IntechOpen

Colorectal Cancer

Diagnosis, Screening and Management

Edited by Jindong Chen

COLORECTAL CANCER - DIAGNOSIS, SCREENING AND MANAGEMENT

Edited by **Jindong Chen**

Colorectal Cancer - Diagnosis, Screening and Management
http://dx.doi.org/10.5772/intechopen.68430
Edited by Jindong Chen

Contributors

Murat Ferhat Ferhatoglu, Abdulcabbar Kartal, Romina Briffa, Simon Langdon, Godfrey Grech, David J Harrison, Octav Ginghina, Cornelia Nitipir, Hamid Elia Daaboul, Mirvat El-Sibai, Racho Ribarov, Igor Richter, Josef Dvořák

Notice

First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Colorectal Cancer - Diagnosis, Screening and Management
Edited by Jindong Chen

p. cm.

Print ISBN 978-1-78923-100-7

Online ISBN 978-1-78923-101-4

eBook (PDF) ISBN 978-1-83881-320-8

Meet the editor

Jindong Chen, PhD, is an AACR active member and chief technology officer in Exploring Health, LLC; a professor in Cancer Biology Section at the Department of Medical Genetics, Zunyi Medical University, China; and a former research associate professor and kidney laboratory codirector at the University of Rochester Medical Center, New York. He earned his PhD degree in Karolinska Institute, Sweden. Then, he served as a research scientist at Van Andel Research Institute, USA, and a senior scientist at the National Cancer Centre Singapore and Duke-NUS University Medical School. He has worked on colorectal carcinoma, breast cancer, and kidney cancer for more than 20 years. He identified two cancer-related genes, NORE1 and LSAMP, and was awarded in the 94th AACR Annual Meeting. He developed several FLCN and HRPT2 knockout mouse models and was awarded in the 100th AACR Annual Meeting. With those models, he conducted a few anticancer drug tests and has been working on antimetastasis drug screening.

Contents

Preface

Colorectal cancer is the third most common cancer in men and the second in women worldwide. Approximately 1.4 million people were diagnosed with colorectal cancer, and 700,000 people die from it each year, which represents a major public health problem. Updated knowledge, information, techniques, and innovative ideals are required for dealing with colorectal cancer.

Here, thanks to international experts for sharing their experience and knowledge on these different aspects in the management of colorectal cancer, this book has this opportunity to offer all physicians treating colorectal cancer, as well as researchers, updated information concerning the biology, diagnosis, screening, and treatment of colorectal carcinoma.

This book provides a detailed evaluation of diagnostic modalities, in-depth analysis of screening for colorectal cancer, recent advances in treatment, and principles and trends in the management of colorectal cancer. This updated knowledge will be an interesting and informative read for any clinician involved in the management of patients with colorectal cancer. In addition, readers such as related physicians, researchers, and colorectal cancer patients are potential beneficiaries of this book.

Finally, I express my deepest thanks to all the authors in this book and InTech teams including Ms. Martina Usljebrka and Mr. Slobodan Momcilovic (publishing process managers). Without their efforts, this book would not have been completed smoothly.

Jindong Chen, MD, PhD
Chief Technology Officer, Exploring Health, LLC
Professor, Cancer Biology Section, Department of Medical Genetics
Zunyi Medical University, China

Chapter 1

Diagnostics in Colorectal Surgery

Murat Ferhat Ferhatoglu and Abdulcabbar Kartal

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.74027

Abstract

The rapid development in radiological examinations has opened a new chapter in colorectal surgery. Unlike classical books, in this section we preferred to use more modern and everyday practical methods such as endoscopy or magnetic resonance imaging or endorectal ultrasonography, rather than sparing less used examinations such as X-rays and barium graphs.

Keywords: colorectal cancer, diagnostic tests, endoscopy, endoultrasonography

1. Endoscopy

1.1. Rectosigmoidoscopy

A rectosigmoidoscopy is an examination of the rectum and pelvic colon with a sigmoidoscope. In this procedure an endoscopic vision equipment is introduced for visualization of the anus, rectum and sigmoid colon. No special preparation is required, except "fleet-enema" applications. It is helpful in the research of hemorrhoidal disease, as well as in the diagnosis of diseases of the rectum and the first portions of the large bowel, for example, Crohn's disease, ulcerative rectocolitis, diverticula, polyps and colorectal cancer.

1.2. Colonoscopy

Colonoscopy is an examination of lower part of the alimentary tract. Colonoscopy is a safe procedure that gives information other tests may not be able to give. Often, people have colonoscopy as a screening test to check for polyps or cancer in the colon or rectum [1].

Indications: Colonoscopy can be performed for both diagnostic and therapeutic indications. Diagnostic indications include screening and surveillance for colon cancer, evaluating signs and symptoms suggestive of possible colonic or distal small bowel disease, assessing a response to treatment in patients with known colonic disease, and evaluating abnormalities found on imaging studies. Therapeutic indications include stricture dilation, stent placement and foreign body removal [2].

Contraindications: Colonoscopy is contraindicated in the following situations.

- When the risks of the colonoscopy outweigh the expected benefits.
- Consent cannot be obtained for a non-urgent procedure.
- A perforation is known or suspected.
- Documented acute diverticulitis.
- Fulminant colitis.

It is important that the expected benefits of colonoscopy be carefully weighed against the risks, particularly in older adults and patients with comorbid illnesses because these patients are at increased risk for serious complications from colonoscopy. A suspected poor preparation is a relative contraindication to colonoscopy.

Important considerations: A high-quality examination requires careful investigation of colonic mucosa. High-quality examination requires appropriate tissue acquisition and endoscopic removal of all polyps less than 2 cm. Removal of polyps larger than 2 cm may require special endoscopic skills.

1.3. Patient preparation

Diet: Patients need to consume a low-residue diet or clear liquids for at least 1 day prior to elective colonoscopy. Liquids that are red can be mistaken for blood in the colon or can obscure mucosal details and should be avoided [3, 4].

Medications: Most medications may be continued up to the time of colonoscopy and are taken with a small sip of water the day of the colonoscopy. Some medications may need to be adjusted prior to colonoscopy, such as medications for diabetes, due to decreased oral intake prior to the procedure. Oral iron should be stopped at least 5 days before the colonoscopy since it makes the residual feces black, viscous, and difficult to purge. Management decisions about antithrombotic agents should be made following discussion with the patient and the clinician prescribing the medications. Aspirin and nonsteroidal anti-inflammatory drugs in standard doses may be continued safely in patients having colonoscopy [5].

Bowel preparation: An adequate bowel preparation is critical for colonoscopy because it permits visualization of the entire colonic mucosa and increases the safety of therapeutic maneuvers. Poor preparation leads to increased procedure time, risk of complications, and

http://dx.doi.org/10.5772/intechopen.74027

probability of missing lesions. It is important to consider the patient's comorbid illnesses and the timing of the preparation when choosing an appropriate preparation or combination of preparations [6].

Sedation assessment: Options for sedation include no sedation, moderate procedural sedation, or deep sedation. Deciding upon the appropriate approach requires an assessment of the patient's sedation needs and risks prior to the colonoscopy. This includes a complete history of factors that might make sedation more difficult such as prior difficulties with sedation, chronic narcotic or benzodiazepine use, diminished mental capacity, and agitation or severe anxiety [7].

Informed consent: Informed consent includes full disclosure with a clear and complete explanation of all portions of the procedure. Discussion of the possible risks of colonoscopy, including frequent and less frequent but severe complications, must occur and be tailored to the specific patient and procedure. Incidences of possible complications should be mentioned. Written documentation of the consent process is mandatory [8].

Equipment: Routine colonoscopy is performed using a high-definition white-light colonoscope. Both adult and pediatric colonoscopes are available. Adult colonoscopes have a diameter of approximately 13 mm, whereas pediatric colonoscopes have a diameter of approximately 11 mm. An ultra-slim colonoscope with a diameter of 9.5 mm may be particularly helpful in patients with tight turns [9]. Various accessories are available that can be passed through the accessory channel of a colonoscope. These include biopsy forceps, brushes, snares, baskets, nets, injection needles, hemostatic clips, and argon plasma coagulation probes (**Figure 1**).

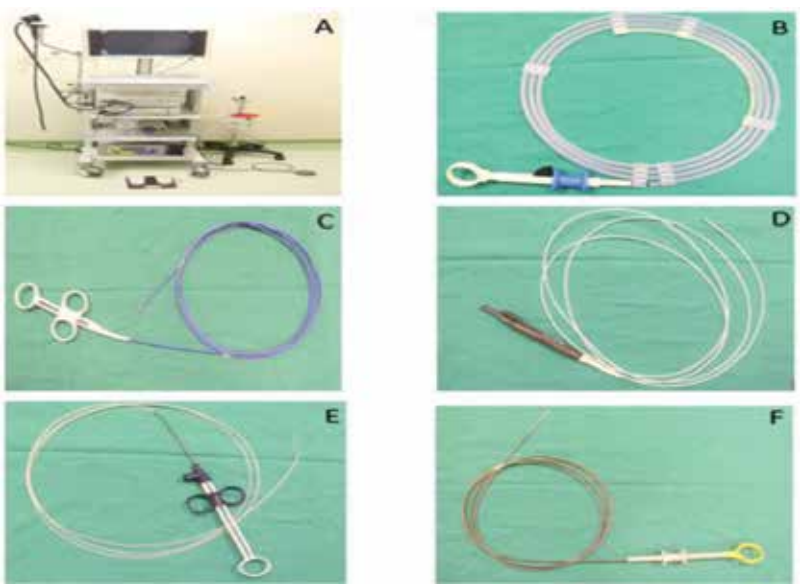

Figure 1. Basic materials used in colonoscopy. A: colonoscope, B: endoscopic clip shooter, C: biopsy forceps, D: endoscopic sclerotherapy needle, E: polypectomy snare, F: foreign body forceps.

Tissue sampling: Visible lesions identified during colonoscopy should be sampled or removed for pathology [10]. Tissue sampling includes biopsies, brushings, and polypectomy. Specimens obtained can be sent for histology, cytology, microbiology, or virology, depending upon the clinical situation.

Polypectomy: Most polyps less than 2 cm in size can be removed endoscopically, as well as many larger polyps. Small polyps may be completely removed using biopsy forceps, while larger polyps require snare resection, with or without electrocautery. Advanced endoscopic mucosal resection and endoscopic submucosal dissection techniques are used for large polyps (greater than 2 cm). Nearly all pedunculated polyps without invasive cancer can be removed endoscopically. If polyps are too numerous for removal, representative samples should be obtained (**Figure 2**).

Photodocumentation and reporting: All colonoscopic procedures should include a complete report detailing the extent of the colon examined, quality of the preparation, and all normal and abnormal findings encountered [11]. Photodocumentation greatly enhances the record and should be included when possible.

1.4. Virtual colonoscopy

Computed tomographic colonography gives a computer-simulated endoluminal vision of air-filled distended colon. This technique uses both spiral or helical CT scan images acquired as an uninterrupted volume of data and employs sophisticated post-processing software to generate images that allow the operator to evaluate a cleansed colon in any chosen direction [12].

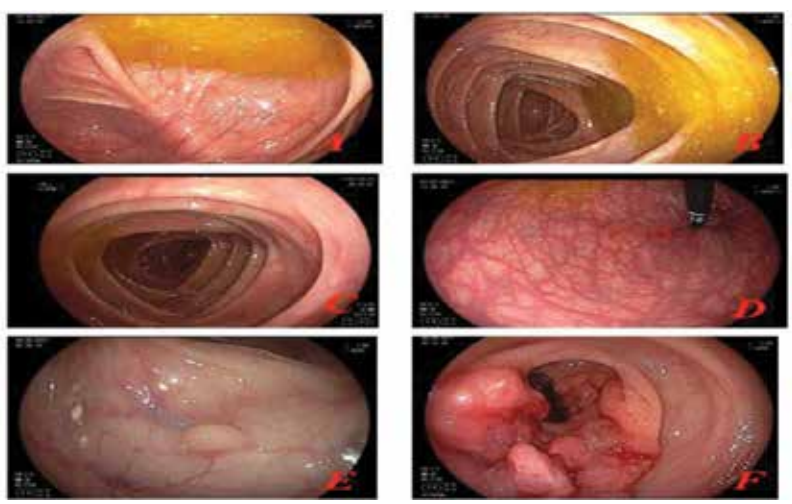

Figure 2. Normal and pathologic images on colonoscopy. A: normal view of caecum, B: normal view of ascending colon, C: normal view of transvers colon, D: reverse image of the rectum, E: sessile polyp of the rectosigmoid junction, F: tumor image at the sigmoid colon.

