



Immunohistochemical Expression of Mesenchymal Epithelial Transition Factor Receptor (C-MET) In Colorectal Carcinoma

Danyal Khan¹, Sara Zia Ullah¹, Fouzia Rauf¹, Sabeen Nasir¹, M. Mumtaz Khan¹

¹Department of Pathology, Peshawar Medical College, Peshawar, Pakistan affiliated with RIPHAH International University, Islamabad, Pakistan

Corresponding Author: Dr. Sara Ziaullah,

Associate Professor, Department of Pathology, Peshawar Medical College, Peshawar, Pakistan. Email: drsarazia@gmail.com

Volume 6, Issue 15, Sep 2024

Received: 15 June 2024

Accepted: 25 Aug 2024

Published: 21 Sep 2024

doi: [10.48047/AFJBS.6.15.2024.14126-14136](https://doi.org/10.48047/AFJBS.6.15.2024.14126-14136)

ABSTRACT

Objectives

To assess the difference in C-MET receptor immunohistochemistry expression between normal and malignant colonic mucosa. To relate the C-MET expression in CRC cases with various clinico-pathologic parameters.

Materials and Methods

An analytical, cross-sectional, retrospective comparative study was conducted. The study took place from August 2020 to August 2021 at the Pathology Department of Peshawar Medical College (PMC). 53 CRC case blocks that were formalin-fixed, paraffin-embedded (FFPE) were selected. Slides were examined histopathologically, and the C-MET receptor was assessed by immunohistochemistry. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 20.0. For categorical variables, Fisher exact and Chi-Square tests were run.

Results

In contrast to the normal intestinal mucosa, which only exhibited high C-MET expression in 28.8% of cases, the majority of tumor cases (60.37%) exhibited high C-MET expression. The difference being statistically significant <0.0001 . Regarding age, gender, laterality, grade, or stage of the tumor, there was no statistically significant variation in C-MET expressions. C-MET expression and tumor histologic subtypes, however, were statistically significantly correlated.

Conclusion

The presence of C-MET overexpression in colorectal carcinoma cases relative to normal colonic mucosa supports the idea that C-MET plays a pathogenic role in colorectal cancer in our group. For CRC subtypes and the lack of lymph node metastasis (N0), C-MET overexpression was statistically significant. C-MET demonstrated a distinct pattern of elevated expression in correlation with increased Tumor Invasion (T3, T4), though not statistically significant. C-MET may serve as drug target.

Keywords

Colorectal Carcinoma, Hepatocyte Growth Factor, C-MET, Immunohistochemistry

INTRODUCTION

According to GLOBOCAN 2020 data, colorectal cancer (CRC) ranks third among most commonly diagnosed cancers, globally. It accounts for 10% of all cases of cancer. Additionally, it ranks as the second most common cause of cancer related deaths ¹. The age-standardized incidence rates of colorectal cancer (CRC) increased globally by 9.5% between 1990 and 2017 ². The incidence of CRC shows a direct relation to the Human Development Index (HDI). The incidence of CRC in developed countries is three times higher compared to developing countries ³. However, it is on a consistent and uniform rise in developing countries ^{3,4}. This change in developing countries seems to arise from the adaptation of risk factors associated with CRC e.g., sedentary lifestyle and animal-source food intake leading to increased body weight ⁵. CRC was the second most common cancer in Pakistan from December 1994 to December 2020, according to statistics provided by Shaukat Khanum Memorial Cancer Hospital and Research Center ⁶.

AJCC, the American Joint Committee on Cancer, states that the important prognostic factors for CRC include serum carcinoembryonic antigen (CEA) levels, TNM stage, microscopic grade, status of surgical margins, and vascular and perineurial invasion ⁷.

The five-year survival rate for CRC is approximately 90 % if the disease is localized and has not spread to surrounding tissues, organs, or any lymph nodes, but if it has spread, the rate declines to 72%. The 5 years survival for patients with distant metastasis is only 14% ⁸.

New tumor markers are being searched the world over, having higher sensitivity and specificity, that are economical as well as convenient to evaluate e.g., serum markers such as DNA of HOTAIR (for HOX transcript antisense RNA) ⁸, lncRNAs (long non-coding RNAs) ⁹ and tissue markers such as Ki67 ¹⁰, PDL ¹¹ are among the widely studied markers showing promising results.

