recovery of Ras wildness, and targeted approach of specific mutations (BRAF inhibitors) amplifications (anti-Her2 treatment) or fusion proteins (NTRK inhibitors) can by guided by the use of NGS. The use of NGS platforms for serial analysis of ctDNA is an important step to better understand the molecular landscape of metastatic colorectal cancer and guide clinical decisions. NGS should be considered a mainstay in clinical practice for the management of CRC patients and health authorities should consider reimbursing its use in the appropriate clinical settings.

Manuscript

Colorectal cancer is the third most diagnosed cancer worldwide and one of the most lethal among neoplasms [1]. The mainstay of treatment for metastatic colorectal cancer consists of backbone fluoropyrimidine based-chemotherapy along with targeted agents. Molecular profiling of metastatic colorectal cancer is applied upon diagnosis with tumors being tested for hotspot mutations in KRAS, NRAS, BRAF genes and also for MMR deficiency as those are prerequisites for therapy selection [2,3]. *RAS* mutationalstatus is an integral driver of the anti-EGFR targeted treatment since only colorectal cancers characterized as *RAS* wild type by molecular profiling derive significant benefit from anti-EGFR monoclonal antibody application (cetuximab, panitumumab)[4]. On the contrary, patients whose tumors are found to carry *RAS* mutations are not responsive to anti-EGFR targeted therapy and may also experience a detrimental effect upon administration of these agents [5].Thus the application of cytotoxic chemotherapy along with an anti-EGFR monoclonal antibody is the treatment of choice in *RAS* wild type metastatic colorectal cancer (approximately 60% of patients), particularly for tumors that are anatomically left sided [2,3]. The same applies in cases where no *BRAF* mutations are discovered with evidence showing that BRAF mutant tumors derive no benefit and are resistant to anti-EGFR therapies [6].

Although this treatment strategy is effective in more than half of the patients with *RAS*-wild type colorectal cancer, the disease will inevitably progress during its course. Thus, the question arises on how to treat patients upon progression, after the initial successful first line combination of anti-EGFR agents with chemotherapy. Some questions to be addressed are: should the anti-EGFR targeted therapy be reintroduced and when? What are the mechanisms of resistance? In which cases should the targeted agents be changed and to what? Which factors should guide the optimal treatment selection?

Recently, a small randomized trial and other phase II studies showed that there are patients with metastatic colorectal cancer, who progressed after the initial clinical benefit on anti-EGFR treatment, may further benefit from re-challenge anti-EGFR treatment [7].

Nonetheless, in other patients, the exposure of cancer cells to anti-EGFR targeted therapy ultimately leads to acquired treatment resistance, probably due to pre-existing *RAS* mutated clones[8] or *RAS* mutation conversion that may occur in up to 48% of patients over the course of treatment [9]. Resistant *RAS* mutated clones may indeed prevail under the selective pressure of applied anti-EGFR targeted therapy while *RAS* wild type clones are being suppressed.

Development of resistance during the course of anti-EGFR treatment may also be explained by the occurrence of other molecular events such as KRAS truncated extracellular domains, *HER2* and *MET*amplification, as well as *PIK3CA* mutations [2,8,10]. Many of these molecular pathways are actually targetable. However, there is also evidence that anti-EGFR resistant cancer clones may decay exponentially during the course of time or during the exposure to non-anti-EGFR treatment [8,11].

The queries on when to recall an anti-EGFR agent, when to use an alternative molecular treatment strategy and which targeted therapy to choose, need to be answered for patients with disease progression.

The initial histological assessment of *RAS* status might not be representative or useful for therapeutic decision-making in further lines of treatment. It is pertinent to consider if a repeat tissue biopsy should be suggested to obtain an updated molecular profile.

Submitting a patient with metastatic disease to serial tissue biopsies is not always feasible, and in general invasive procedures are needed with the potential risk of complication while the appropriate infrastructure should be available. However, the major question that emerges is whether the obtained tissue can be considered representative of the overall metastatic burden of disease, as tissue heterogeneity is a well recognized pitfall of this approach [12].