Indications: Potential indications for virtual colonoscopy include the following:

- Screening for colorectal cancer: virtual colonography is an method for colorectal cancer screening in asymptomatic patients over the age of 50 years. There is consensus that CT colonography should not be used for screening in patients at increased risk for colorectal cancer (e.g., history of adenomas, inflammatory bowel disease, familial colorectal cancer syndrome).
- Evaluation for synchronous colorectal cancer: in patients with a colorectal cancer in whom a complete colonoscopy cannot be performed due to the inability to pass the colonoscope beyond an obstructing tumor, a CT colonography can rule out a proximal synchronous colorectal cancer (**Figure 3**).
- Evaluation of patients with signs or symptoms suggestive of colorectal cancer: while colonoscopy is the preferred initial diagnostic test in patients with signs or symptoms of a colorectal cancer as it permits biopsy of the lesion, a CT colonography may be performed in patients with an incomplete or failed colonoscopy or in whom a colonoscopy is contraindicated [13, 14].
- Evaluation of with signs or symptoms suggestive of divertiküler disease.

Contraindications: The following situations are relative contraindications for CT colonography:

- Active colonic inflammation
- Symptomatic colon-containing abdominal wall hernia
- Recent acute diverticulitis
- Recent colorectal surgery
- Recent deep endoscopic biopsy/polypectomy/mucosectomy
- Known or suspected colonic perforation
- Symptomatic or high-grade bowel obstruction

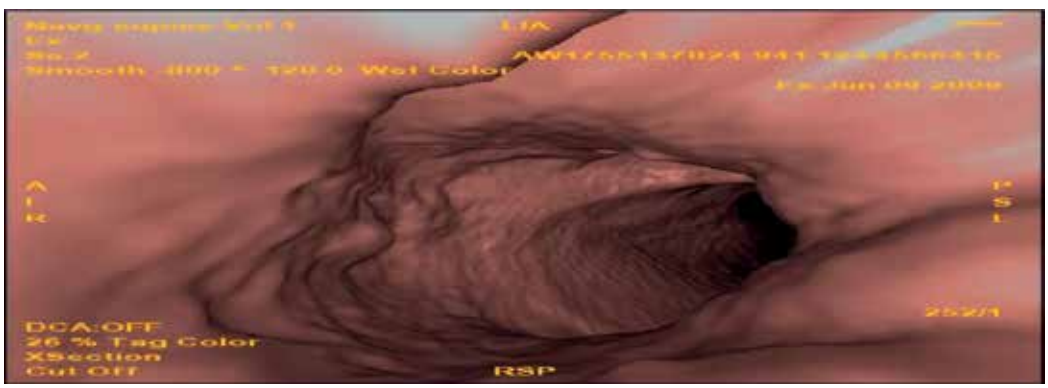

Figure 3. In the image of three-dimensional endoluminal CT colonography, a 9 mm diameter polyp.

Patient preparation: Patient preparation consists of dietary restriction with a low-residue diet and clear liquids for 24 h or more and bowel preparation with a laxative. Patient preparation is critical for computed tomographic (CT) colonography as stool can obscure underlying polyps or mass lesions, and in some cases, can simulate polyps. Several regimens (e.g., polyethylene glycol, phospho soda, magnesium citrate) have been used. It is important to consider the patient's comorbid illnesses when choosing an appropriate preparation or combination of preparations.

Even with the use of cathartic colon preparations, retained fluid in the lumen may obscure or mimic small polyps. Residual material is therefore tagged with oral administration of water-soluble contrast alone (typically with several meals prior to the examination) or in combination with a low-volume barium contrast agent. The contrast-enhanced residual material can then be differentiated from the surrounding colonic mucosa. While bowel preparation is required at the present time, CT colonography without a cathartic bowel preparation is also being evaluated [15].

1.5. Procedure

Technique and data acquisition: Following placement of a thin and flexible rectal catheter, the colon is distended with air or carbon dioxide throughout its length. Carbon dioxide has an improved patient tolerance as compared with air due to more rapid post-procedure absorption. Distension is also facilitated by use of smooth muscle relaxants, such as glucagon or hyoscine, which reduce peristalsis. Colonic distension is evaluated on the computed tomographic (CT) table immediately prior to image acquisition by reviewing the planar CT scout image in order to ensure technical adequacy of the resultant acquisition.

Following this, an uninterrupted volume of data is then acquired through the abdomen in several seconds during a single breath-hold. Because of the presence of stool, fluid, or bowel spasm, data are often acquired in both the supine and prone positions in order to redistribute fluid and colonic gas, thereby facilitating polyp detection. Scanning parameters are designed to cover a large volume of data with thin slices in order to optimize subsequent image reformation.

Intravenous injection of iodinated contrast medium is reserved for patients with known colorectal cancer in order to improve staging, in patients with symptoms of colorectal cancer, or in whom the extracolonic organs need to be further evaluated.

Image processing and reconstruction: Once image data are acquired, post-processing is performed on a computer using a variety of commercially available software packages [16]. The data are then used to render multiplanar reformatted images (in coronal, sagittal, and axial planes), mucosal relief profiles, or hybrid surface-shaded or volume-rendered endoluminal perspectives (picture).

1.6. Capsule endoscopy

Capsule endoscopy is a noninvasive diagnostic method and designed for imaging of the small intestine, which is hard to visualize. Images of esophagus, stomach, and proximal colon can

also be obtained. Images have a 1:8 amplification and resolution is higher than conventional endoscopes. This better images allow visualization of individual villi. Capsule endoscopy gives the concept of physiological endoscopy since the capsule moves in a passive state, does not inflate the bowel, and get images of the mucosa in the collapsed state. Capsule endoscopy is usually used for the diagnosis of small intestine disorders.

1.6.1. Small bowel capsule endoscopy

Indications: Primary indication is suspected intestinal bleeding, Crohn's disease and intestinal tumors. Capsule endoscopy can be used to diagnose small bowel injury due to the use of nonsteroidal anti-inflammatory drugs (NSAIDs), to evaluate abdominal pain of unclear etiology, to investigate for polyps in patients with familial polyposis syndromes and small bowel malignancies in patients with Lynch syndrome, and celiac disease [17–19].

Contraindications: The contraindication of the procedure are listed below:

Dementia, gastroparesis, an esophageal stricture or swallowing disorders, those patients who are inoperable or refuse surgery, partial or intermittent small bowel obstruction, patients who have defibrillators or pacemakers and pregnant women [20, 21].

Procedure: The video capsule (PillCam SB, EndoCapsule, and MiRo capsule) is swallowed with water after 12 h fasting. Following ingestion of the video capsule, clear liquids can be taken after 3 h, and foods can be taken 5 h later. Capsules are disposable and are excreted with defecation. The sensor arrays are removed 8–12 h after ingestion, and the recorded images are downloaded and being processed on Workstation computers. The recorders obtain about 50,000 images in duration of 8–24 h. Review of the video, selection of images, and production of a report may take 30–90 min [22–24].

1.6.2. Colon capsule endoscopy

A colon capsule for the screening of colorectal cancer has been approved by the US Food and Drug Administration and by the European Medicines Agency. Guidelines suggest that colon capsule endoscopy is a suitable and good alternative to colonoscopy for colorectal cancer in average-risk patients. However, it is not recommend for patients at increased risk for colon cancer or for patients with alarm symptoms [25].

Like conventional colonoscopy, a bowel preparation should be given to patients. The evening prior to the examination, patients should take about 3 L of polyethylene glycol. The morning of the procedure, the patient drinks another liter of polyethylene glycol between 6:00 and 7:00 am, and then the capsule is ingested at 8:00 am. Additional drugs (phospho soda and bisacodyl) can be given during the procedure for increasing transit of the capsule [25].

The colon capsule can be used to screen for colon cancer and polyps. Unfortunately, colonic capsule endoscopy cannot allow for biopsy polyp removal. Colonoscopy is required for lesions detected during the colon capsule endoscopy, subsequently for further evaluation and/or treatment.

2. Magnetic resonance imaging

Magnetic resonance imaging (MRI) is one of the most important methods for local staging of patients with rectal cancer. There are three different modalities of MRI as body coil MRI, endorectal coil MRI and pelvic phased-array coil MRI. Body coil MRI is not superior to CT scan in staging and it is insufficient in local staging.

ERC-MRI is used to provide images of the rectum and the area surrounding the rectum with a probe inserted into the rectum through the anal canal. ERC-MRI is an important method for staging of the anal canal and rectal cancers and diagnosis of anorectal fistulas and abscesses. ERC-MRI can make T-staging with an accuracy of 70–90% particularly for T1–T2 and early T3 tumors [26]. Despite this, likelihood of success in evaluation of T3 and T4 tumors decreases due to narrow field of view and implementation difficulty of ERC-MRI. Additionally, endorectal coil MRI cannot visualize the mesorectal fascia and it is an important disadvantage. Likelihood of success is less in conditions where the patient is noncompliant and not tolerating anal coil insertion, in tumors with a longitudinal length of more than 5 cm, tumors invading ¾ of the lumen, tumors located above the level of 10 cm from the anal verge [27].

More detailed images were obtained by using pelvic phased-array coil MRI (PP-MRI) which was developed recently and providing high-resolution images. Thus, local staging of rectal tumor resulted in a higher accuracy rate. Obtaining a wide angled image is its superiority to endorectal coil MRI.

Distension of rectal lumen and rectal wall with use of preoperative intrarectal contrast material such as water or gel, air insufflation, premedication with spasmolytic agents improve the quality of both of ERC-MRI and PP-MRI [28].

T-staging: The accuracy rate of staging performed with PP-MRI is markedly higher compared to CT. Excellent imaging of layers of the rectal wall, mesorectum and mesorectal fascia particularly in middle and upper rectal cancers improved description of T3 tumors.

It is very important to be able to determine the distance between the mesorectal fascia and tumor in detail as mm for local recurrence. This distance shows compliance reaching 95% with pathological measurement performed in specimen removed by using PP-MRI and total mesorectal excision. This feature enabled us to make preoperative substaging of T3 tumors [29]. In T3 tumors, if tumor invasion into the mesorectum is <5 mm then tumor is classified as T3a and if it is >5 mm then tumor is classified as T3b. While less number of lymph node involvement was observed in T3a tumors, lymph node involvement was seen in a more aggressive manner in T3b tumors. PP-MRI fell behind endoanal ultrasonography to show the relationship between the tumor and the anal sphincter muscles particularly in patients requiring intersphincteric resection.

Besides, endoanal ultrasonography is more successful than PP-MRI in discrimination between T1 tumor and T2 tumor in lower rectal cancers compared to PP-MRI.

N-staging: Regardless of T stage, N positivity shows locally advanced tumor and there is also a higher risk of local recurrence. These patients are candidates for neoadjuvant hormone

therapy. Distinguishing tumor and involved lymph node from reactive lymph node is important. PP-MRI has a success rate reaching 85% in evaluation of lymph node.

3. Contraindications for MRI

Absolute	Relative
1. Presence of pacemaker	1. Pregnancy
2. Cochlear implant	2. Claustrophobia
3. Metallic implant/object in the eyeball	3. Metallic implant/object in the soft tissue
4. If communication cannot be established with patient	4. Prosthetic heart valve
	5. Dental implant
	6. Intrauterine device
	7. Monitored patient
	8. Permanent makeup and tattoo

4. Endorectal ultrasonography

ERUS is being increasingly commonly used as a method in preoperative staging of rectal cancer. It is an ultrasonic method enabling simultaneous investigation of rectal wall layers and perirectal tissues in a 360° axis with a probe inserted into the rectum. A probe within a balloon inflated with water and inserted into the rectum detect the sound waves echoes of its own level by continuously rotating 360°. Axial length of tumor, its extension into rectal wall layers (T) and lymph nodes in perirectal tissues (N) are detected by moving the probe forward and backward through the anal canal and rectum (**Figure 4**). The structures of anal sphincters are established at the level of anal canal. The relationship between the tumor and anal sphincter is determined. However, inability to visualize the mesorectal fascia is the most important disadvantage. Therefore, it causes errors in staging of advanced T3 and T4 tumors. Besides, the lymph nodes far from the rectum may not be determined. Additionally, since higher staging can be made an erroneously, care should be exercised during interpretation of locally advanced cancer or desmoplastic tumors [30].

The other negative aspects of ERUS are as followings: inability to use in obstructive tumors, decline in diagnostic accuracy of T-staging in patients undergoing preoperative radiotherapy due to increased echogenicity of the rectal wall [31].

Lymph node staging by ERUS is problematic. It is difficult to determine whether lymph node is metastatic or not. Sensitivity and specificity of ERUS in determining lymph node are approximately 55 and 78%; respectively [33].

