Am J Transl Res 2023;15(9):5785-5790
www.ajtr.org /ISSN:1943-8141/AJTR0152053

Case Report

Precision oncology strategy in cetuximab-resistant ERRFI1-mutant colon cancer: case report of disease progression on afatinib

Olesya Kuznetsova[1,2], Alexandra Lebedeva[1], Alexandra Kavun[1], Ekaterina Belova[1,3], Mikhail Fedyanin[2,4,5], Vladislav Mileyko[1], Maxim Ivanov[1,6], Dmitry Nosov[7]

[1]OncoAtlas LLC, 119049 Moscow, Russia; [2]N.N. Blokhin Russian Cancer Research Center, 115478 Moscow, Russia; [3]Lomonosov Moscow State University, 119991 Moscow, Russia; [4]State Budgetary Institution of Health Care of The City of Moscow "Moscow Multidisciplinary Clinical Center Kommunarka" of The Department of Health of The City of Moscow, 142770 Moscow, Russia; [5]Federal State Budgetary Institution "National Medical and Surgical Center Named After N.I. Pirogov" of The Ministry of Health of The Russian Federation, 105203 Moscow, Russia; [6]Moscow Institute of Physics and Technology, 141701 Dolgoprudny, Russia; [7]The Central Clinical Hospital of The Administrative Directorate of The President of The Russian Federation, 121359 Moscow, Russia

Received July 5, 2023; Accepted September 11, 2023; Epub September 15, 2023; Published September 30, 2023

Abstract: Despite the existence of effective first and second line therapy options for patients with colorectal cancer, heavily treated patients have limited additional therapies. Genomic profiling is a promising tool for guiding subsequent treatment selection. Here, we describe the results of treating a colorectal cancer patient with molecularly-matched therapy based on the results of genomic profiling. The patient received a combination of afatinib and bevacizumab due to the presence of ERRFI1 variant. To our knowledge, this is the first report on the effect of EGFR inhibitors in patients with ERRFI1-altered RAS/BRAF wild-type colorectal adenocarcinoma.

Keywords: Colorectal cancer, ERRFI1 mutation, EGFR TKI therapy, precision medicine, molecular tumor board

Introduction

Colorectal cancer (CRC) is one of the leading causes of death worldwide, accounting for 6% of all cancer-related deaths. Over a million cases are diagnosed every year [1]. While localized CRC is associated with a relatively good prognosis and a 90% 5-year survival, prognosis for patients with metastatic disease is poor, with a 5-year survival rate of 14% [1].

Systemic therapy for advanced or metastatic CRC includes chemotherapy, targeted therapy, and immune checkpoint inhibitors. Targeted agents used for the treatment of CRC include anti-EGFR monoclonal antibodies, HER2 inhibitors, BRAF and MEK inhibitors, and immune checkpoint inhibitors include anti-PD-1/PD-L1 monoclonal antibodies. EGFR inhibitors, specifically anti-EGFR monoclonal antibodies, play a central role in the treatment of CRC, and are indicated on the basis of specific molecular characteristics of the tumor. EGFR-directed monoclonal antibodies cetuximab and panitumumab, in combination with chemotherapy, are indicated for the treatment of advanced RAS/BRAF wild-type CRC. EGFR tyrosine kinase inhibitors, on the other hand, are not routinely used for treating CRC. Other standard of care therapeutic agents are indicated based on the presence of BRAF V600E, MSI, or the tumor mutational burden [2]. Tumor-agnostic targeted therapy, including TRK inhibitors for NTRK fusion-positive tumors, or specific RET inhibitors for RET fusion-positive tumors, may also be used. However, fusions are rarely detected in CRC [3].

The MAPK, WNT, and PI3K signaling pathways are the most frequently upregulated pathways in CRC. Potentially targetable alterations in the KRAS, PIK3CA, AKT, ERBB2, MET genes, as well as mutations in genes involved in homologous recombination can be found in metastatic CRC

[3]. ERRFI1 is a negative regulator of the EGFR family [4]. Mutations in this gene are infrequent across all solid tumors, including CRC. *In vitro* studies and several case reports have suggested that EGFR inhibitors may be effective against ERRFI1 mutations in several cancer types [5-7]. However, the effect of EGFR inhibitors against CRCs harboring mutations in ERRFI1 has not been studied.