The advent of liquid biopsies may be a game changer in this setting. Circulating tumor DNA (ctDNA) is a small fraction of the total free DNA found in the peripheral blood of patients with metastatic cancer [13]. Direct and real time genetic information regarding the tumor can be obtained through a single blood aspiration and subsequent molecular analysis [14].

Metastatic colorectal cancers have a high prevalence of ctDNA and in some cases the percentage of detection is almost 100% [14]. Thus, ctDNA analysis might represent more accurately the tumor heterogeneity rather than a tissue biopsy of a single metastatic site. Concordance between tumor tissue and plasma ctDNA for RAS status ranges between 78% and 85% [15,16]. Approximately 7.5% - 9% of RAS wild-type cases by tissue analysis could be RAS mutant by ctDNA analysis [16,17]. Changes in ctDNA levels during the course of treatment is significantly correlated with tumor shrinkage, and patients with a ctDNA decrease by 80% or more after treatment have been reported to have longer progression-free survival [16].

Moreover, considering the high cost of targeted anti-EGFR treatments, the use of ctDNA Next Generation Sequencing (NGS) may be considered as a valuable cost-effective tool for patient

monitoring both during and after the courseof anti-EGFR therapy since it may promptly identify tumor mutations that will render the anti-EGFR administration vain.

NGS analyses of ctDNA also give the possibility to unearth new targetable gene mutations across patients displaying either primary or delayed resistance due to molecular conversion after the exposure to an anti-EGFR line of treatment. Throughout the literature, there is limited evidence regarding this hypothesis. Most studies comprise of small patient series, limiting the validity of their results. However, the same conclusion is drawn in most of them[9, 12, 18-20].

In fact, a recent meta-analysis of four small trials (E-RECHALLENGE, CRICKET, JACCRO CC-08, JACCRO CC-09)[9,18-21] [Table] showed that anti-EGFR rechallenge may be successfully reintroduced in further lines of treatment among patients who continue to display RAS wild type profile, when compared to patients experiencing tumor molecular conversion. Anti-EGFR re-challenge treatment among patients with plasma ctDNA RAS wild type status was associated with a consistent benefit in progression free survival (HR = 0.40, 95% CI 0.22-0.70; p = 0.001) and overall survival (HR 0.37, 95% CI 0.16-0.85; p = 0.02) when compared to its use among patients with plasma ctDNA RAS mutation, and ctDNA RAS wild type patients also performed statistically better in term of disease control rate, risk for disease progression and risk for death at different time points analyzed [21]. This is of particular importance considering that half of the anti-EGFR pretreated patients may display *RAS* wildness directly after progression so long as they are onanti-EGFR treatment holiday for a period of time of about four to six months [8, 11,22].

Thus, the burning question is what to do with the remaining fifty percent of patients not recovering from a molecular wildness. Circulating tumor DNA analysis with NGS techniques bridges the gap in the knowledge of the molecular signature of potentially targetable tumor through time lapse offering analysis of a broad panel of genes with cost-effectiveness and in a time saving manner. The improved sensitivity of contemporary ctDNA sequences devices [Illumina HiSeq 2500, , Ion torrent, Roche GS junior, Roche GS FLX, Qiagen GeneReader etc.] provides a significant tool for the identification of newly acquired mutations or loss of known ones. Hence, these platforms can further guide the therapeutic decision with the timely identification of potentially targetable mutations such as BRAF*, HER2*, MET or even *PIK3CA* [23].

BRAF Mutations: BRAF mutated tumors display an aggressive and rapidly lethal course of disease, chemoresistance and anti-EGFR resistancedespite RAS wildness. [6]. BRAF-inhibition

by itself has had disappointing results, but combining BRAF-inhibition with EGFR-inhibition may improve outcome. The BEACON trial recently underscored the overall survival benefit of combined treatment with encorafenib plus cetuximab and binimetinib among patients with BRAF V600 mutation [24].

HER2-gene amplification: colorectal cancer patients having HER2-gene amplification also display poor disease course and express a marked resistance toanti EGFR antibody therapy [25]. Its identification is of importance since anti-HER2treatment with trastuzumab, pertuzumab, lapatinib, T-DM1, trastuzumab-deruxtecan, or tucatinib has demonstrated an ability to change the course of the diseases in the recent Heracles, Heracles-B, MyPatway, TRIUMPH, MOUNTAINER, DESTINY trials[26-31] and could be offered to patients in the context of clinical trials.