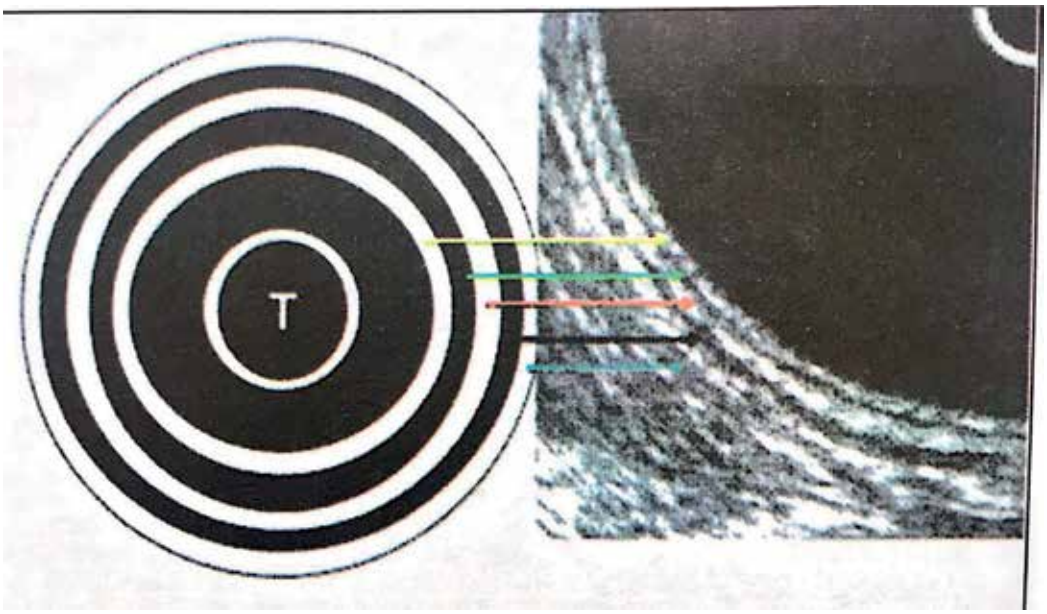

Figure 4. Rectal layers. Yellow arrow: mucosal surface, green arrow: mucosa, red arrow: submucosa, black arrow: proper muscle layer, blue arrow: perirectal fatty tissue.

The accuracy rate of ERUS in determining perirectal lymph node is about 70 and 75%. If sonographic appearance of lymph node is round in shape and its size is greater than 1 cm, it is suggestive of a malignancy. Malignant lymph nodes are hypoechoic and hypervascular. Lymph nodes with a diameter of greater than 0.5 cm are 50–70% malignant, the probability of malignancy is less than 20% if its diameter is less than 4 mm [32].

When ERUS, MRI and CT are compared for staging of rectal cancer, a marked superiority of ERUS and MRI is observed in T and N-staging compared to CT. While the accuracy rates of CT, ERUS and endorectal coil MRI in T-staging were reported to be 73, 87 and 84%, respectively; the accuracy rates of CT, ERUS and endorectal coil MRI in N-staging were reported to be 66, 74 and 82%, respectively [33].

Also localization and size of tumor affect the accuracy rates of methods. While the results with ERUS are better in tumors located within the 1/3 lower part of the rectum, PP-MRI provides a higher rate of accuracy in the middle and upper rectum. While ERUS is better in T1 and T2, a more detailed evaluation can be performed with PP-MRI in T3 and T4. Currently, since importance of the distance of the tumor to the mesorectal fascia became evident, the value of PP-MRI in local staging increased. Because, ERUS cannot visualize the mesorectal fascia. This is an important part of evaluation which will be performed before decision-making process for neoadjuvant chemotherapy. Performing PP-MRI in the preoperative assessment of rectal tumors should be considered mandatory. However, there is a risk for higher staging (due to desmoplastic reaction). This might cause initiation of unnecessary neoadjuvant chemotherapy in some patients. ERUS is superior in assessment of anal sphincter involvement.

In conclusion, when these two stagings are performed together the accuracy rate in local staging increases, in other words these two methods are complementary. Reporting higher or lower stage T is in question for both methods. This can be observed much more in PP-MRI for discrimination of T2/T3 and in ERUS for discrimination of T3/T4. In recent years, tumor extension can be determined well with development of three-dimensional ERUS.

Author details

Murat Ferhat Ferhatoglu* and Abdulcabbar Kartal

*Address all correspondence to: ferhat.ferhatoglu@okan.edu.tr

Department of General Surgery, Faculty of Medicine, Okan University, Istanbul, Turkey

References

[1] Bjorkman DJ, Popp JW Jr. Measuring the quality of endoscopy. Gastrointestinal Endoscopy. 2006;**63**:1

[2] Rex DK, Schoenfeld PS, Cohen J, et al. Quality indicators for colonoscopy. Gastrointestinal Endoscopy. 2015;**81**:31

[3] Faigel DO, Eisen GM, Baron TH, Dominitz JA, Goldstein JL, Hirota WK, et al. Preparation of patients for GI endoscopy. Gastrointestinal Endoscopy. 2003;**57**:446-450

[4] Park DI, Park SH, Lee SK, Baek YH, Han DS, Eun CS, et al. Efficacy of prepackaged, low residual test meals with 4L polyethylene glycol versus a clear liquid diet with 4L polyethylene glycol bowel preparation: A randomized trial. Journal of Gastroenterology and Hepatology. 2009;**24**:988-991

[5] ASGE Standards of Practice Committee, Anderson MA, Ben-Menachem T, Gan SI, Appalaneni V, Banerjee S, Cash BD, et al. Management of antithrombotic agents for endoscopic procedures. Gastrointestinal Endoscopy. 2009;**70**:1060-1070

[6] Chokshi RV, Hovis CE, Hollander T, Early DS, Wang JS. Prevalence of missed adenomas in patients with inadequate bowel preparation on screening colonoscopy. Gastrointestinal Endoscopy. 2012;**75**:1197-1203

[7] Standards of Practice Committee of the American Society for Gastrointestinal Endoscopy, Lichtenstein DR, Jagannath S, Baron TH, Anderson MA, Banerjee S, Dominitz JA, et al. Sedation and anesthesia in GI endoscopy. Gastrointestinal Endoscopy. 2008;**68**:815-826

[8] Standards of Practice Committee, Zuckerman MJ, Shen B, Harrison ME, Baron TH, Adler DG, Davila RE, et al. Informed consent for GI endoscopy. Gastrointestinal Endoscopy. 2007;**66**:213-218

[9] Shumaker DA, Zaman A, Katon RM. A randomized controlled trial in a training institution comparing a pediatric variable stiffness colonoscope, a pediatric colonoscope, and an adult colonoscope. Gastrointestinal Endoscopy. 2002;**55**:172-179

[10] Faigel DO, Eisen GM, Baron TH, Dominitz JA, Goldstein JL, Hirota WK, et al. Tissue sampling and analysis. Gastrointestinal Endoscopy. 2003;**57**:811-816

[11] Williams JE, Faigel DO. Colonoscopy reports and current state of performance measures. Gastrointestinal Endoscopy Clinics of North America. 2010;**20**:685-697

[12] Levine MS, Yee J. History, evolution, and current status of radiologic imaging tests for colorectal cancer screening. Radiology. 2014;**273**:160-180

[13] Levin B, Lieberman DA, McFarland B, Andrews KS, Brooks D, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: A joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. Gastroenterology. 2008;**134**: 1570-1595

[14] U.S. Preventive Services Task Force. Screening for colorectal cancer: U.S. Preventive Services Task Force recommendation statement. Annals of Internal Medicine. 2008;**149**: 627-637

[15] Kim B, Park SH, Hong GS, Lee JH, Lee JS, Kim HJ, et al. Iohexol versus diatrizoate for fecal/fluid tagging during CT colonography performed with cathartic preparation: Comparison of examination quality. European Radiology. 2015;**25**:1561-1569

[16] Summers RM, Yao J, Pickhardt PJ, Franaszek M, Bitter I, Brickman D, et al. Computed tomographic virtual colonoscopy computer-aided polyp detection in a screening population. Gastroenterology. 2005;**129**:1832-1844

[17] Petroniene R, Dubcenco E, Baker JP, Ottaway CA, Tang SJ, Zanati SA, et al. Given capsule endoscopy in celiac disease: Evaluation of diagnostic accuracy and interobserver agreement. The American Journal of Gastroenterology. 2005;**100**:685-694

[18] Cobrin GM, Pittman RH, Lewis BS. Increased diagnostic yield of small bowel tumors with capsule endoscopy. Cancer. 2006;**107**:22-27

[19] Gastineau S, Viala J, Caldari D, Mas E, Darviot E, Le CC, et al. Contribution of capsule endoscopy to Peutz-Jeghers syndrome management in children. Digestive and Liver Disease. 2012;**44**:839-843

[20] Bandorski D, Höltgen R, Stunder D, Keuchel M. Capsule endoscopy in patients with cardiac pacemakers, implantable cardioverter defibrillators and left heart assist devices. Annals of Gastroenterology. 2014;**27**:3-8

[21] Stanich PP, Kleinman B, Betkerur K, Mehta Oza N, Porter K, Meyer MM. Video capsule endoscopy is successful and effective in outpatients with implantable cardiac devices. Digestive Endoscopy. 2014;**26**:726-730

[22] de Franchis R, Avgerinos A, Barkin J, Cave D, Filoche B, ICCE. ICCE consensus for bowel preparation and prokinetics. Endoscopy. 2005 Oct;**37**(10):1040-1045

[23] Marshall CA, Cave DR. Preparation for video capsule endoscopy: A clear choice? Gastrointestinal Endoscopy. 2017;**85**:194-195

[24] Holden JP, Dureja P, Pfau PR, Schwartz DC, Reichelderfer M, Judd RH, et al. Endoscopic placement of the small-bowel video capsule by using a capsule endoscope delivery device. Gastrointestinal Endoscopy. 2007;**65**:842-847

[25] Spada C, Hassan C, Galmiche JP, Neuhaus H, Dumonceau JM, Adler S, Epstein O, et al. European Society of Gastrointestinal Endoscopy. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2012;**44**:527-536

[26] Matsuoka H, Nakamura A, Masaki T, et al. Comparison between endorectal coil and pelvic phased array coil MRI in patients with anorectal tumor. American Journal of Surgery. 2003;**185**:328-332

[27] Drew PJ, Farouk R, Turnbull LW, et al. Preoperative magnetic resonance staging of rectal cancer with an endorectal coil and dynamic gadolinium enhancement. The British Journal of Surgery. 1999;**86**:250-254

[28] Iafrate F, Laghi A, Paolantonia P, et al. Preoperative staging of rectal cancer with MR ımaging: Correlation with surgical and histopathologic findings. Radiographics. 2006;**26**: 701-714

[29] Brown G, Radcliffe AG, Newcombe RG, et al. Preoperative assessment of prognostic factors in rectal cancer using high-resolution magnetic resonance imaging. The British Journal of Surgery. 2003;**90**:355-364

[30] Karantanas AH, Yarmenitis S, Papanikolaou N, Gourtsoyiannis N. Preoperative imaging, staging of rectal cancer. Digestive Diseases. 2007;**25**:20-32

[31] Beynon J, Mortensen NJ, Foy DM, et al. Preoperative assessment of local invasion in rectal cancer: Digital examination, endoluminal sonography or computed tomography? The British Journal of Surgery. 1986;**73**:1015-1017

[32] Bipat S, Glas AS, Slors FJ, et al. Rectal cancer: Local staging and assessment of lymph node involvement with endoluminal US, CT, and MR imaging a metaanalysis. Radiology. 2004;**232**:773-783

[33] Merkel S, Mansmann U, Siassi M, et al. The prognostic inhomogeneity in pT3 rectal carcinomas. International Journal of Colorectal Disease. 2001;**16**:298-304

Chapter 2

Liquid Biopsy for Colorectal Cancer Screening, A Modern Approach for Patients Stratification and Monitoring

Octav Ginghina and Cornelia Nitipir

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72701

Abstract

Despite great advances have been made in oncologic approaches, the morbidity and mortality caused by colon cancer are still overwhelming. Particularly, the intra- and inter-tumour heterogeneity makes accurate sampling challenging and often leads to failure of even modern therapeutic strategies. Moreover, tumour molecular genotype can suffer alterations over time, triggering suboptimal therapeutic outcomes as a result of irrelevant information provided by histological biopsies. Daily, tumour cells shed into the bloodstream at the early stages of the disease. These circulating tumour cells (CTCs) can be detected and analysed after enrichment, providing this way valuable information in real time. Furthermore, apoptotic and/or necrotic tumour cells discharge DNA fragments into the circulating bloodstream. Elevated levels of these so-called circulating tumour DNA (ctDNA) fragments can be identified in the peripheral blood of patients as compared to healthy individuals. In this view, the detection and characterization of the CTCs and ctDNA are a real-time "liquid biopsy" that has been developed for accurate tumour monitoring and molecular characterization. This modern non-invasive analytical approach allows consecutive sampling to monitor CTC number and tumour genetic changes over time without the need of tissue biopsy. Consequently, "liquid biopsies" can be used to screen for cancer, stratify patients to the optimum treatment and to monitor the patient's response to treatment or identify treatment resistance. This chapter offers an overview of the following approaches with respect to liquid biopsies: CTCs and ctDNA. Some of the analytical techniques and challenges in the detection of these rare events will also be presented here.