The tyrosine kinase receptor C-MET is one of these markers ^{12, 13}. The ligand for the C-MET receptor is called hepatocyte growth factor, or HGF. Another name for it is scatter factor ^{8,14, 15}. In the intracellular area, several tyrosine residues undergo auto-phosphorylation as a result of HGF/C-MET interactions. Among the regular activities of the HGF/C-MET signaling pathway are cellular differentiation, proliferation, and angiogenesis^{16,17}. Because it has been linked to tumor cell proliferation, survival, invasion, and metastasis, the deregulation of the HGF/C-MET pathway plays a part in the etiology of cancer ^{8,15}. Numerous solid tumors in humans, including ovarian ¹⁸, head and neck ¹⁹, gastric ²⁰, and colorectal ²¹, have been shown to overexpress C-MET.

Multiple studies have shown that its overexpression is associated with advanced stage ²² and poor prognosis ²¹. Targeted therapy against C-MET has shown promising results in hepatocellular carcinoma ^{23 24}, lung adenocarcinoma ²⁵, and myeloma ²⁶. Some of these therapies are now FDA-approved ^{27,28}.

Although studies have been done on tissue markers such as Ki67 ²⁹, BRAF (30), CDX-2 ³¹, and serum markers such as CEA ^{32,33} no data is available on C-MET's prognostic or therapeutic role. This study is designed as a roadmap for further research on C-MET in our population. We compared the immunohistochemical expression of the C-MET receptor in normal colonic mucosa and neoplastic colonic mucosa and correlated the immunohistochemical C-MET expression of CRC with various clinico-pathologic parameters like subtypes, grade, stage, etc.

MATERIAL AND METHODS

This cross-sectional comparative study was carried out in the pathology department of Peshawar Medical College in Peshawar. On October 4, 2019 (# Prime/IRB/2019/182), the Institutional Review Board of Peshawar Medical College, Riphah International University, approved this study. From 2014 to 2021, 53 diagnosed cases of colorectal cancer (CRC; Group B) and normal colonic

mucosa (Group A) were obtained from the PMC Diagnostic Laboratories, Histopathology Division's electronic archives using formalin fixed paraffin embedded (FFPE) blocks.

Histopathologic examination

We examined slides stained with hematoxylin and eosin for tumor subtype, grading, AJCC staging, vascular invasion, and perineural invasion.

Immunohistochemistry

IHC analysis was performed using a rabbit monoclonal primary antibody from Bio SB against total C-MET (SP44). IHC staining was performed using a universal kit from Bio SB in accordance with the manufacturer's instructions.

Evaluation of Immunohistochemical Expression

Two pathologists who worked independently and were code-blind to the slide categories assessed the C-MET staining intensity at 400x magnification. The stain was concentrated mainly in cytoplasm and cell membrane. The immunohistochemistry scoring was done semi-quantitatively according to criteria designed by Li et al., 2016⁹. The IHC evaluation method is depicted in Table 1.

1: IHC Evaluation Method by Li et., 2016

Score	Proportion	Intensity
0	No Positive Tumour Cells	No Staining
1	<10% Positive tumour cells	Weak Staining, Light Yellow
2	10–30% Positive tumour cells	Moderate Staining, Yellowish Brown
3	>30% Positive Tumour Cells	Strong Staining, Brown
Staining Index Calculation = Staining Intensity× Tumour Cell Staining Proportion		
≥4	High C-MET Expression	
<4	Low C-MET Expression	

Statistical Analysis

The factors that were recorded included age, gender, tumor grade, tumor invasion site, lymph node metastatic (N), vascular invasion, perineural invasion, tumor invasion (T) of colon cancer, and C MET expression in both CRC and normal colonic mucosa.

A statistical analysis was conducted using data entered into the Statistical Package for Social Sciences (SPSS) version 20.0. We estimated the mean and standard deviation for the continuous variable of age. It was necessary to stratify data for factors such age, site, and tumor invasion (T).

For categorical variables such as gender, tumor site, grade of colorectal cancer, and C-MET expression, frequency and percentage were finally computed.

A chi-square test was employed to assess the difference in C-MET expression between the normal and malignant colonic mucosa. A similar analysis was done on other categorical variables, such as tumor invasion, grade, stage, vascular invasion, and perineural invasion, using the Chi-square test and Fisher exact tests.

For statistical significance, a p-value of 0.05 or less was required.