Mismatch repair deficiency / microsatellite instability (dMMR/MSI-h): The subset of patients harboring dMMR (MLH1, MSH2, MSH6, PMS2) /MSI high and those with Lynch syndrome derives a substantial progression-free survival benefit from the use of immunotherapy with pembrolizumab in Keynote-177 trial [32], and early promising results are also available from the use of ipilimumab and nivolumab combination in Check Mate-142 study [33].

Neurotrophin receptor kinase (NTRK) fusion: Positive results in term of disease control have been evidenced even among the small number of patients enrolled in the studies investigating entrectinib and larotrectinib, which are highly selective NTRK inhibitors, opening a new field of research for metastatic colorectal cancer [34,35].

Drug tailored to *PIK3CA* and *KRAS G12C* mutations are actually available and are under evaluation offering a new potentially promising field for the treatment of resistant disease.

According to the European Society for Medical Oncology scale of clinical actionability for molecular targets (ESCAT), which defines six levels of clinical evidence for molecular targets, patients with these molecular targets are classified as tier II (investigational drugs, magnitude of tumor activity is not known) and tier III (data are available in other tumor types). They are likely to benefit from a targeted drug. However, the treatment should be considered within a prospective clinical registry or a prospective clinical trial, ensuring the analysis and publishing of the outcome results. [36]

Nonetheless, the way we should interpret allele frequencies of relevant mutations found in ctDNA, their clinical relevance and the positivity thresholds need to be clarified. Would a low allele frequency of Ras mutations be sufficient to re-initiate anti-EGFR therapy? What about

the clinical significance of each mutation found? Many questions about the clinical application of NGS technologies are still unanswered and represent food for thought and evolving research field.

In conclusion, ctDNA NGS molecular profiling among *RAS* wild type colorectal cancer patients experiencing progression during or after anti-EGFR treatment should be considered a mainstay inclinical practice, since it canidentify both patients who may benefit from an anti-EGFR rechallenge but also new targetable treatment pathways for those patients displaying *RAS* conversion or primary anti-EGFR resistance. The use of NGS platforms for serial analysis of ctDNA is also an important step for better comprehension of the molecular landscape of metastatic colorectal cancer. It might shed light upon everyday clinical practice queries regarding the optimal treatment choice and sequence of available therapies.

References

[1]. Global Cancer Observatory. Retrieved from https://gco.iarc.fr/

[2].Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer special articles Annals of Oncology. Ann Oncol.2016;27:1386–1422.

[3].Yoshino T, Arnold D, Taniguchi H, et al. Pan-Asian adapted ESMO consensus guidelines for the management of patients with metastatic colorectal cancer: a JSMO-ESMO initiative endorsed by CSCO, KACO, MOS, SSO and TOS. Ann Oncol. 2018; 29(1):44-70. doi: 10.1093/annonc/mdx738.

[4]. Van Cutsem E, Lenz HJ, Köhne CH, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J ClinOncol. 2015; 33(7):692-700. doi: 10.1200/JCO.2014.59.4812.

[5]. DouillardJY, Siena S, Cassidy J, et al. Final resultsfrom PRIME: randomized phase III study of panitumumabwith FOLFOX4 for first-line treatment of metastatic colorectal cancer. Ann Oncol. 2014;25(7):1346-1355. doi: 10.1093/annonc/mdu141.

[6]. Pietrantonio F, Petrelli F, Coinu A, et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. Eur J Cancer.2015; 51: 587–594.

[7]. Mauri G, PizzutiloEG, Amatu A, et al. Retreatment with anti-EGFR monoclonal antibodies in metastatic colorectal cancer: Systematic review of different strategies. Cancer Treat Rev. 2019;73:41-53. doi: 10.1016/j.ctrv.2018.12.006.

[8].Morelli MP, Overman MJ, Dasari A, et al. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. Ann Oncol. 2015;26(4):731–736.