Keywords: colorectal cancer, liquid biopsy, circulating tumour cells, ctDNA, tumour heterogeneity

1. Introduction

Uncontrolled division and growth of human cells and subsequent invasion to other tissue via the circulatory and the lymphatic systems are commonly known as cancer.

One of the cancer types known to affect the gastrointestinal (GI) tract and rated third most commonly diagnosed form irrespective of gender is colorectal cancer (CRC).

In a context outlined by alarming figures of both prevalence (with a lifetime CRC risk of 5.1%) and high mortality rate, CRC being second in line among cancer-related causes of deaths in both genders, advances in therapy have become particularly significant; thus, in addition to established liver resection, outcomes in survival rate have been greatly improved in recent years due to better means for earlier detection, advances in chemotherapy and therapies based on biological agents.

Largely studied nowadays, factors influencing the likelihood of developing CRC are a close family history of genetic changes in that respect (20% of cases), notably associated with certain genetic syndromes such as the Lynch syndrome (hereditary nonpolyposis colorectal cancer, HNPCC) in ca. 3% of cases [1] and familial adenomatous polyposis with its variant the Gardner syndrome (in a further ca. 1% of CRC instances).

HNPCC-related CRC risk factors include early onset (ca. 44 average age) triggered by an autosomal dominant inheritance, development in 70% of cases in splenic flexure proximity as well as a surplus of synchronous (18% of all patients) and metachronous CRC (45% of patients) following segmental resection or hemicolectomy. In addition to a CRC cause, Lynch syndrome frequently also results in other carcinoma types such as ovary and endometrial, other gastrointestinal location (stomach, small intestine), pancreas as well as transitional carcinoma in the renal pelvis and the ureter [2].

This high metastatic disease potential is the main cause of CRC lethal outcome and may be attributed to the contribution of a specific gene (I(MACCI)); already isolated [3], this is a transcriptional factor able to influence the expression of the hepatocyte growth factor and therefore associated with proliferation of CRC cells, scattering and new tissue invasion and further tumour growth and metastasis, as shown in cell cultures and animal studies in mice. MACCI close involvement as contributor to occurrence of metastasis makes it a novel target for CRC approach, which needs confirmation by further studies and clinical trials [4].

Contribution of genetic factors in CRC development combines with action of epigenetic ones at cell level.

However important the role of genetic features, most CRC may rather be the outcome of chronic intestinal inflammation preceding tumour development, gut microbiota and such environmental factors as life style (diet included) and food and environmental-borne mutagens [5, 6].

Among chronic intestinal inflammations responsible for CRC risk, inflammatory bowel disease (under 2% of CRC cases every year [7]), Crohn's disease and ulcerative colitis need special

mention for being the frequent cause of tumour growth [8], the risk growing with the severity of inflammation and the duration of the disorder [9]. Statistically, in that respect, 10 years' duration of Crohn's disease results in CRC in 2% of patients, and the risk increases four times and nine times for 20 and 30 years' durations, respectively [7]. On the other hand, a history of over 30 years of ulcerative colitis results in development of some type of cancer precursor or CRC in ca. 16% patients [7].

2. Tumour progression

As regards CRC development, it typically originates in benign, premalignant or malignant polyps occurring at the level of the colon or rectum epithelial lining (as, for instance, hyperplastic polyps, tubular adenoma or colorectal adenocarcinoma, respectively). Such abnormalities are the result of inherited or acquired oncogenic and inactivating mutations revealed by complex genome scale analysis, which has shown the existence of hypermutated and non-hypermutated CRC tumour categories [10].

Among non-hypermutated types, one commonly occurring mutation affects the Wnt signalling pathway, leading to increased signalling activity and emerging at the level of the intestinal crypt stem cell [11].

Most frequently, the mutated CRC-related gene is the *APC* gene. APC protein prevents β-catenin protein accumulation. The absence of APC leads to β-catenin accumulation and translocation to the cell nucleus, where it binds to the DNA, thus activating transcription of proto-oncogenes. Although normally playing an important role in stem cell renewal and differentiation, when inappropriately expressed and highly accumulated, these proto-oncogene products can induce cancer.

In addition to the absence of the APC protein, high β-catenin-related CRC may also be determined by β-catenin (CTNNB1) mutation, blocking its very own breakdown, or occurrence of mutations in other APC similarly operating genes (e.g., AXINI, AXIN2, NKDI, TCF71.2) [12].

Besides deficient Wnt signalling pathways, realization of the cancer potential requires additional mutations. Usually, action of Wnt pathway defects is prevented by intervention of the cell division monitoring p53 protein, a product of the TP53 gene, which normally eliminates flawed cells. Thus, a mutation arising in the TP53 gene may reverse the potential from benign epithelial tumour cell into invasive epithelial cell.

If not affecting the p53-encoding gene, mutations may instead target a different protein playing a protective role, i.e., the BAX12 but also ARDI A, CTNNB I, SOX9, FAM123B and ATM.

On the other hand, hypermutated tumours progress through specific genetic events and display MSH3, MSH6, TGFBR2, ACVR2A, SLC9A9, BRAF and TCF71.2 mutated forms.

Whichever the tumour type, all these genes are involved in the Wnt and TGF-β signalling pathways, leading to higher MYC activity, as major CRC factor [13]. Role of "field defects"/"field cancerisation", the concept first emerged in the early 1950s to refer to an area of the epithelium

featuring a preconditioning responsible for cancer predisposition of the area in question [14]. Despite unclear origins at the time of their introduction, the terms define premalignant tissue as potential sites for new cancer.

In time, research has progressively emphasized the importance of "field defects" in advance to CRC, and the assumption was confirmed by studies showing almost the exclusive use of discrete neoplastic foci for in vitro research and well-defined tumours for in vivo studies [15] in all cancer research.

In addition, as research further indicated, the majority of somatic mutations occurring at tumour level emerged during development of apparently normal cells [16], at the site of "field defects" (and therefore in preneoplastic stage).

A further addition to terminology refers to the term "aetiologic field effect", based on the "field defect" concept and referring to molecular and pathologic changes in preneoplastic cells at molecular level. The term also covers the extent to and manner in which exogenous environmental factors as well as molecular changes in the local microenvironment influence neoplastic progression throughout [17].

3. Epigenetic factors in tumour growth

However important mutation-induced genetic alterations, epigenetic alterations are significantly more common in CRC and involve hundreds of genes. As revealed by research, (Vogelstein and colleagues) oncogene mutations and suppressor mutations (both known as "driver mutations") are rather limited in average CRC forms (1–2 and 1–5, respectively), although accompanied by an estimated 60 additional so-called "passenger" mutations [18].

Common types of epigenetic cancer-related alterations modifying gene expression levels by action on the different types of RNA (miRNAs) may involve abnormal methylation of DNA in tumour suppressor promoters [19], such as reduced expression of miR-137 because of methylation of the miR-137-encoding DNA sequence in the CpG island [20]. Altered miR-137 expression triggers drastic (2- to 20-fold) alteration of mRNA expression of the target genes and related slighter changes in expression of proteins produced by the genes.

There are further microRNAs, of comparable numbers of target genes, which undergo even more frequent epigenetic alterations of field defects in the colon, resulting in specific CRC forms [21].

As common is direct hyper-/hypo-methylation of CpG islands of protein-encoding genes as well as histone alterations or modification of chromosomal architecture, with influence on gene expression [22]. Research has recently outlined the potential of early epigenetic decline in expression of DNA repair enzyme as cause of cancer characteristic genomic and epigenomic instability [18].

4. Colorectal cancer (CRC)

4.1. CRC clinical manifestations

Although CRC clinical signs vary with tumour location in the intestine as well as with the presence of metastases, medical practice has outlined certain warning signs and symptoms now considered typical, such as loss of appetite, weight loss, vomiting and/or nausea, rectal bleeding and anaemia in the over 50 age group [23], severe and persistent constipation and modified stools (accompanied by blood elimination and/or diminished thickness) [24]; weight loss and changed bowel habit may be considered a warning only if accompanied by bleeding [22].

4.2. CRC diagnosis

4.2.1. Diagnostic steps

Typically, CRC may be diagnosed by the sampling of colon areas suspected of tumour development during procedures suitable for the lesion site, i.e. colonoscopy or sigmoidoscopy.

Once the tumour is confirmed, the level of the disease needs to be determined, which is generally done by a CT scan involving the chest, abdomen and pelvis, but also by position imaging tomography and MRI, for certain cases.

The next diagnostic step is to determine the stage of the tumour, based on the TNM cancer staging system (where T stands for primary tumour stage, N for the presence of regional lymph nodes and M for remote metastasis). Staging criteria include the extent of initial tumour spreading, the presence and site of lymph nodes and the metastasis level [25].

4.2.2. Microscopic examination

Adenocarcinoma is a malignant tumour of the epithelium, whose source lies in the superficial glandular epithelial cells of the colon and cecum lining. This tumour invades the colon/cecum wall and further progressively permeates the respective layers (first the muscularis mucosae, then the submucosa and lastly the muscularis propria). Tumour cells in question are organized as irregular tubular, multistratified structures, featuring multiple lumens and decreased stroma (in a back-to-back growth pattern). In addition, in some cases, tumour cell lacks of cohesion may be observed, as well as a secretion of mucus pervading the interstitium and resulting in extensive mucus/colloid (optically "empty" spaces) pools (forming the so called "mucinous (colloid)"), poorly differentiated adenocarcinoma. Mucus remaining within the walls of the tumour cell drives the nucleus towards the cell membrane, and the "signet-ring cell" emerges.

In fact, differentiation may vary in adenocarcinoma, contingent on cellular pleomorphism, glandular architecture and muco-secretion of the predominant pattern; thus, three variants of adenocarcinoma may be observed as regards the degree of cell differentiation: well differentiated, moderately differentiated and poorly differentiated [26].

CRC cell characteristics may be determined by analysis of tissue samples harvested by biopsy or during surgery. The pathology report provides data on cell type and grade. In CRC, the most common cell (98% of cases) is adenocarcinoma, but other types may also occur in rare cases (squamous cell carcinoma and lymphoma) [27].

4.2.3. Immunochemistry

It is generally considered that more than half of CR adenomas and up to 90% of CRC tumours present overexpression of the COX (cyclooxygenase)-2, normally absent from healthy colon tissue but acting as fuel for abnormal cell growth [28].

The cancer variant may be determined by histologic examination.

4.2.4. Macroscopic examination

In order to predict the likely course of tumour progression and adequate management, macroscopic examination looks closely at the site of the tumour in the intestine; thus, tumour development on the ascending colon and cecum (the right side of the large intestine) is most often exophytic (growing outwards from the bowel wall), which may in infrequent cases result in faecal obstruction, accompanied by anaemia.

Tumours growing on the left bowel side are largely peripheral and may result in obstruction of the bowel lumen and thinner stools [27].

4.3. CRC prevention

One key approach to CRC (as for other cancers, in fact) and unanimously recognized as such is prevention; closer surveillance and healthier lifestyle can essentially contribute to CRC prevention.

Therefore, research has greatly been focused on effective means in that respect, in all areas of intervention.

As regards lifestyle, diets are currently recommended to include more significant amounts of vegetables, fruits and whole grains and decrease consumption of white flour products, sugars and red meat.

As in other areas of healthcare, physical exercise has been proved to be beneficial, though less significant for preventing or reducing colon cancer risk [29, 30]. However, avoiding prolonged sitting as a daily routine is important [31].

Medication has also been the target of research, which has shown the potential of aspirin and celecoxib to reduce CRC danger in high-risk groups as determined by assessment of family medical history and other personal risk factors, though not in average risk ones [32–35].

Calcium supplementation is currently under study as well, not with sufficient evidence yet.

As for protection factors, in vitro studies have shown that intake and blood levels of vitamin D act as one, as have lactic acid bacteria, due to their antioxidant activity, immunomodulation

as well as promotion of programmed cell death, proliferative effects and epigenetic alteration of cancer cells [36].

Screening is an important and effective means for prevention and early detection of cancer in general and CRC in particular, the more so as most CRC cases (>80%) originate in adenomatous polyps [37].