RESULTS

Clinicopathologic Parameters

53 FFPE blocks of both normal colorectal mucosa and colorectal cancer were included in the study. Thirty-five (66%) cases belong to male gender and 18 (44%) to female gender. There were 2:1 men to women. Patients with colorectal cancer (CRC) had an average age of 47.96 years (SD: 15.28). Ages 18 to 80 made up the range. Of the cases, most (n = 32, 60.3%) included individuals under 50 years old.

The most prevalent CRC subtype was "Not otherwise specified" (NOS), with n = 37 (69.8%). It was followed by "Mucinous type," with n = 12(22.6%), and 4 cases (7.5%) with Signet ring cell type.

The majority of CRCs (n = 35, 64%) were well differentiated, with moderately differentiated cases (n = 10, 18.8%) and poorly differentiated cases (n = 8, 15.1%). The most prevalent tumor invasion (T) category was "T3" (n=32, 60.37%), which was followed by "T4" (n=12, 22.64%), T2 (n=8), and T1 (n=1, 1.8%).

In comparison to instances with lymph node metastases (n=26, 49.1%), no lymph node involvement was observed in (n=27, 50.9 %) of the cases. Cases in Stage N1 Stage N2 (n = 14, 26.41%) were higher than in stage N2 (n=12, 22.6%). Ten cases (18.8%) had perineural invasion, while only 22 cases (41.5%) demonstrated vascular invasion.

C-MET Expression Comparison between Tumor and Normal Colonic Mucosa

Most of the CRC cases (n=32, 60.37%) showed 'high C-MET expression' (Score ≥ 4) while 21 CRC cases (39.62%) showed low C-MET expression (Score < 4). Among the normal colorectal mucosa cases, the majority (n=43, 81.13 %) showed low C-MET expression while 15 (28.8%) showed 'high C-MET expression'. There was a statistically significant difference because the p-value was less than 0.00001.

Table 2: Comparison of C-MET expression in Neoplastic Mucosa and Normal Mucosa

	Low Expression	High Expression	Total	P Value <0.00001
Normal Colonic Mucosa	43 (81.13%)	10 (18.86%)	53 (100%)	
Tumor	21 (39.62%)	32 (60.37%)	53 (100%)	

Correlation of C-MET Expression with Clinico-pathological Parameters in Colorectal Carcinoma

Female CRC cases showed a clear trend for C-MET overexpression (77.77% High Staining) as compared to the male population (51.42% high staining) however, Statistical analysis revealed that the difference (p-value = 0.063) was not significant.

Among patients below 50 years (Total 32/53), 59.37% of cases (n=19/32) showed 'high C-MET expression' while 40.62 % (n=13/32) showed 'low expression'. Among patients above 50 years (n=21/53), 61.9% showed high expression while 8/21 (38.09%) showed low expression. Nevertheless, there was no statistically significant difference (p-value = 0.853) in the expression of C-MET between cases under and over 50.

Comparing C-MET expression in right and Left CRC, 12/21 (57.14%) Right-sided CRC cases showed high C-MET expression while 9/21 (42.85%) showed low C-MET expression. Out of 32 Left sided CRC cases 20/32 (62.5%) showed high C-MET expression while 12/32 (37.5 %) cases showed low C-MET expression. Right-sided and left-sided CRC cases did not differ in C-MET expression in a way that was statistically significant (p-value = 0.69).

Among 37 cases of CRC 'Not Otherwise Specified' (NOS), 27/37 (72.97%) showed high C-MET expression while 10/37 (27.02%) showed low expression. Among 12 cases of Mucinous Subtype, 5/12 (41.66%) showed high C-MET expression while 7/12 (58.33%) cases showed low C-MET expression. All 4 (100%) Signet Ring Cell Carcinomas cases showed low C-MET expression. This difference in C-MET expression among subtypes of CRC was statistically significant (p-value = 0.004)

Among 37 Well differentiated CRC cases, 23/31 (62.16%) showed high C-MET expression while 14/37 (37.83%) showed low C-MET expression. Among 16 high-grade (Moderate and Poorly Differentiated) CRC cases 9/16 (56.25%) cases showed high expression while 7/16 (43.75%) of the cases showed low C-MET expression. Thus, the p-value (p-value 0.68), which compares C-MET expression in high- and low-grade CRC cases, did not reveal any significant differences.