[9].Cremolini C, Rossini D, Dell'Aquila E, et al. Rechallenge for Patients With RAS and BRAF Wild-Type Metastatic Colorectal Cancer WithAcquiredResistance to First-line Cetuximab and Irinotecan: A Phase 2 Single-Arm Clinical Trial. JAMAOncol. 2019;5(3):343-350. doi: 10.1001/jamaoncol.2018.5080.

[10].Tougeron D, Lecomte T, Pages JC, et al. Effect of low-frequency KRAS mutations on the response to anti-EGFR therapy in metastatic colorectal cancer. Ann Oncol . 2013;24(5):1267–1273.

[11]. Parseghian CM, Loree JM, Morris VK, et al. Anti-EGFR-resistant clones decay exponentially after progression: implications for anti-EGFR re-challenge.Ann Oncol. 2019;30(2):243-249.

[12]. Boonstra PA, Wind TT, van Kruchten M, et al. Clinical utility of circulating tumor DNA as a response and follow-up marker in cancer therapy. Cancer Metastasis Rev. 2020; 39(3):999-1013. doi: 10.1007/s10555-020-09876-9.

[13]. Diaz LA, Bardelli A. Liquid Biopsies: Genotyping Circulating Tumor DNA. J ClinOncol . 2014;32(6):579–586.

[14]. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. SciTransl Med. 2014; 6(224):224ra24. doi: 10.1126/scitranslmed.3007094.

[15] Normanno, N. *et al.* RAS testing of liquid biopsy correlates with the outcome of metastatic colorectal cancer patients treated with first-line FOLFIRI plus cetuximab in the CAPRI-GOIM trial. *Ann. Oncol.* **29**, 112–118

[16] Hsu, H. C. *et al.* Targeted sequencing of circulating tumor DNA to monitor genetic variants and therapeutic response in metastatic colorectal cancer. *Mol. Cancer Ther.* **17**, 2238–2247

[17] Lim, Y., Kim, S., Kang, JK. *et al.* Circulating tumor DNA sequencing in colorectal cancer patients treated with first-line chemotherapy with anti-EGFR. *Sci Rep* **11,** 16333 (2021).

[18]. Tsuji A, Eto T, Masuishi T, et al. Phase II study of third-line cetuximabrechallenge in patients with metastatic wild-type K-RAS colorectal cancer who achieved a clinical benefit in response to first-line cetuximab plus chemotherapy (JACCRO-08). Ann Oncol. 2016; 27 (Suppl 6): vi149–vi206. doi:10.1093/annonc/mdw370.58.

[19]. Tsuji A, Nakamura M, Watanabe T, et al. Phase II study of third-line panitumumabrechallenge in patients with metastatic wild-type KRAS colorectal cancer who achieved a clinical benefit in response to first-line panitumumab plus chemotherapy. (JACCRO-09) Ann Oncol. 2018;29 (suppl 5):v68–v69,P-243. doi.org/10.1093/annonc/mdy151.242.

[20].Osawa H, Shinozaki E, Nakamura M, et al. Phase II study of cetuximab rechallenge in patients with ras wild-type metastatic colorectal cancer: E-rechallenge trial. Ann Oncol. 2018;29(Suppl 8):viii161. doi.org/10.1093/annonc/mdz338.112.

[21] Vlachou MS, Mauri D, Zarkavelis G, Ntellas P, Tagkas C, Gkoura S, Pentheroudakis G. Plasma ctDNA RAS status selects patients for anti-EGFR treatment rechallenge in metastatic

colorectal cancer: a meta-analysis. Exp Oncol. 2021 Sep;43(3):252-256. doi: 10.32471/exp-oncology.2312-8852.vol-43-no-3.16592. PMID: 34591420.

[22]. Toledo RA, Cubillo A, Vega E, et al. Clinical validation of prospective liquid biopsy monitoring in patients with wild-type RAS metastatic colorectal cancer treated with FOLFIRI-cetuximab. Oncotarget . 2017;8(21):35289–35300.

[23]. Kastrisiou M, Zarkavelis G, Pentheroudakis G, Magklara A. Clinical Application of Next-Generation Sequencing as A Liquid Biopsy Technique in Advanced Colorectal Cancer: A Trick or A Treat? Cancers (Basel). 2019;11(10):1573.