As mentioned above, screening is also a very important means for cancer diagnosis before the emergency of actual symptoms (by 2–3 years) [25].

Close relatives of HNPCC patients need accurate and structured screening, according to a well-designed programme and schedule [38], as in certain countries such as Canada, the United Kingdom, Australia and the Netherlands [39–41].

Therefore, these should undergo a first routine colonoscopy at the age of 25, which, as a routine, should be repeated every 3 years, in the case of negative results, and every year should an adenoma be found. In cases where routine colonoscopy reveals the presence of cancer, subtotal colectomy needs to be performed.

In addition, for women, ovarian ultrasound and endometrial biopsy need to be performed as early as 25 years old.

Screening tests have been devised and researched, current practice now relying mostly on colonoscopy (both standard and virtual via CT scan), faecal occult blood testing, multitarget stool DNA screening and flexible sigmoidoscopy [25].

Although with proven efficacy in other respects, sigmoidoscopy is the only procedure able to provide screening of the right side of the colon, the site for almost half (42%) of malignancies [42].

Equally effective, standard colonoscopy is less costly than virtual colonoscopy via CT scan and avoids the additional risk of exposure to radiation and also able to eliminate any potential abnormal growth found [25].

In the 50–75 age group with standard risk factors, screening should include faecal occult blood testing or immunochemical testing every 2 years; an alternative is performance of sigmoidoscopy every 10 years, to the detriment of colonoscopy [43].

For patients with familial adenomatous polyposis, the high-malignancy risk may be offset by total proctocolectomy, ensuring elimination of the risk of both colon and rectal cancers [44].

4.4. CRC management

Given CRC's incurable character, therapeutic decisions in that respect can only be directed to either cure or as a palliative, largely depending on tumour stage [45] but also on other factors as well, such as the patient's health status and even preferences.

4.4.1. Therapy

Surgery can be a means leading to cure but in early stages only, whereas at later stages, when the metastatic disease has also been initiated, the curative potential of surgery decreases, and

palliation (alleviation of tumour-related symptoms and patient's comfort and quality of life) becomes prevalent [25].

For the very first stage, one colonoscopy intervention can suffice to eliminate cancer [46], while the curative potential of surgery decreases with the tumour stage.

Therefore, one stage further, in localized cancers, cure may still be attempted through ample removal associated with ensuring adequate margins, which can be achieved laparoscopically or more often by open laparotomy [25], with colon reconnection, or by colostomy [46].

In the stage of a few emerging metastases, those in the lungs or liver may be eliminated.

In certain cases at this stage, surgery may be preceded by chemotherapy, in an attempt to minimize the tumour before removal.

If recurrence occurs, this mainly involves the lungs and liver [25].

4.4.2. Chemotherapy

This is administered in cases beyond stage 1 CRC, given the curing potential of surgery. No chemotherapy is also customary in CRC stage II; on condition no such risk factors as threats from negative lymph node sampling or the presence of a T4 tumour are present.

Chemotherapy is also not feasible in patients with identified abnormal mismatched repair genes.

On the contrary, chemotherapy is a must and an integral therapy component in stage II and stage IV CRC25, characterized by cancer spreading to remote organs or the lymph nodes; the use of the chemotherapeutic agents oxaliplatin, fluorouracil or capecitabine is instrumental in increasing life expectancy, with the disadvantage of debatable chemotherapy benefits in the case of cancer-free lymph nodes.

Turn to palliative care becomes necessary where CRC has become extensively metastatic or may not be resected, opening the alternative for several different chemotherapy medications [25], including, oxaliplatin, fluorouracil, capecitabine, irinotecan and tegafur/uracil [47, 48].

4.4.3. Radiotherapy

Given bowel sensitivity to radiation, patient with colon cancer treatment cannot benefit from addition of radiation to chemotherapy, although this may be effective for rectal cancer [25]. The same was for chemotherapy; radiotherapy may be used as neoadjuvant and adjuvant in certain rectal cancer stages only [49].

4.4.4. Palliation

For patients with incurable CRC forms, palliative care, though not a promising cure, may bring the benefit of better quality of the patient's life both directly and indirectly, via the life of their families, lessening symptoms and anxiety and also reducing the need of hospital admission [50].

Palliation is typically symptom directed and consists of procedures designed to improve symptoms or minimize the possibility of complications such as abdominal pain, tumour bleeding and/or bowel obstruction [51], thus contributing to improved quality of life.

Such procedures may include surgery, for elimination of cancer tissue to some extent, without attempting to cure, placement of a stent or performing a bypass of part of the bowel.

Non-surgical palliative care approaches include pain medication and/or radiotherapy aiming to reduce the tumour size [52].

4.4.5. Follow-up

The main purpose of follow-up is to obtain the earliest identification of later metachronous lesions, i.e., metastases or tumours not originating from the initial cancer [53].

As an underlying measure for cancer survivors, exercise as a mainstay of lifestyle may be useful as secondary therapy, as shown by results indicating important reduction in 8-oxo-dG in the urine of patients after taking moderate exercise for 2 weeks of following primary therapy [54].

4.5. CRC prognosis

The most commonly used prognosis criterion is the 5-year survival rate, which is under 60% for CRC in Europe, whereas this is the cause of death for one third of CRC patients [25] in most developed countries. The reason for these unexpectedly low outcomes despite evident progress in new therapeutic means and their improved availability worldwide is mainly CRC late identification (stage IV already present in 20% of patients seeking medical attention), with potentially resectable isolated liver metastasis in ca. 25% of these patients. Of these 25%, one third of patients undergoing resection achieve 5-year survival [55, 56].

5. Liquid biopsy

Despite the major advances in cancer therapies, the morbidity and mortality associated with this disease are still enormous. Tumour heterogeneity holds the main responsibility underlying inefficient treatment and failure of current therapeutic strategies, including the targeted therapies. For efficiency reasons, the molecular targeted therapies require constant monitoring of the tumour genome, but harvesting consecutive tissue biopsies is very difficult and inconvenient for medical and economic reasons. Therefore, the lack of real-time information regarding tumour heterogeneity during the disease evolution most commonly results in the treatment failure and requires the development of novel approaches. In this view, liquid biopsies offer a tool for real-time screening of disease particularities, stratify patients for the best treatment and also monitor the response of the treatment. Due to their non-invasive nature, liquid biopsies can be used for repeated sampling to monitor tumour genetic alterations over time, avoiding this way consecutive tissue biopsies. Liquid biopsies analyse circulating tumour cells, cell-free tumour DNA and/or exosomes, known as tumour-circulating markers.

5.1. Circulating tumour cells (CTCs)

Circulating tumour cells (CTCs) have been identified during the 1800s and presumed responsible for the metastatic process [57]. These cells are of epithelial origin and shed from the tumours in the peripheral blood of patients where they can be enriched, detected and analysed.

The detection of CTCs in the peripheral blood of patients with cancer holds a great promise for the future development of efficient anticancer therapies. However, due to the very low concentrations of CTCs in the peripheral blood (one tumour cell for millions of normal blood cells), their detection and identification still remain challenging and require high analytical sensitivity and specificity methods, which usually consist in a combination of enrichment and detection [58].

CTC enrichment strategies include a wide range of technologies based on those CTC particularities that can discriminate them out of the normal haematopoietic cells. Concrete CTCs can be detected based on physical properties such as size, density, electric charges, deformability or biological properties such as cell surface marker expression and viability. CTC separation based on their physical properties holds the great advantage of being done without labelling the cells. Some of these methods include density gradient centrifugation, filtration, photoacoustic flow cytometry, microfluidics, etc. [59, 60].

Nevertheless, the biological properties of the CTCs hold a major role in their identification, mainly based on immunobead assays. These assays use antibodies targeting tumour-associated antigens (positive selection) or leukocyte-specific antigens such as CD45 (negative selection) in order to detect and separate CTCs from the blood cells. The positive selection usually targets the epithelial cell adhesion molecule (EpCAM). Subsequently, CTCs are confirmed with antibodies against cytokeratins (CKs) [59]. Among the current EpCAM-based technologies, the US Food and Drug Administration approved CellSearch® system (Veridex) which is the current "gold standard" for all new CTC-detection methods. According to this standard, CTCs are nucleated cells that express the epithelial cell adhesion molecule and cytokeratins but lack the expression of the common leukocyte CD45 marker ($EpCam^+$_$CK18/19^+$_$DAPI^+$_$CD45^-$ cells).

Interestingly, some CTCs undergo the epithelial to mesenchymal transition (EMT) and loose critical epithelial markers. Capturing CTCs' lacking EpCAM expression requires the use of antibody cocktails against a panel of epithelial cell surface antigens such as HER2, MUC-1, EGFR and folate-binding protein receptor and against mesenchymal or stem cell antigens such as c-MET, N-cadherin and CD318 [61].

Regardless of the enrichment method, the isolated CTCs still contain a significant number of normal blood cells, and therefore CTCs should be next identified by a method that can discriminate between malignant cells and normal blood cells. The CellSearch® system as well as other assays is based on the fluorescent staining of the cells for the following markers: CKs (positive marker), the common leukocyte antigen CD45 (negative marker) and a nuclear dye (4,6-diamidino-2-phenylindole, DAPI).

Functional EPISPOT (for EPithelial ImmunoSPOT) assay has been introduced for CTC analysis in order to detect only the viable CTCs, able to produce metastases [62].

Other alternatives to immunologic assays of viable CTC-detection target specific mRNAs. A commercially available RNA-based CTC assay is the AdnaTest™ (AdnaGen), which uses nonquantitative RT-PCR to identify cells that express the transcripts of tumour-specific genes after immunomagnetic capture of MUC-1, HER2 and EpCAM cells [63].

5.2. Circulating tumour DNA (ctDNA)

Cell-free DNA (cfDNA) is a powerful tool for its potential use in a wide range of clinical fields such as cancer research [64, 65], non-invasive prenatal testing [66] and transplant rejection diagnostics [67]. Most cfDNA in plasma is highly fragmented (150–180 bp) [68] with a higher prevalence of tumour-associated mutations in the shorter fragments [69]. In patients suffering from cancer, a fraction of the cfDNA is tumour-derived and is known as circulating tumour DNA (ctDNA).

cfDNA reaches the systemic circulation by various pathologic or normal physiologic mechanisms [70]. However, with respect to solid tumours, the ctDNA is usually released as a result of necrosis or autophagy [71]. Notably, unlike apoptosis, necrosis generates larger DNA fragments [72]. Cancer patients generally have much higher levels of cfDNA than healthy individuals [73, 74]. ctDNA carries genomic and epigenomic alterations according to the tumour genomic alterations (copy number variation, point mutations, microsatellite instability, degree of integrity, loss of heterozygosity, rearranged genomic sequences, DNA methylation, etc.) [75]. Only on the basis of these biological characteristics, ctDNA can be discriminated from normal cfDNA. Consequently, after its validation ctDNA could be used as a specific biomarker that provides personalized information to detect residual disease or monitor tumour progression during therapy.

Due to the high degree of fragmentation as well as the small fraction of ctDNA within the cfDNA, the analysis of ctDNA is challenging and requires highly sensitive techniques. Classical methods of analysis include qRT-PCR, fluorescence and spectrophotometric approaches [76–78]. Digital droplet PCR has been developed as a high sensitive tool to detect ctDNA [79]. This technique consists in a droplet-based system [80, 81], a microfluidic platform [82, 83] and the so-called BEAMing strategy [84, 85]. Additionally, next-generation sequencing technology is currently used in plasma DNA analysis in order to identify ctDNA alterations [86–88].

6. Conclusions

There is increasing evidence that circulating tumour markers such as CTCs and ctDNA offer real-time information regarding cancer progression and tumour genotype in the view of a better systemic therapy management with direct impact on patient's disease prognosis. Additionally, future characterization of these circulating markers could contribute to approach-specific-targeted therapies to a certain population of cancer patients.

Acknowledgements

This work was done under the project PN-IIIP2-2.1-PTE-2016-0149/19PTE - TUMFLOW, financed by UEFISCDI.