Here, we report a case of progressive disease following EGFR tyrosine kinase inhibitor therapy combined with bevacizumab in a patient with metastatic colorectal adenocarcinoma harboring a suspected deleterious variant in the ERRFI1 gene. The investigators obtained a written statement of informed consent from the patient prior to publication.

Case description

A 30-year-old male patient was diagnosed with a stage IV CRC (sigmoid) in August 2021. At the time of diagnosis, CT imaging identified massive carcinomatosis, and bowel obstruction was suspected. The patient underwent omentectomy and preventive colostomy. During surgery multiple lesions of colon adenocarcinoma up to 5 mm over the surface of parietal and visceral peritoneum were observed. Histology revealed a poorly differentiated mucinous adenocarcinoma - microsatellite stable (MSS), KRAS/NRAS/BRAF wild-type. The patient started first line chemotherapy with cetuximab combined with FOLFOXIRI in September 2021. Stable disease (SD) according to Response Criteria for Solid Tumors (RECIST) version 1.1 was achieved at a maximum effect after 4 cycles. No clinically significant adverse effects were observed during 12 cycles of therapy. In November 2021, with the aim of broadening possible treatment options, comprehensive genomic profiling (FoundationOne CDx, Foundation Medicine, MA, USA) was performed using the patient's histologic samples (peritoneal metastasis). As the patient had an ongoing response to treatment, no additional decisions were made at the time.

In March 2022, CT revealed enlargement of peritoneal lesions and an increase of effusion in the abdominal cavity. These changes met the RECIST 1.1 criteria for disease progression (PD). Subsequently, the patient was referred to a local Molecular Tumor Board (MTB) to discuss further treatment strategy. Genomic profiling revealed no oncogenic mutations in KRAS, NRAS or BRAF, consistent with the initial results. The following alterations were identified: MYC amplification (equivocal), RICTOR amplification, TP53 splicing mutation (c.559+1G>A), ERRFI1 p.P320L, ESR1 p.H6Y, MCL1 p.V146L, RAD21 amplification, SETD2 p.G2299R, as well as TSC2 p.E92V. The tumor mutational burden was low (3 mutations per megabase), and the tumor was microsatellite stable, as estimated by NGS. MYC amplifications are possible targets for CDK4/6 inhibitors when combined with PARP inhibitors based on *in vitro* studies [8]; but since the amplification was equivocal, this biomarker was not further discussed. RICTOR amplifications have long been proposed to be associated with efficacy for mTOR inhibitors, but the results of this approach have been discouraging [9]. RICTOR amplification is considered ESCAT tier IV alteration [10]. Notably, both MYC and RICTOR amplifications may be associated with resistance to EGFR-targeting agents; however, the evidence supporting this notion is limited. SETD2 alterations can be targeted by AKT or immune checkpoint inhibitors [11, 12], but the variant observed was of unknown clinical significance. Thus, this alteration was designated ESCAT X in the context of AKT and immune checkpoint inhibitors. The TSC2 missense variant was considered of possible germline origin; the variant was classified as benign due to high frequency in the general population, and therefore was considered a tier X alteration [13].

Another alteration observed was a ERRFI1 missense variant p.Pro320Leu. Variant allele frequency (VAF) was not reported, and therefore it could not be reliably established whether the variant was somatic or germline. The variant has not been functionally characterized in the literature, nor has it been described in any knowledge base. This alteration affects the EGFR/ERBB binding domain (EBD) of ERRFI1, which interacts with the tyrosine kinase domain of EGFR/ERBB [14]. The position was predicted to be highly conserved across species. Moreover, the alteration was predicted to be deleterious by several in silico predictors (SIFT, PolyPhen, CADD, PrimateAI, VEST4; SIFT - 0, PolyPhen - 1, CADD - 25.7). Therefore, it was considered as a POTENTIALLY loss-of-function deleterious variant. Since no other promising