[24]. Kopetz S, Grothey A, Yaeger R, et al. Encorafenib, Binimetinib, and Cetuximab in *BRAF* V600E-Mutated Colorectal Cancer. N Engl J Med. 2019;381(17):1632-1643. doi: 10.1056/NEJMoa1908075.

[25]. Sartore-Bianchi A, Amatu A, Porcu L, et al. HER2 Positivity Predicts Unresponsiveness to EGFR-Targeted Treatment in Metastatic Colorectal Cancer. Oncologist. 2019;24(10):1395-1402. doi: 10.1634/theoncologist.2018-0785.

[26].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;17(6):738-746. doi: 10.1016/S1470-2045(16)00150-9. Epub 2016 Apr 20. Erratum in: Lancet Oncol. 2016 Oct;17 (10):e420. PMID: 27108243.

[27]. Sartore-Bianchi A, Lonardi S, Martino C, et al. Pertuzumab and trastuzumabemtansine in patients with HER2-amplified metastatic colorectal cancer: the phase II HERACLES-B trial. ESMO Open. 2020;5(5):e000911. doi: 10.1136/esmoopen-2020-000911.

[28]. Meric-Bernstam F, Hurwitz H, RaghavKPS, 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;20(4):518-530. doi: 10.1016/S1470-2045(18)30904-5.

[29]. Siena S, Di Bartolomeo M, Pratap Singh Raghav K, et al. A phase II, multicenter, open-label study of trastuzumabderuxtecan (T-DXd; DS-8201) in patients (pts) with HER2-expressing metastatic colorectal cancer (mCRC): DESTINY-CRC01. ASCO Virtual Meeting, 29-31 May 2020. J ClinOncol 38: 2020 (suppl; abstr 4000).doi:10.1200/JCO.2020.38.15_suppl.4000.

[30]. Strickler JH, Zemla T,Ou F-S, et al. Trastuzumab and tucatinib for the treatment of HER2 amplified metastatic colorectal cancer (mCRC): Initial results from the MOUNTAINEER trial. Annals of Oncology (2019) 30 (suppl_5): v198-v252. DOI:https://doi.org/10.1093/annonc/mdz246.005.

[31]. Nakamura Y, Okamoto W, Sawada K, et al.TRIUMPH Study: A multicenter phase II study to evaluate efficacy and safety of combination therapy with trastuzumab and pertuzumab in patients with HER2-positive metastatic colorectal cancer (EPOC1602). Ann Oncol. (2017) 28 (suppl_5): v158-v208. 10.1093/annonc/mdx393

[32]. Andre T, ShiuKK, Kim TW, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high/mismatch repair deficient metastatic colorectal cancer: The phase 3 KEYNOTE-177 study.J ClinOncol 38: 2020 (suppl; abstr LBA4).

[33]. Lenz H-J, Lonardi S, Zagonel V, et al: Nivolumab plus low-dose ipilimumab as first-line therapy in microsatellite instability-high/DNA mismatch repair deficient metastatic colorectal cancer: Clinical update. JCO 2020 38:4_suppl, 11-11

[34]. Doebele RC, Drilon A, Paz-Ares L, et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials. Lancet Oncol. 2020 Feb;21(2):271-282. doi: 10.1016/S1470-2045(19)30691-6. Epub 2019 Dec 11. Erratum in: Lancet Oncol. 2020 Feb;21(2):e70. Erratum in: Lancet Oncol. 2020 Jul;21(7):e341. Erratum in: Lancet Oncol. 2020 Aug;21(8):e372.

[35]. Hong DS, DuBois SG, Kummar S, et al. Larotrectinib in patients with TRK fusion-positive solid tumours: a pooled analysis of three phase 1/2 clinical trials. Lancet Oncol. 2020;21(4):531-540. doi: 10.1016/S1470-2045(19)30856-3.

[36].Mateo J, Chakravarty D, Dienstmann R, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol. 2018;29(9):1895-1902. doi: 10.1093/annonc/mdy263.