Author details

Octav Ginghina[1,2*] and Cornelia Nitipir[3]

*Address all correspondence to: sciencecontactemail@gmail.com

1 Department of Surgery, "Sf. Ioan" Emergency Clinical Hospital, Bucharest, Romania

2 Department II, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy Bucharest, Bucharest, Romania

3 Department of Clinical Oncology, Elias Emergency Clinical Hospital, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

References

[1] Watson AJ, Collins PD. Colon cancer: A civilization disorder. Digestive Diseases. 2011;**29**(2):222-228

[2] Lynch HT, Lynch J. Clinical implications of advances in the molecular genetics of colorectal cancer. Tumori. 1995;**81**(Suppl):19-29

[3] Stein U, Walther W, Artlt F, et al. MACCI, a newly identified key regulator of HGF-MET signaling predicts colon cancer metastasis. Nature Medicine. 2008;**15**(1):59-67

[4] Stein J. MACCI-a novel target for solid cancers. Expert Opinion on Therapeutic Targets. 2013;**17**(9):1039-1052

[5] Yang K, Kurihara N, Fan K, Newmark H, Rigas B, Bancroft L, Corner G, Livote E, Lesser M, Edelmann W, et al. Dietary induction of colonic tumors in a mouse model of sporadic colon cancer. Cancer Research. 2008;**68**:7803-7810

[6] Rustgi AK. The genetics of hereditary colon cancer. Genes & Development. 2007;**21**: 2525-2538

[7] Triantafillidis JK, Nasioulas G, Kosimidis PA. Colorectal cancer and inflammatory bowel disease: Epidemiology, risk factors, mechanisms of carcinogenegis and prevention strategies. Anticancer Research. 2009;**29**(7):2727-2737

[8] Jawad N, Direkze N, Leedham SJ. Inflammatory bowel disease and colon cancer. Recent Results in Cancer Research. 2011;**185**:00115

[9] Xie J, Itzkowitz SH. Cancer in inflammatory bowel disease. World Journal of Gastroenterology. 2008;**14**(3):378-389

[10] Muzny DM, Bainbridge MN, Chang K, et al. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;**487**(7407):330-337

[11] Ionov Y, Peinado MA, Malkhosyan S, et al. Ubiquitous somatic mutations in simple repeated sequence reveal a new mechanism for colonic carcinogenesis. Nature. 1993;**363** (6429):558-561

[12] Markowitz SD, Betagnolli MM. Molecular origin of cancer: Molecular basis of colorectal cancer. The New England Journal of Medicine. 2009;**361**(25):2449-2460

[13] Saughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium;clinical implication of multicentric origin. Cancer. 1953;**6**(5):963-968

[14] Rubin H. Fields and field cancerization: The preneoplastic origins of cancer: Asymptomatic hyperplastic fields are precursors of neoplasia, and their progression to tumors can be tracked by saturation density in culture. BioEssays. 2011;**33**(3):224-231

[15] Vogelstein B, Papadopoulos N, Velculescu VF, et al. Cancer genome landscapes. Science. 2013;**339**(6127):1546-1558

[16] Lochhead P, Chan AT, Nishihara R, et al. Etiologic field effect: Reappraisal of field effect in cancer predisposition and progression. Modern Pathology. 2014;**28**:14-29

[17] Bernstein C, Prasad AR, Nfonsam V, et al. DNA damage, DNA repair and cancer. In: Chen C, editor. New Research Directions in DNA Repair. In-Tech. 2013. ISBN: 978-51-114-6

[18] Schuebel KE, Chen W, Cope L, et al. Comparing the DNA hypermethylome with gene mutations in human colorectal cancer. PLoS Genetics. 2007;**3**(9):e157

[19] Balaguer F, Link A, Lozano JJ, et al. Epigenetic silencing of miR-137 is an early event colorectal carcinogenesis. Cancer Research. 2010;**70**(16):6608-6618

[20] Deng G, Kakar S, Kim YS. MicroRNA-124a and microRNA-34b/c are frequently methylated in all histological types of colorectal cancer and polys, and in the adjacent normal mucosa. Oncology Letters. 2011;**2**(1):175-180

[21] Schnekenburger M, Diederich M. Epigenetic offer new horizons for colorectal cancer prevention. Current Colorectal CancerReports. 2012;**8**:66-81

[22] Astin M, Griffin T, Neal RD, et al. The diagnostic value of symptoms for colorectal cancer in primary care: A systematic review. British Journal of General Practitioners. 2011;**61**(586):231-243

[23] Alpers DH, Kallo AN, Kaplowtz N, et al. In: Yamada T, editor. Principles of Clinical Gastroenterology. Chichester: Wiley Blackwell; 2008. p. 381. ISBN: 978-1-4051-6910-3

[24] Terzic J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology. 2010;**138**:2101-2114.e5

[25] Kopetz S, Chang GJ, Overman MJ, Eng C, Sargent, DJ, Larson DW, Grothey A, Vauthey JN, Nagorney DM, McWilliams RR. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. Journal of Clinical Oncology. 2009;**27**:3677-3683

[26] Triantafillidis JK, Nasioulas G, Kosmidids PA. Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies. Anticancer Research. 2009;**29**(7):2727-2737

[27] Sostres C, Gargallo CJ, Lanas A. Aspirin, cyclooxygenase inhibition and colorectal cancer. World Journal of Gastrointestinal Pharmacology and Therapeutics. 2014;**5**(1):40-49

[28] Campos FG, Logullo Waitzberg AG, Kiss DR, et al. Diet and colorectal cancer evidence for etiology and prevention. Nutrición Hospitalaria. 2005;**20**(1):18-25

[29] Harriss DJ, Atkinson G, Batterham A, et al. Colorectal Cancer, Lifestyle, Exercise and Research Group. Lifestyle factors and colorectal cancer risk (2): A systematic review and meta-analysis of associations with leisure – time physical activity. Colorectal Disease. 2009;**11**(7):689-701

[30] Biswas A, PI O, Faulkner GE, et al. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: A systematic review and meta-analysis. Annals of Internal Medicine. 2015;**162**(2):123-132

[31] Cooper K, Squires H, Carrol C, et al. Chemoprevention of colorectal cancer: Systematic review and economic evaluation. Health Technology Assessment. 2010;**14**(32):1-206

[32] Agency for Healthcare Research and Quality. Aspirin or Anti Inflammatory Drugs for the Primary Prevention of Colorectal Cancer. United States Department of Health & Human Services; 2010/2011

[33] Weingarten MA, Zalmanovici A, Yaphe J, et al. Dietary calcium supplementation for preventing colorectal cancer and adenomatous polyps. Cochrane Database of Systematic Reviews. 2008;**1**:CD003548

[34] Ma Y, Zhang P, Wang F, et al. Association between vitamin D and risk of colorectal cancer: A systematic review of prospective studies. Journal of Clinical Oncology. 2011; **29**(28):3775-3782

[35] Zhong L, Zhang X, Covasa M. Emerging role of lactic acid bacteria in protection against colorectal cancer. World Journal of Gastroenterology. 2014;**20**(24):7878-7886

[36] What I Do to Reduce My Risk of Colorectal Cancer. Centers for Disease Control and Prevention; April 2, 2014. Retrieved March 5, 2015. https://www.cdc.gov/cancer/colorectal/basic_info/prevention.htm

[37] Westergaard H. Colorectal cancer the role of screening and surveillance. Journal of Investigative Medicine. 1996;**44**:216-227

[38] NHS Bowel Cancer Screening Programme. Available from: www.cancerscreening.nhs.uk

[39] Home-Bowel Cancer Australia. Available from: www.bowelcanceraustralia.org

[40] Bevolkingsonderzoek darmkanker. Available from: www.rivm.nl

[41] Siegel RL, Ward EM, Jemal A. Trends in colorectal cancer incidence rates in the United States by tumor location and stage, 1992-2008. Cancer Epidemiology Biomarker and Prevention. 2012;**21**(3):411-416

[42] Bacchus CM, Dunfield L, Connor GS, et al. Task Force on Prevention Health Care. Recommendations on screening for colorectal cancer in primary care. CMAJ: Canadian Medical Association Journal de1'Association medicale canadienne. 2016;**188**(5):340-348

[43] Moslein G, Pistorius S, Saeger H, et al. Preventive surgery for colon cancer in familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer syndrome. Langerbecks. Archives of Surgery. 2003;**388**(1):9-11

[44] Stein A, Atanackovic D, Bokemeyer C. Current standards and new trends in the primary treatment of colorectal cancer. European Journal of Cancer. 2011;**47**(Suppl 3):S312-S314

[45] Colon Cancer Treatment (PDQ®). National Cancer Institute (NCI). May 12, 2014. Retrieved June 29, 2014. https://www.cancer.gov/types/colorectal/hp/colon-treatment-pdq

[46] Bruera G, Ricevuto E. Intensive chemotherapy of metastatic colorectal cancer: weighing between safety and clinical efficacy. Evaluation of Masi G, Loupakis F, Salvatore L, et al. Bevacizumab with FOLFOXIRI (irinotecan, oxaliplatin, fluorouracil, and folinate) as first-line treatment for metastatic colorectal cancer: a phase 2 trial. The Lancet Oncology. 2010;**1111**:845-852

[47] Devita Jr VT, Theodore SL, et al. Devita, Hellmanand Rosenberg's Cancer: Principles & Practice of Onocology. 8th ed. Philadelphia: Wolters Kluwer/Lippincott William's Wilkins; 2008. p. 1258

[48] Higginson IJ, Evans CJ. What is the evidence that palliative care teams improve outcomes for cancer patients and their families. Cancer Journal. 2010;**16**(5):423-435

[49] Wasserberg N, Kauffan HS. Palliation of colorectal cancer. Surgical Oncology. 2007;**16**(4):299-310

[50] Amersi F, Stamos MJ, Ko CY. Palliative care for colorectal cancer. Surgical Oncology Clinics of North America. 2004;**13**(3):467-477

[51] National Comprehensive Cancer Network (PDF). Available from: www.ncc.org

[52] Betof AS, Dewhist MW, Jones LW. Effects and potential mechanisms of exercise training on cancer progression. A translational perspective. Brain, Behavior, and Immunity. 2013;**30**:S75-S87

[53] Figueredo A, Rumble BR, Maroun J, et al. Follow-up of patients with curatively resected colorectal cancer: A practice guideline. BMC Cancer. 2003;**3**(26):1-23

[54] Qaseem A, Denberg TD, Hopkins RH, et al. Screening for colorectal cancer: A guidance statement from the American College of Physicians. Annals of Internal Medicine. 2012;**156**(5):378-386

[55] Available from: https://web.archive.org/web/200660925051637/ http:// www.cancer.org/docroot/PRO/content/PRO_1_1_Cancer_Statistics_2006_Presentation. asp

[56] Simmonds PC, Primrose JN, Colquitt JL, et al. Surgical resection of hepatic metastases from colorectal cancer: A systematic review of published studies. British Journal of Cancer. 2006;**94**(7):982-999

[57] Recamier JCA. L'histoire de le meme maladie. Gabor. 1956;**1829**:110

[58] Alix-Panabieres C, Pantel K. Circulating tumor cells: Liquid biopsy of cancer. Clinical Chemistry. 2013;**59**(1):110-118

[59] Alix-Panabières C, Schwarzenbach H, Pantel K. Circulating tumor cells and circulating tumor DNA. Annual Review of Medicine. 2012;**63**:199-215

[60] Parkinson DR, Dracopoli N, Gumbs Petty B, Compton C, Cristofanilli M, Deisseroth A, et al. Considerations in the development of circulating tumor cell technology for clinical use. Journal of Translational Medicine. 2012;**10**:138

[61] Pecot CV, Bischoff FZ, Mayer JA, Wong KL, Pham T, Bottsford-Miller J, et al. A novel platform for detection of CKand CKCTCs. Cancer Discovery. 2011;**1**:580-586

[62] Alix-Panabières C. EPISPOT assay: Detection of viable DTCs/CTCs in solid tumor patients. Recent Results in Cancer Research. 2012;**195**:69-76

[63] Andreopoulou E, Yang LY, Rangel KM, Reuben JM, Hsu L, Krishnamurthy S, et al. Comparison of assay methods for detection of circulating tumor cells (CTCs) in metastatic breast cancer (MBC): AdnaGen AdnaTest BreastCancer select/detect™ versus Veridex CellSearch™ system. International Journal of Cancer. 2012;**130**:1590-1597

[64] Luo J, Shen L, Zheng D. Diagnostic value of circulating free DNA for the detection of EGFR mutation status in NSCLC: A systematic review and meta-analysis. Scientific Reports. 2014;**4**:6269

[65] Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Science Translational Medicine. 2014;**6**:224ra24

[66] Song K, Musci TJ, Caughey AB. Clinical utility and cost of non-invasive prenatal testing with cfDNA analysis in high-risk women based on a US population. The Journal of Maternal-Fetal & Neonatal Medicine. 2013;**26**:1180-1185

[67] Macher HC, Suárez-Artacho G, Guerrero JM, et al. Monitoring of transplanted liver health by quantification of organ-specific genomic marker in circulating DNA from receptor. PLoS One. 2014;**9**:e113987

[68] Jiang P, Chan CW, Chan KC, et al. Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**:E1317-E1325

[69] Diehl F, Li M, Dressman D, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:16368-16373