The majority of the advanced stage (T3, T4) cases showed a strong expression of C-MET (29/44, 65.9%) while the rest of the cases (15/44, 34.1%) showed a low C-MET expression. Most of the low-stage (T1, T2) cases (6/9, 66.66%) showed weak C-MET expression while a lesser number (3/9, 33.33%) showed strong C-MET expression. However, the results were not statistically significant with a p-value of 0.06.

In our investigation, there was a statistically significant difference in C-MET expression between tumor cases with no lymph node metastases (N0) and cases with positive lymph node involvement (N1/N2).

Table 3: Relation between gender and the C-MET expression in CRC cases

	Low Expression	High Expression	Total	P Value 0.063
Male	17 (48.57 %)	18 (51.42%)	35 (100%)	
Female	4 (22.22%)	14 (77.77%)	18 (100%)	

Table 4: Relation between patient age and C-MET expression in CRC cases

	Low Expression	High Expression	Total	P Value 0.853
Age <50	13 (40.62 %)	19 (59.37 %)	32 (100%)	
Age >50	8 (38.09 %)	13 (61.9 %)	21 (100%)	

Table 5: Relation between the location and C-MET expression in CRC mucosa cases

	Low Expression	High Expression	Total	p Value
--	----------------	-----------------	-------	---------

Right Colon	9 (42.85%)	12 (57.14%)	21 (100%)	0.696
Left Colon	12 (37.5%)	20 (62.5%)	32 (100%)	

Table 6: Relation of C-MET Expression of CRC cases with different Subtypes of Colorectal Carcinoma

CRC Subtype	Low Expression	High Expression	Total	P Value 0.004
NOS	10 (27.02%)	27 (72.97)	37 (100%)	
Mucinous Type	7 (58.33%)	5 (41.66%)	12 (100%)	
Signet Ring Cell Type	4 (100%)	0	4 (100%)	

Table 7: Relation of C-MET expression in CRC cases with Grades of CRC

	Low Expression	High Expression	Total	P Value 0.686
Well Differentiated	14 (37.83%)	23 (62.16%)	37 (100%)	
Moderately / Poorly Differentiated	7 (43.75%)	9 (56.25%)	16 (100%)	

Table 8: Relation of C-MET expression with Tumor Invasion

	Low Expression	High Expression	Total	P Value 0.06
T1, T2	6 (66.66%)	3 (33.33%)	9 (100%)	
T3, T4	15 (34.1%)	29 (65.9%)	44 (100%)	

Table 9: Relation of C-MET expression in CRC cases with Lymph Node Status (N)

	Low Expression	High Expression	Total	P Value 0.03
N0	7 (25.92%)	20 (74.07%)	27 (100%)	
N1, N2	14 (53.84%)	12 (46.16%)	26 (100%)	

DISCUSSION

C-MET expression in Colorectal Carcinoma

Variable C-MET expression outcomes in colorectal cancer are reported by several research in the literature. In our study

According to Table 2, the C-MET Expression level was substantially greater (60.3%) in CRC cases than in normal colonic mucosa (18.8%). Our findings are consistent with those of several research.

In China, Liu et al. (2012) found that C-MET expression was higher in colorectal cancer (73.3%) than in the mucosa of the normal colon (40.0%)³⁴.

Armstrong et al., 2021 from the UK observed C-MET expression in 280 cases and reported significantly higher expression in CRC samples 30.80% compared to normal tissue samples (12.25%)³⁵. Garouniatis et al., 2013 from Greece and De Oliveira et al., 2009 from Brazil showed C-MET positivity in 71.58 % and 79% of the cases respectively^{22,36}.

Contrary to our results Lee et al., 2018 from Korea observed C-MET expression in only 15.3% of cases among 255 patients of CRC (21). Canadian researchers Yoon et al., 2020 found that normal colonic mucosa had somewhat greater C-MET levels than CRC³⁷. According to Abou-Bakr et al. (2021), C-MET expression was 50% positive (38). These variations in C-MET expression suggest that etiology varies geographically.

C-MET Expression and Clinicopathologic Parameters

Our observation is that C-MET expression is not statistically significant in relation to the gender or age of the patients. However, there was higher Expression of C-MET in female patients Vs males (77.77 Vs 51.42%). As per our research, Abou-Bakr et al., 2021, Liu et al., 2012, Lee et al., 2018, and Garouniatis et al., 2013 discovered no correlation between the patient's age or gender and C-MET expression^{21,34,36,38}.