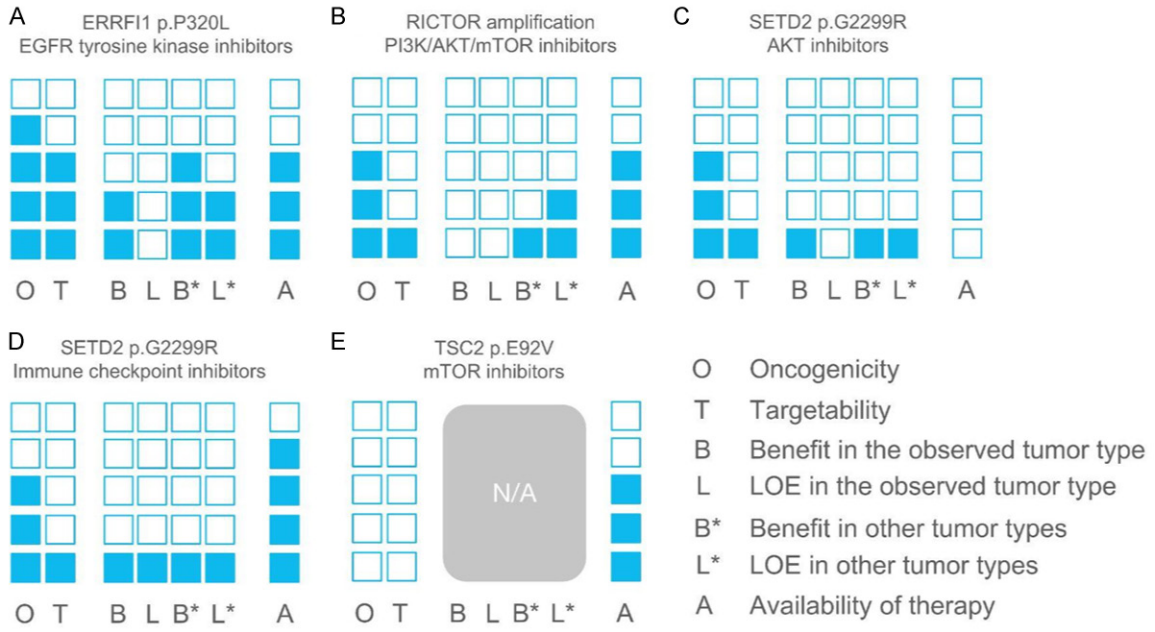

Figure 1. Evidence blocks that support the use of EGFR TKI for this patient (A), and evidence blocks for other potentially targetable alterations discussed within the Molecular Tumor Board (B-E). Columns O and T reflect the molecular biologist's overall opinion, and are as follows: O - Level of confidence of activating/deleterious (for oncogenes/tumor suppressor genes respectively) effect of the alteration, and T - Feasibility of targeting this biomarker for this patient. Columns B-L* reflect the knowledge base supporting rationale for targeting this biomarker based on published data (including results of clinical studies, retrospective studies, case reports, and biological evidence). Specifically, the columns are as follows: B - Anticipated magnitude of benefit for the biomarker-drug pair in patients' tumor type; L - Level of evidence supporting biomarker-drug pair in patient's tumor type; B* - Best magnitude of benefit for the biomarker-drug pair anticipated in a different tumor type; L* - Level of evidence supporting biomarker-drug pair in a different tumor type. Finally, column A represents the availability of the drug, considering the on-label and off-label indications for use, as well as available clinical trials.

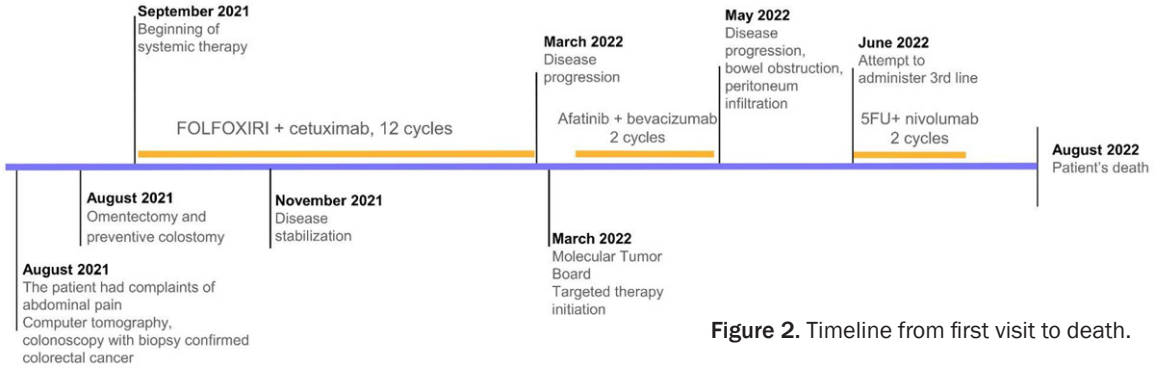

Figure 2. Timeline from first visit to death.