[70] Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Research. 2001;**61**(4):1659-1665

[71] Roninson IB, Broude EV, Chang BD. If not apoptosis, then what? Treatment-induced senescence and mitotic catastrophe in tumor cells. Drug Resistance Updates. 2001;**4**(5):303-313. DOI: 10.1054/drup.2001.0213

[72] Wang BG, Huang HY, Chen YC, Bristow RE, Kassauei K, Cheng CC, et al. Increased plasma DNA integrity in cancer patients. Cancer Research. 2003;**63**(14):3966-3968

[73] Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nature Medicine. 2008;**14**(9):985-990. DOI: 10.1038/nm.1789

[74] Kohler C, Barekati Z, Radpour R, Zhong XY. Cell-free DNA in the circulation as a potential cancer biomarker. Anticancer Research. 2011;**31**(8):2623-2628

[75] Marzese DM, Hirose H, Hoon DS. Diagnostic and prognostic value of circulating tumor-related DNA in cancer patients. Expert Review of Molecular Diagnostics. 2013;**13**(8):827-844. DOI: 10.1586/14737159.2013.845088

[76] Bjorkman L, Reich CF, Pisetsky DS. The use of fluorometric assays to assess the immune response to DNA in murine systemic lupus erythematosus. Scandinavian Journal of Immunology. 2003;**57**(6):525-533

[77] Tuaeva NO, Abramova ZI, Sofronov VV. The origin of elevated levels of circulating DNA in blood plasma of premature neonates. Annals of the New York Academy of Sciences. 2008;**1137**:27-30. DOI: 10.1196/annals.1448.043

[78] Chen Z, Feng J, Buzin CH, Liu Q, Weiss L, Kernstine K, et al. Analysis of cancer mutation signatures in blood by a novel ultra-sensitive assay: Monitoring of therapy or recurrence in non-metastatic breast cancer. PLoS One. 2009;**4**(9):e7220. DOI: 10.1371/journal.pone.0007220

[79] Vogelstein B, Kinzler KW. Digital PCR. Proceedings of the National Academy of Sciences of the United States of America. 1999;**96**(16):9236-9241

[80] Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, et al. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Analytical Chemistry. 2011;**83**(22):8604-8610. DOI: 10.1021/ac202028g

[81] Pekin D, Skhiri Y, Baret JC, Le Corre D, Mazutis L, Salem CB, et al. Quantitative and sensitive detection of rare mutations using droplet-based microfluidics. Lab on a Chip. 2011;**11**(13):2156-2166. DOI: 10.1039/c1lc20128j

[82] Forshew T, Murtaza M, Parkinson C, Gale D, Tsui DW, Kaper F, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Science Translational Medicine. 2012;**4**(136):136ra68. DOI: 10.1126/scitranslmed.3003726

[83] Wang J, Ramakrishnan R, Tang Z, Fan W, Kluge A, Dowlati A, et al. Quantifying EGFR alterations in the lung cancer genome with nanofluidic digital PCR arrays. Clinical Chemistry. 2010;**56**(4):623-632. DOI: 10.1373/clinchem.2009.134973

[84] Dressman D, Yan H, Traverso G, Kinzler KW, Vogelstein B. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(15):8817-8822. DOI: 10.1073/pnas.1133470100

[85] Higgins MJ, Jelovac D, Barnathan E, Blair B, Slater S, Powers P, et al. Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. Clinical Cancer Research. 2012;**18**(12):3462-3469. DOI: 10.1158/1078-0432.CCR-11-2696

[86] Ignatiadis M, Dawson SJ. Circulating tumor cells and circulating tumor DNA for precision medicine: Dream or reality? Annals of Oncology. 2014;**25**(12):2304-2313. DOI: 10.1093/annonc/mdu480

[87] Haber DA, Velculescu VE. Blood-based analyses of cancer: Circulating tumor cells and circulating tumor DNA. Cancer Discovery. 2014;**4**(6):650-661. DOI: 10.1158/2159-8290.CD-13-1014

[88] Lanman RB, Mortimer SA, Zill OA, Sebisanovic D, Lopez R, Blau S, et al. Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. PLoS One. 2015;**10**(10):e0140712. DOI: 10.1371/journal.pone.0140712

Chapter 3

Treatment Strategies in Colorectal Cancer

Hamid Elia Daaboul and Mirvat El-Sibai

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71620

Abstract

Colorectal cancer is known to be one of the most commonly diagnosed cancers worldwide. It maintains a high mortality rate despite the newest methodological therapeutic approaches adopted in various academic establishments. The treatment modalities in colorectal cancer follow the degree of disease progression based on staging information. Earliest the cancer is diagnosed, the highest the possibility to be cured. Different strategies are being involved in treating colorectal cancer, starting from simple endoscopic polypectomy to remove a potential malignant polyp, to wider surgical intervention to get rid of a primary unmetastasized tumor, to other concomitant radio-chemotherapy combinations to reduce a bulky tumor rendering it operable, ending in more sophisticated chemotherapeutical regimens combined with targeted drugs to shrink the metastatic lesions and prolong survival rate. Different new treatments are being investigated with a sole aim to preserve the patient's quality of life and extend life span.

Keywords: colorectal cancer, colorectal polyps, chemotherapy, targeted therapy, immunotherapy, Lynch syndrome, familial adenomatous polyposis syndrome

1. Introduction

Colorectal cancers (CRC) are considered the third most commonly diagnosed cancers in the world. The incidence and mortality rates vary worldwide from lowest in Africa and Asia to highest in Australia, North America, and Europe. The etiology is mainly due to changes in dietary habits, from low-fiber ingestion to high-fat diet, increased body mass index (BMI), low physical activity, cigarette smoking, alcohol consumption, diabetes mellitus, ulcerative colitis, Crohn's disease, some inherited syndromes (familial adenomatous polyposis syndrome and nonpolyposis colorectal cancer or Lynch syndrome (LS), MUTYH-associated and Turcot-associated polyposis syndromes, Peutz-Jeghers syndrome, Juvenile polyposis syndrome, and Cowden syndrome), in addition to radiation therapy for another abdominal

cancer [1–3]. Early diagnosis is necessary to get full remission, and screening has proved to be fundamental in decreasing the mortality rate. The treatment of CRC is multidisciplinary and implies a collaboration of many therapeutic teams including surgical, chemotherapy, as well as radiotherapy experts. In the following chapter, we will be studying the different treatment modalities and strategies approved and administered worldwide, according to CRC stages.

The staging classification of CRC has been conceived according to collaboration between the Union for International Cancer Control (UICC) and the seventh edition of the American Joint Committee on Cancer (AJCC-7), taking into consideration the Dukes' staging with its modifications by Astler-Coller (MAC) and Kirklin system (**Table 1**).

Stage	UICC/AJCC			Dukes	MAC
0	Tis	N0	M0	—	—
I	T1	N0	M0	A	A
	T2	N0	M0	A	B1
II A	T3	N0	M0	B	B2
B	T4a	N0	M0	B	B2
C	T4b	N0	M0	B	B3
III A	T1–T2	N1–N1c	M0	C	C1
	T1	N2a	M0	C	C1
B	T3–T4a	N1–N1c	M0	C	C2
	T2–T3	N2a	M0	C	C1–C2
	T1–T2	N2b	M0	C	C1
C	T4a	N2a	M0	C	C2
	T3–T4a	N2b	M0	C	C2
	T4b	N1–N2	M0	C	C3
IV A	Any T	Any N	M1a	D	D
B	Any T	Any N	M1b	D	D

Tis, Tumor confined to the mucosa; T1, tumor invades the submucosa; T2, tumor invades the muscularis propria; T3, tumor invades subserosa or beyond, without invading other organs; T4, tumor invades nearby organs (T4a, without perforation of visceral peritoneum; T4b, with perforation of visceral peritoneum); N1, metastasis to one to three regional lymph nodes (RLNs) (N1a, metastasis to one RLN; N1b, metastasis to two to three RLNs; N1c, metastasis into areas of fat near lymph nodes but not the nodes themselves); N2, metastasis to four or more RLNs (N2a, metastasis to four to six RLNs; N2b, metastasis to seven or more RLNs); M1, distant metastases present (M1a, metastasis to distant organ, as the liver or lung, or distant set of lymph nodes; M1b, metastasis to distant organs, to distant set of lymph nodes, or to distant parts of the peritoneum as the lining of the abdominal cavity).

Table 1. Anatomic AJCC-7 staging for CRC.

2. Treatment of CRC by stage

2.1. Treatment of stage 0 CRC

In stage 0 colorectal cancer, the tumor is still confined to the inner lining of the colon (T in situ). A surgical removal of the cancer is all that is needed. A polypectomy or a colonoscopic local excision is usually sufficient. Partial colectomy is required in case of bigger tumors.

2.2. Treatment of malignant polyps

2.2.1. Definition, classification, and staging

Malignant polyps are adenomas that have been identified histologically, after endoscopic excision, to be adenocarcinomas which have invaded through the muscularis mucosa into the basic submucosa (pT1) [4]. They can occur sporadically or as part of a polyposis syndrome. They can be classified endoscopically by their size and morphology and histologically as favorable (low risk) and unfavorable (high risk). In 1985, Haggitt reconceived the Japanese society classification and the Paris endoscopic classification into a new one taking into consideration the level of invasion depth (**Table 2**).

Despite the big advantage and wide use of Haggitt's classification in assessing the quality of resection of endoscopic polypectomies, the sessile, flat, or depressed lesions yet were not successfully evaluated using this classification. In the early 1990s, Kikuchi succeeded in quantifying the grade of vertical and horizontal submucosal invasion, dividing the invasion into three levels (**Table 3**).

Morphologically, polyps are known to be either pedunculated or sessile. Pedunculated polyps are usually attached to the colonic mucosa via a stalk of variable length, while sessile polyps are devoid of stalk, are flattened in shape, and overlay the mucosa with less separation of the adenomatous epithelium part from the underlying layers of the colon [5].

Histologically, polyps can be divided into low-risk versus high-risk features (**Table 4**) [4].

Level	Location of carcinoma
0	Carcinoma in situ or confined to the mucosa. Not invasive
I	Carcinoma invading through the muscularis mucosa into the submucosa but limited to the head of the polyp
II	Carcinoma invading the level of the neck of the polyp
III	Carcinoma invading in any part of the stalk of the polyp
IV	Carcinoma invading into the submucosa of the bowel wall below the stalk of the polyp but above the muscularis propria

Table 2. Haggitt's classification according to the level of invasion.

Submucosal level	Submucosal invasion
Sm1*	Characterizes lesions that are limited to the upper third of the submucosal layer
Sm1a	Submucosal invasion under one fourth of tumoral width
Sm1b	Submucosal invasion between one fourth and a half of the tumoral width
Sm1c	Horizontal affection of the superior third of the submucosa over half of the tumoral width
Sm2	Characterizes lesions that are limited to the middle third of the submucosal layer
Sm3	Characterizes lesions that are limited to the lower third of the submucosal layer

*Sm1 lesions are further subdivided into three categories (a, b, and c) with regard to the degree of horizontal involvement of the upper submucosal layer (B), to the horizontal involvement of the total lesion (A). B/A ratios of 0.25, 0.25–0.5, and >0.5 correspond to a, b, and c, respectively.

Table 3. Kikuchi's classification according to submucosal invasion level.

Low-risk features (favorable)	High-risk features (unfavorable)
• Pedunculated (levels 1–3 according to Haggitt classification)	• Tumor budding
• Well-differentiated adenocarcinoma	• Poorly differentiated adenocarcinoma (grade 3)
• Free resection margin (2 mm)	• Positive, indeterminate, or <1 mm resection margin
• En bloc resection	• Piecemeal removal
• Neither lymphatic nor vascular invasion	• Presence of either lymphatic or vascular invasion
• Submucosal invasion Sm < 1 mm	• Submucosal invasion Sm* >1 mm

*While Sm1a + b lesions have a very low risk for metastasis, the malignant potential increases with depth of submucosal invasion [6].

Table 4. Polyp classification according to histological criteria.