A statistically significant difference was found among CRC Subtypes and C-MET expression (Table 6). The adenocarcinoma NOS type show high expression in most cases. Liu et al. (2012), Lee et al. (2018), and Abou-Bakr et al. (2021) observed no significant difference between tumor subtype and C-MET expression, which is contrary to our observation^{21,34,38}.

In our investigation, we could not find any statistically significant correlation between C-MET expression and CRC grades. Our findings are in line with the studies by Lee et al., 2018, Abou-Bakr et al., 2021, and Garouniatis et al., 2013^{21, 34, 36, 38}. In contrast to the study by Liu et al., 2012, which demonstrated a statistically significant difference between C-MET expression and various grades. According to his research, the percentage of C-MET positivity was highest for poorly differentiated cases (92.6%) as compared to moderately differentiated (60%) and well-differentiated cases (75%).

In our study population, we observed that the higher stage (T3, T4) showed a stronger intensity of C-MET expression. However, the results were not statistically significant. This clear trend in favor of a higher T stage indicates that C-MET expression may be associated with tumor progression and poorer prognosis. Liu et al., 2012 from China and De Oliveira et al., 2009 showed a statistically significant difference between C-MET expression and Tumor Depth of Invasion with weaker expression for Lower Stages and Higher Expression for higher stages^{22,34}. However, no discernible variation in C-MET expression was found between the stages of colorectal cancer (CRC) by Garouniatis et al., 2013 from Greece and Abou-Bakr et al., 2021 from Egypt^{36, 38}. Multicenter studies with a larger cohort of CRC patients will help establish a relation between stage and C-MET in our population.

According to our research, there is a statistically significant difference in C-MET expression between tumor patients with no lymph node metastasis (N0) and cases with positive lymph node metastases (N1/N2). The study by Liu et al., 2012 showed contrasting results where N1 and N2 showed higher C-MET expression as compared to N0³⁴. However, a study by Garouniatis et al., 2013 and Yoon et al., 2020 showed no significant difference between lymph node metastasis status and C-MET expression^{36,37}.

CONCLUSION

C-MET immunohistochemical expression difference among CRC cases and normal colonic mucosa was statistically significant which favors the role of C-MET in the pathogenesis of CRC

in our population. C-MET overexpression was statistically significant for subtypes of CRC and the absence of Lymph Node Metastasis (N0). Although it was not statistically significant, C-MET showed a clear trend of high expression for higher Tumor Invasion (T3, T4). Different age groups, tumor locations, tumor grades, and vascular and perineural invasion did not statistically significantly affect C-MET expression.

LIMITATIONS

Our study was not able to include a bigger sample size due to time and budget constraints.

RECOMMENDATIONS

A larger cohort on C-MET expression in CRC including follow-up and patient survival can better represent the scope of the study in our population. It can also give more significant results regarding C-MET expression in relation to Tumor Invasion and Lymph Node Metastasis. Further case-control studies on C-MET targeting immunotherapy will add to our knowledge of how C-MET-related immunotherapy may help our patients. Other antibodies that work closely with C-MET like EGFR can be included in future studies.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION:

Danyal Khan: Chief Investigator

Sara Zia Ullah: Supervisor, Role in the literature and article review

Fozia Rauf: Co-Supervisor, Role in the literature review, SPSS, and article review

Sabeen Nasir: Proofreading and final approval

M. Mumtaz Khan: Proofreading and final approval

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.* 2021 May;71(3):209–49.
2. GBD 2017 Colorectal Cancer Collaborators. The global, regional, and national burden of colorectal cancer and its attributable risk factors in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol.* 2019 Dec;4(12):913–33.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018 Nov;68(6):394–424.
4. Fidler MM, Soerjomataram I, Bray F. A global view on cancer incidence and national levels of the human development index. *Int J Cancer.* 2016 Dec 1;139(11):2436–46.
5. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin.* 2020 May;70(3):145–64.
6. Mahmood S, Faraz R, Yousaf A, Hussain A, Zafar A, Kiran F, et al. COLLECTIVE CANCER REGISTRY REPORT-DECEMBER 1994 TO DECEMBER 2020: SHAUKAT KHANUM MEMORIAL CANCER HOSPITAL & RESEARCH CENTER, PAKISTAN.
7. American Joint Committee on Cancer prognostic factors consensus conference - Compton - 2000 - Cancer - Wiley Online Library [Internet]. [cited 2024 Apr 18]. Available from: [https://acsjournals.onlinelibrary.wiley.com/doi/full/10.1002/\(SICI\)1097-0142\(20000401\)88:7%3C1739::AID-CNCR30%3E3.0.CO%3B2-T](https://acsjournals.onlinelibrary.wiley.com/doi/full/10.1002/(SICI)1097-0142(20000401)88:7%3C1739::AID-CNCR30%3E3.0.CO%3B2-T)
8. Zhang YW, Vande Woude GF. HGF/SF-met signaling in the control of branching morphogenesis and invasion. *J Cell Biochem.* 2003 Feb 1;88(2):408–17.
9. Liu H, Li J, Koirala P, Ding X, Chen B, Wang Y, et al. Long non-coding RNAs as prognostic markers in human breast cancer. *Oncotarget.* 2016 Mar 1;7(15):20584–96.