alterations were detected following genomic profiling (**Figure 1**), the MTB suggested to target the ERRFI1 alteration by pan-ERBB inhibitors (such as afatinib, neratinib, etc.) in combination with the anti-VEGF monoclonal antibody bevacizumab. This alteration-drug pair was designated ESCAT IV.

The patient started afatinib + bevacizumab treatment in April 2022, but in May 2022 he presented with bowel obstruction. During exploratory laparotomy and ileostomy, total infiltration of peritoneum was observed, and disease progression was confirmed. After his general condition improved, the patient started another line of systemic treatment with 5-fluorouracil and nivolumab. However, following the second cycle of this regimen, the patient quickly deteriorated and passed away shortly (**Figure 2**).

Table 1. Summary of previously published case reports supporting the rationale for targeting ERRFI1 alterations by EGFR-directed targeted therapy

Tumor type	Drug	Therapy effect	Source
Cholangiocarcinoma	Erlotinib	PR (-58%)	[5]
NSCLC (wt for other common driver mutations)	1. EGFR TKI (not specified) 2. EGFR mAb	1. PR 2. Secondary response	[7]
NSCLC (wt for other common driver mutations)	EGFR TKI (not specified)	PR	[7]

PR - partial response, NSCLC - non-small cell lung cancer, EGFR - epidermal growth factor receptor, TKI - tyrosine kinase inhibitor, mAb - monoclonal antibodies, wt - wild type.

Discussion

Here, we report a case of unsuccessful molecularly-matched treatment of a patient with colorectal adenocarcinoma with a suspected deleterious variant of the ERRFI1 gene predicted to be deleterious by multiple computational algorithms.

NGS produces large amounts of genomic data, which are complex and requires thorough interpretation of the findings. While some of those findings have a strong predictive effect in relation to certain antitumor therapies, the use of others requires further validation. In oncology, rare genomic findings, such as ERRFI1 loss of function mutations, represent an exciting area for the investigation of treatment efficacy from both a biological and clinical standpoint.

ERRFI1 (also known as MIG6, RALT or Gene 33) is a negative regulator of the EGFR family. ERRFI1 binds directly to EGFR, therefore inhibiting EGFR catalytic activity and hindering dimerization. Additionally, ERRFI1 inhibits autophosphorylation of EGFR and ERBB2, and mediates lysosomal degradation of EGFR [15, 16]. Other studies show that ERRFI1 acts as an immediate early response gene induced by growth factors, including EGF, suggesting its likely tumor suppressor role [17]. Since this gene encodes for a negative regulator of the EGFR signaling pathway, we hypothesized that loss of function alterations of the ERRFI1 gene could be associated with a benefit of EGFR-targeting therapy. This association was supported by *in vitro* studies [6], as well a handful of case reports [5, 7] (**Table 1**). CRC is frequently EGFR-dependent due to high prevalence of EGFR overexpression [18], giving the additional rationale for the application of EGFR-targeting therapy. Taken together, these considerations guided treatment selection for this patient.

Our patient's case highlights the importance of correct representation of genomic findings in genomic profiling reports. The ERRFI1 p.P320L variant was found in the 'Variants of unknown significance' section of the report, along with several other variants that were designated irrelevant from a clinical standpoint. Variant allele frequencies, as well as other technical features essential for correct interpretation, such as sequencing coverage, were not reflected in the original genomic profiling report. Additionally, somatic/germline origin was not reported for any of the variants, thus complicating further decision making. While tumor-only sequencing does not allow for precise determination of variant origin, computational tools, as well as additional factors such as population frequency and representation in specific knowledge bases allow for high-confidence prediction regarding variant origin (germline or somatic) [19].