10. Melling N, Kowitz CM, Simon R, Bokemeyer C, Terracciano L, Sauter G, et al. High Ki67 expression is an independent good prognostic marker in colorectal cancer. *J Clin Pathol*. 2016 Mar;69(3):209–14.
11. PD-L1 expression can be regarded as prognostic factor for survival of non-small cell lung cancer patients after chemoradiotherapy - PubMed [Internet]. [cited 2024 Apr 23]. Available from: <https://pubmed.ncbi.nlm.nih.gov/29322798/>
12. Petterson SA, Dahlrot RH, Hermansen SK, K A Munthe S, Gundesen MT, Wohlleben H, et al. High levels of C-MET is associated with poor prognosis in glioblastoma. *J Neurooncol*. 2015 May;122(3):517–27.
13. Cooper CS, Park M, Blair DG, Tainsky MA, Huebner K, Croce CM, et al. Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature*. 1984 Sep 6;311(5981):29–33.
14. Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, et al. Identification of the hepatocyte growth factor receptor as the C-MET proto-oncogene product. *Science*. 1991 Feb 15;251(4995):802–4.
15. Graveel CR, Tolbert D, Vande Woude GF. MET: A Critical Player in Tumorigenesis and Therapeutic Target. *Cold Spring Harb Perspect Biol*. 2013 Jul;5(7):a009209.
16. Rosário M, Birchmeier W. How to make tubes: signaling by the Met receptor tyrosine kinase. *Trends Cell Biol*. 2003 Jun;13(6):328–35.
17. Blumenschein GR, Mills GB, Gonzalez-Angulo AM. Targeting the hepatocyte growth factor-cMET axis in cancer therapy. *J Clin Oncol Off J Am Soc Clin Oncol*. 2012 Sep 10;30(26):3287–96.
18. Yamamoto S, Tsuda H, Miyai K, Takano M, Tamai S, Matsubara O. Gene amplification and protein overexpression of MET are common events in ovarian clear-cell adenocarcinoma: their roles in tumor progression and prognostication of the patient. *Mod Pathol Off J U S Can Acad Pathol Inc*. 2011 Aug;24(8):1146–55.
19. Chau NG, Perez-Ordóñez B, Zhang K, Pham NA, Ho J, Zhang T, et al. The association between EGFR variant III, HPV, p16, C-MET, EGFR gene copy number and response to EGFR inhibitors in patients with recurrent or metastatic squamous cell carcinoma of the head and neck. *Head Neck Oncol*. 2011 Feb 27;3:11.
20. Hack SP, Bruey JM, Koeppen H. HGF/MET-directed therapeutics in gastroesophageal cancer: a review of clinical and biomarker development. *Oncotarget*. 2014 May 24;5(10):2866–80.
21. Lee SJ, Lee J, Park SH, Park JO, Lim HY, Kang WK, et al. C-MET Overexpression in Colorectal Cancer: A Poor Prognostic Factor for Survival. *Clin Colorectal Cancer*. 2018 Sep;17(3):165–9.
22. De Oliveira ATT, Matos D, Logullo AF, DA Silva SRM, Neto RA, Filho AL, et al. MET Is highly expressed in advanced stages of colorectal cancer and indicates worse prognosis and mortality. *Anticancer Res*. 2009 Nov;29(11):4807–11.
23. Shang R, Song X, Wang P, Zhou Y, Lu X, Wang J, et al. Cabozantinib-based combination therapy for the treatment of hepatocellular carcinoma. *Gut*. 2021 Sep;70(9):1746–57.
24. Preclinical trials for prevention of tumor progression of hepatocellular carcinoma by LZ-8 targeting C-MET dependent and independent pathways - PubMed [Internet]. [cited 2024 Apr 23]. Available from: <https://pubmed.ncbi.nlm.nih.gov/25607934/>
25. Jenkins RW, Oxnard GR, Elkin S, Sullivan EK, Carter JL, Barbie DA. Response to Crizotinib in a Patient With Lung Adenocarcinoma Harboring a MET Splice Site Mutation. *Clin Lung Cancer*. 2015 Sep;16(5):e101-104.