Several hypotheses might be generated in relation to why our patient did not benefit from the treatment with afatinib. While EGFR-targeting monoclonal antibodies significantly improve outcomes of metastatic CRC patients, treatment with EGFR tyrosine kinase inhibitors generally fails to improve progression-free survival and response rates in KRAS wild type metastatic CRC, and is associated with increased toxicity [20]. Different alterations in EGFR, and possibly in EGFR-related genes, could, however, influence treatment outcome. Mutations in the EGFR gene preventing cetuximab binding [21] have been reported as a mechanism of acquired, but not primary, resistance to cetuximab. Other studies have suggested that CRC patients harboring EGFR mutations might benefit from EGFR-directed therapy that is routinely used in clinical practice [22]. When analyzing population frequency of this variant, we found that this variant was present in major popula-

tion databases (such as gnomAD, ExAC, etc.) with a frequency of 0.000284. This could indicate that this variant may be of germline origin. To further understand whether the fact that this variant occurs in the general population is enough to declare this variant germline, we analyzed population frequencies of other ERRFI1 mutations that were found in the MSK-IMPACT project (59 unique variants in total) (accessed by cBioPortal). 16 of 59 (27%) variants have been previously detected and were classified as germline variants in various population projects, suggesting that this variant could indeed be somatic. Nonetheless, the information regarding variant origin should clearly be reflected in the original genomic report for every detected variant, irrespective of clinical relevance.

Conclusion

This case suggests that EGFR-targeting therapy may be ineffective for RAS wild-type colorectal cancer patients in the presence of ERRFI1 mutations. To our knowledge, this is the first report of the effect of EGFR inhibition in patients with ERRFI1-altered colorectal adenocarcinoma.

Disclosure of conflict of interest

Olesya Kuznetsova, Alexandra Lebedeva, Alexandra Kavun, Ekaterina Belova, Vladislav Mileyko and Maxim Ivanov are currently employed by OncoAtlas LLC.

Address correspondence to: Alexandra Lebedeva, OncoAtlas LLC, 1/4A Leninsky Ave., 119049 Moscow, Russian. E-mail: lebedeva.alexandra.2015@post.bio.msu.ru

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-249.

[2] National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Colon Cancer Version 2.2022 - October 27, 2022. Accessed 21 November 2022.

[3] Mosele F, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP, Normanno N, Scarpa A, Robson M, Meric-Bernstam F, Wagle N, Stenzinger A, Bonastre J, Bayle A, Michiels S, Bièche I, Rouleau E, Jezdic S, Douillard JY, Reis-Filho JS, Dienstmann R and André F. 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: 1491-1505.

[4] Reschke M, Ferby I, Stepniak E, Seitzer N, Horst D, Wagner EF and Ullrich A. Mitogen-inducible gene-6 is a negative regulator of epidermal growth factor receptor signaling in hepatocytes and human hepatocellular carcinoma. Hepatology 2010; 51: 1383-90.

[5] Borad MJ, Champion MD, Egan JB, Liang WS, Fonseca R, Bryce AH, McCullough AE, Barrett MT, Hunt K, Patel MD, Young SW, Collins JM, Silva AC, Condjella RM, Block M, McWilliams RR, Lazaridis KN, Klee EW, Bible KC, Harris P, Oliver GR, Bhavsar JD, Nair AA, Middha S, Asmann Y, Kocher JP, Schahl K, Kipp BR, Barr Fritcher EG, Baker A, Aldrich J, Kurdoglu A, Izatt T, Christoforides A, Cherni I, Nasser S, Reiman R, Phillips L, McDonald J, Adkins J, Mastrian SD, Placek P, Watanabe AT, Lobello J, Han H, Von Hoff D, Craig DW, Stewart AK and Carpten JD. Integrated genomic characterization reveals novel, therapeutically relevant drug targets in FGFR and EGFR pathways in sporadic intrahepatic cholangiocarcinoma. PLoS Genet 2014; 10: e1004135.

[6] Hou J, Bhinge KN and Wang L. Abstract 2782: the effect of ERRFI1 as a novel AKT regulator on cell proliferation and response to therapy is cell context dependent. Cancer Res 2014; 74: 2782.

[7] Schneider J, Jain P, Schalper K, Lovly C, Gardner F, Ross J, Schrock A, Ali S, Miller V and Velcheti V. MA 15.06 ERBB receptor feedback inhibitor-1 alterations in non-small cell lung cancer. J Thorac Oncol 2017; 12: S1863.

[8] Yi J, Liu C, Tao Z, Wang M, Jia Y, Sang X, Shen L, Xue Y, Jiang K, Luo F, Liu P and Cheng H. MYC status as a determinant of synergistic response to olaparib and palbociclib in ovarian cancer. EBioMedicine 2019; 43: 225-237.

[9] Zhao D, Jiang M, Zhang X and Hou H. The role of RICTOR amplification in targeted therapy and drug resistance. Mol Med 2020; 26: 20.

[10] Mateo J, Chakravarty D, Dienstmann R, Jezdic S, Gonzalez-Perez A, Lopez-Bigas N, Ng CKY, Bedard PL, Tortora G, Douillard JY, Van Allen EM, Schultz N, Swanton C, André F and Pusztai L. 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: 1895-1902.

[11] Chen Y, Zheng X, Xiong J, Guan Y, Li Y, Gao X, Lin J, Fei Z, Chen L, Chen L, Chen G, Yi X, Cao W, Ai X, Zhou C, Li X, Zhao J, Yan X, Yu Q and Chen C. 79P SETD2 a potential tissue-agnostic predictive biomarker for ICIs in solid tumors. Ann Oncol 2021; 32: S390.

[12] Terzo EA, Lim AR, Chytil A, Chiang YC, Farmer L, Gessner KH, Walker CL, Jansen VM and Rathmell WK. SETD2 loss sensitizes cells to PI3Kβ and AKT inhibition. Oncotarget 2019; 10: 647-659.

[13] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K and Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405-424.

[14] Zhang X, Pickin KA, Bose R, Jura N, Cole PA and Kuriyan J. Inhibition of the EGF receptor by binding of MIG6 to an activating kinase domain interface. Nature 2007; 450: 741-744.

[15] Descot A, Hoffmann R, Shaposhnikov D, Reschke M, Ullrich A and Posern G. Negative regulation of the EGFR-MAPK cascade by actin-MAL-mediated Mig6/Errfi-1 induction. Mol Cell 2009; 35: 291-304.

[16] Frosi Y, Anastasi S, Ballarò C, Varsano G, Castellani L, Maspero E, Polo S, Alemà S and Segatto O. A two-tiered mechanism of EGFR inhibition by RALT/MIG6 via kinase suppression and receptor degradation. J Cell Biol 2010; 189: 557-571.

[17] Guha U, Chaerkady R, Marimuthu A, Patterson AS, Kashyap MK, Harsha HC, Sato M, Bader JS, Lash AE, Minna JD, Pandey A and Varmus HE. Comparisons of tyrosine phosphorylated proteins in cells expressing lung cancer-specific alleles of EGFR and KRAS. Proc Natl Acad Sci U S A 2008; 105: 14112-7.

[18] Zhou J, Ji Q and Li Q. Resistance to anti-EGFR therapies in metastatic colorectal cancer: underlying mechanisms and reversal strategies. J Exp Clin Cancer Res 2021; 40: 328.

[19] Kalatskaya I, Trinh QM, Spears M, McPherson JD, Bartlett JMS and Stein L. ISOWN: accurate somatic mutation identification in the absence of normal tissue controls. Genome Med 2017; 9: 59.

[20] Chan DLH, Segelov E, Wong RS, Smith A, Herbertson RA, Li BT, Tebbutt N, Price T and Pavlakis N. Epidermal growth factor receptor (EGFR) inhibitors for metastatic colorectal cancer. Cochrane Database Syst Rev 2017; 6: CD007047.

[21] Montagut C, Dalmases A, Bellosillo B, Crespo M, Pairet S, Iglesias M, Salido M, Gallen M, Marsters S, Tsai SP, Minoche A, Seshagiri S, Serrano S, Himmelbauer H, Bellmunt J, Rovira A, Settleman J, Bosch F and Albanell J. Identification of a mutation in the extracellular domain of the epidermal growth factor receptor conferring cetuximab resistance in colorectal cancer. Nat Med 2012; 18: 221-223.

[22] Kim N, Cho D, Kim H, Kim S, Cha YJ, Greulich H, Bass A, Cho HS and Cho J. Colorectal adenocarcinoma-derived EGFR mutants are oncogenic and sensitive to EGFR-targeted monoclonal antibodies, cetuximab and panitumumab. Int J Cancer 2020; 146: 2194-2200.