26. Lath DL, Buckle CH, Evans HR, Fisher M, Down JM, Lawson MA, et al. ARQ-197, a small-molecule inhibitor of C-MET, reduces tumour burden and prevents myeloma-induced bone disease in vivo. *PloS One*. 2018;13(6):e0199517.
27. Singh H, Brave M, Beaver JA, Cheng J, Tang S, Zahalka E, et al. U.S. Food and Drug Administration Approval: Cabozantinib for the Treatment of Advanced Renal Cell Carcinoma. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2017 Jan 15;23(2):330–5.
28. Kazandjian D, Blumenthal GM, Chen HY, He K, Patel M, Justice R, et al. FDA approval summary: crizotinib for the treatment of metastatic non-small cell lung cancer with anaplastic lymphoma kinase rearrangements. *The Oncologist*. 2014 Oct;19(10):e5-11.
29. Nabi U, Nagi AH, Sami W. Ki-67 proliferating index and histological grade, type and stage of colorectal carcinoma. *J Ayub Med Coll Abbottabad JAMC*. 2008;20(4):44–8.
30. Wasti H, Shawana S, Shafique S, Shahid Y, Saleem K, Kumar S. To Correlate the Expression of KRAS and BRAF V600e with Histological Grades and Variants in Tissue Samples of Colorectal Carcinoma. *J Gandhara Med Dent Sci*. 2023 Apr 1;10:52–6.
31. CDX-2 expression in colorectal cancer: A single centre study from Pakistan | Journal of Rawalpindi Medical College. 2023 Jan 21 [cited 2024 Apr 18]; Available from: <https://www.journalrmc.com/index.php/JRMC/article/view/1206>
32. Hayat M, Haider G, Hussain S, Kerio P, Bai R, Akbar S, et al. Patterns of serum CEA levels in different clinico-pathological variables of colorectal cancer. *J Fatima Jinnah Med Univ*. 2020 Jul 15;14(2):68–71.
33. (PDF) Evaluation of carcino-embryonic antigen (CEA) and diagnostic significance in adult patients suffering from colorectal carcinoma (CRC) and GIT malignancies [Internet]. [cited 2024 Apr 25]. Available from: https://www.researchgate.net/publication/260694122_Evaluation_of_carcino-embryonic_antigen_CEA_and_diagnostic_significance_in_adult_patients_suffering_from_colorectal_carcinoma_CRC_and_GIT_malignancies
34. Liu Y, Li Q, Zhu L. Expression of the hepatocyte growth factor and C-MET in colon cancer: correlation with clinicopathological features and overall survival. *Tumori*. 2012;98(1):105–12.
35. Armstrong GR, Khot MI, Tiernan JP, West NP, Perry SL, Maisey TI, et al. The utility of C-MET as a diagnostic tissue biomarker in primary colorectal cancer. *Int J Exp Pathol*. 2021 Jun;102(3):172–8.
36. Garouniatis A, Zizi Zerbetzoglou A, Rizos S, Kostakis A, Nikiteas N, Papavassiliou A. FAK, CD44v6, C-MET and EGFR in colorectal cancer parameters: Tumour progression, metastasis, patient survival and receptor crosstalk. *Int J Colorectal Dis*. 2012 Jun 26;28.
37. Yoon JY, Wang JY, Roehrl MHA. A combined FAK, C-MET, and MST1R three-protein panel risk-stratifies colorectal cancer patients. *Transl Oncol*. 2020 Jul 30;13(11):100836.
38. The Prognostic Significance of C-MET and p53 Immunohistochemical Expression in Gastric and Colorectal Carcinomas | Open Access Macedonian Journal of Medical Sciences [Internet]. [cited 2024 Apr 26]. Available from: <https://oamjms.eu/index.php/mjms/article/view/5910>