2.2.2. Treatment

All of the aforementioned classifications are mandatory for accurate assessment of the degree of malignancy and aggressiveness of the resected polyp for rational clinical decision. Studies have shown polyps smaller than 5 mm in diameter, have negligible risk of malignancy, and are easily managed by standard techniques of endoscopic snare removal. Protruding polyps (Haggitt levels I, II, or III) with favorable histological features should be subjected to local excision or endoscopic polypectomy. Haggitt level IV lesions with favorable histology are considered low risk and can be favorably managed with endoscopic polypectomy provided margins are safe (>2 mm). Haggitt level IV protruding polyps and/or polyps exhibiting unfavorable features should be surgically excised due to the high incidence of lymph node metastasis. Excision can be performed either through traditional open approach or via more conservative laparoscopic techniques [7]. For sessile non-protruding polyps, a wider excision should be reconsidered requiring endoscopic mucosal resection (EMR) or endoscopic submucosal dissection

(ESD) [4]. Endoscopic mucosal resection is more specific for removal of sessile polyps limited to the mucosa and submucosa (Sm1a + b) and is typically used for complete excision of lesions up to 2 cm [8]. Endoscopic submucosal dissection is usually adopted for larger gastrointestinal lesions, where it more easily promotes the en bloc resection, yet it carries greater risk of perforation (31%) and late bleeding (15%) [9]. Lesions with a deep level of invasion (Sm1c, Sm2, or Sm3) or rectal lesions (specifically those of the distal third) showed higher incidence of lymph node metastasis 12–25% and should be treated by a definitive oncologic segmental resection due to the high risk of regional lymph node involvement.

2.2.3. Surveillance

Local recurrence is basically common in managed malignant polyps. Regular endoscopic follow-up is recommended to detect any disease recurrence; however, the duration of subsequent surveillance varies [10, 11]. In favorable histological criteria, protruding (levels I, II, or III), and noninvasive Sm1a + b polyps, it is recommended that a colonoscopy be carried out 3 months after the polypectomy [12, 13]. Further regular checkup is advised within 1, 3, and 5 years [14]. In malignant pedunculated polyps with unfavorable histological criteria, the risk of relapse or residual lesions reached 39% in treated patients. These patients are also found to have distant metastasis on follow-up, even 5 years after surgery [15]. Accordingly, in addition to the regular endoscopic surveillance, monitoring the serum level of carcinoembryonic antigen (CEA) and imaging techniques as computerized tomography or magnetic resonance imaging would enable early detection of disease recurrence. According to the American Cancer Society and the US Multi-Society Task Force on Colorectal Cancer's guidelines, shorter follow-up intervals are recommended in case of senility, positive family history, or hereditary nonpolyposis colorectal cancer (HNPCC). Furthermore, endoscopic ultrasound or flexible sigmoidoscopy at 3- to 6-month intervals for the first 2 years after polypectomy can be considered for detecting early curable recurrences.

2.3. Treatment of stage I CRC

Stage I CRC includes T1 and T2, where cancer is still limited to and has not yet invaded the layers of the colon into other nearby organs. T1 cancers are usually parts of polyps that were discussed hereinabove. For T2 cancers, the standard of care consists of partial colectomy with regional lymph node dissection. A laparoscopic-assisted colectomy can be an acceptable choice for patients who are not candidates for open colectomy.

Stage I adenocarcinoma of the rectum is relatively rare, and a surgical removal of the cancer is usually curable. For the low-risk stage I rectal cancer, both endoscopic resection and transanal excision can be used. Transanal endoscopic microsurgery (TEM) is a transanal operation suitable for small tumors and not too far from the anus. It involves wide excision of all layers of the invaded rectum with the surrounding tissue to secure negative margins. If the cancer is located in the upper part of the rectum, a low anterior resection (LAR) is recommended, where the incision takes part across the abdomen to remove the affected rectum along with some surrounding tissue and lymph nodes, and followed by anorectal anastomosis. If the cancer occupies the lower part of the rectum (alongside the anus), an abdominoperineal resection

(APR) with permanent colostomy is advised, when the distance between tumor and anus is too short to allow safe anastomosis. No additional therapy is needed after these operations, unless the surgeon finds the cancer with high-risk features. Then, an adjuvant concomitant chemoradiotherapy is appropriate with 5-fluorouracil (5-FU) or capecitabine [16].

2.3.1. Surveillance

Regular follow-up testing after the end of treatment aims at seizing any early disease recurrence. Colonoscopy should be repeated 1 year after therapy completion. In case of normal results, the next checkup should be after 3 years and then after 5 years. In case of finding any advanced adenoma (polyps with ruffled structure, larger than 1 cm, or with high-grade dysplasia), colonoscopy should be repeated within 1 year [17].

2.4. Treatment of stage II CRC

2.4.1. Assessing risk factors

The role of adjuvant chemotherapy remains undetermined in stage II CRC. Surgical intervention should aim at a wide resection of the tumor with the involved bowel segment, all together with cutting out of the lymphatic system draining that part. The resection should include at least 5 cm colon segment of either side of the resected tumor. For adequate tumor staging (II or III), and to determine and eliminate any possible lymph node metastases (pN), at least 12 lymph nodes should be excised and subjected to histological analysis. Partial colectomy may be the only needed treatment for low- and medium-risk stage II CRC patients. High-risk patients should be subjected to chemotherapy if one of the following risk factors was identified:

- High pT4 stage (T4 or tumor invading into adherent organs)
- Suboptimal lymph node resection (less than 12)
- Presence of lymphovascular or perineural invasion
- Bowel obstruction or perforation
- Poorly differentiated histology
- High carcinoembryonic antigen (CEA) marker level
- Positive margins

Various additional risk factors are being implied in assessing the additive benefit to the high-risk factors in stage II colorectal cancer using adjuvant chemotherapy.

One of the most promising risk factors is the microsatellite instability (MSI)/mismatch repair (MMR), which is regarded as a good prognostic factor. Microsatellites are short, tandemly repeated DNA sequences in the genome that are susceptible to errors of DNA replication in the presence of a defective mismatch repair (MMR) system. They are detected in about 15% of all colorectal cancers and can be used to determine stage II patients who are at very low risk of recurrence and with low benefit of adjuvant chemotherapy [18, 19]. Moreover, it has been

established in a multivariate analysis that microsatellite instability was significantly associated with survival advantage independently of any other prognostic factors (hazard ratio (HR) 0.42; 95% confidence interval 0.27–0.67; $p < 0.001$) [20].

Another potential predictive colorectal marker is the allelic deletion of chromosome 18q, or the loss of heterozygosity (LOH) of chromosome 18, which is considered as a bad prognostic factor. The 18q loci hold several genes that are highly related to apoptosis and carcinogenesis. Patients (stage II or III) presenting 18qLOH were found to have less disease-free survival and overall survival than those with retained chromosome 18 (DFS 44% versus 64%, $p = 0.002$; OS 50% versus 69%, $p = 0.005$) [21].

Another prognostic marker in CRC is the expression of guanylyl cyclase C (GCC) in resected lymph nodes. GCC is a protein that is usually expressed by intestinal cells but universally overexpressed in colorectal cancer. GCC is an intestinal tumor-suppressing receptor which regulates epithelial homeostasis. Silencing of GCC contributes to tumorigenesis by reflecting dysregulation of the cell cycle and DNA repair [22]. The presence of GCC in resected lymph nodes reflects the detection of prognostically important occult metastases [23].

The Kirsten rat sarcoma (*KRAS*) oncogene is a proto-oncogene involved in the normal tissue signaling pathways. *KRAS* mutation can occur via a single amino acid substitution or a single nucleotide substitution. The resulting protein is implicated in various malignancies, including colorectal cancer [24]. Even though the British QUASAR trial in 2007 did not succeed to show any significant difference in overall survival between fluorouracil-treated and folinic acid–treated observation groups in stage II CRC [25], the risk of disease recurrence was found significantly higher for *KRAS*-mutant than *KRAS* wild-type tumors (28% versus 21%), and the risk of recurrence appeared larger in *KRAS*-mutant rectal than colon tumors [26].

The tumor suppressor TP53, or genome guardian, is another important predictive prognostic factor in CRC. TP53 is the most commonly mutated gene in human cancers, and its prevalence in CRC comprises 34% of the proximal colon tumors where it is mostly related to lymphatic invasion and 45% of the distal colorectal tumors where it is majorly correlated with lymphovascular invasion [27]. Clinical studies have shown that CRC patients with mutant p53 are more 5-fluorouracil-based chemotherapy resistant and have poorer prognosis than those with wild-type p53 [28].

The transforming growth factor beta (TGF-β) signaling pathway plays a central but paradoxical role in the predisposition and progression of colorectal cancer. TGF-β acts as a potent tumor suppressor in normal intestinal epithelial cells by inhibiting cell proliferation and inducing apoptosis. However, mutations in the genes encoding for TGFB receptor 2 (TGFBR2), with high levels of microsatellite instability, promote colon tumorigenesis by perturbing the function of TGF-β signaling pathways and stimulating the proliferation and invasion of poorly differentiated and metastatic colon cancer cells [29, 30].

Thymidylate synthase (TS) is an enzyme implicated in the formation of thymidine, one of DNA nucleotides. It catalyzes the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). This role in nucleotide metabolism has made TS an important target of many chemotherapeutic agents such as 5-FU and the new folate-based TS inhibitors

(raltitrexed and pemetrexed). Elevated intracellular TS levels have been implicated in emerging resistance to fluoropyrimidines and other TS inhibitors due to the increase in transcription and translation roles of TS. Therefore, high TS expression in early-stage CRC patients is correlated to a poorer overall survival in both chemotherapy-treated and chemotherapy-untreated patients following surgery [31].

2.4.2. Choice of chemotherapy

5-Fluorouracil remains the backbone chemotherapy in treating CRC. In MOSAIC study, patients with stage II or III disease were randomly assigned to receive adjuvant FOLFOX4 or 5-FU/leucovorin (LV). In stage II disease, no improvement in DFS or OS was noted in 899 patients upon adding oxaliplatin to 5-FU (DFS HR = 0.84, p = 0.258; OS HR = 1.00, p = 0.986). Moreover, in patients with high-risk stage II disease, the estimated 10-year overall survival was 75.4% in FOLFOX arm versus 71.7% in 5-FU/leucovorin arm (p = .058) [19]. Similar results were obtained with the NSABP C-07 trial, where patients were randomized to receive either bolus 5-FU/LV alone or with oxaliplatin. While the addition of oxaliplatin to 5-FU/LV improved DFS, no benefit in OS was observed at all [32]. Furthermore, the QUASAR study investigated the role of adjuvant 5-FU in disease recurrence in "average-risk" patients (patients without any high-risk feature). As a result, 5-FU decreased the risk of recurrence compared to observation alone (relative risk (RR) for colon cancer = 0.78, p = 0.004; RR for rectal cancer =0.68, p = 0.004). And, the risk of death was improved in treated patients (RR = 0.84; p = 0.046), with an absolute survival benefit of 3.6% [25]. A major predictive prognostic factor in stage II CRC is microsatellite instability. As known, microsatellites are repeated DNA sequences in the genome. They are very susceptible to errors in DNA replication and especially in case of a defective mismatch repair (MMR) system, where they really can substitute it [19]. In colon cancer, the high level of MSI is associated with mutations in the MMR system. Based on findings from over 7000 patients classified as MSI-high (MSI-H), MSI-low (MSI-L), or MSI-stable (MSS) colon cancers, those with MSI-H had a better prognosis compared to those with MSI-L or MSS tumors by 15% [33]. Another important predictive factor in stage II CRC is 18qLOH. Loss of heterozygosity of chromosome 18 is highly associated with decreased overall survival [21, 34, 35]. The ECOG 5202 trial aimed at stratifying patients according to the molecular prognostic factors, MSI and 18qLOH. The recommendations were for stage II patients with low-risk (MSI-H or with either MSS or MSI-L together with 18qLOH retention) observation without any treatment. However, for those with high-risk observation (either MSS or MSI-L with 18qLOH), chemotherapy with FOLFOX is suggested [19].

As a conclusion for stage II CRC adjuvant treatment, the following algorithm is reasonable (**Table 5**).

2.4.3. Access to radiotherapy

According to Johns Hopkins colorectal health team, radiotherapy can be used adjuvantly in case of pT4, where the lesion is fixed and adherent to the abdominal wall or bladder, as it provides a lower chance of recurrence. Similarly, in case of rectal cancer, neoadjuvant chemoradiotherapy is indicated in order to shrink the tumor size prior to surgery and to avoid colostomy if

Low risk (with MSI-H or with either MSS or MSI-L and retention of 18qLOH)	Observation
Average risk (with MSS)	Observation or fluoropyrimidine* as single agent (optional)
High risk**:	• Fluoropyrimidine as single agent
• (with MSI-H and 18qLOH retention)	• FOLFOX or CAPOX***
• (with MSS or MSS-L and 18qLOH)	

*Fluoropyrimidines are a class of antimetabolites that are converted in the body to 5-fluorouracil. These include 5-fluorouracil, capecitabine, doxifluridine, tegafur, and carmofur.

**The high-risk factors are mentioned hereinabove in the text.

***FOLFOX denotes folinic acid/5-FU/oxaliplatin; CAPOX denotes capecitabine/oxaliplatin.

Table 5. Stage II CRC adjuvant treatment algorithm.

possible. Radiotherapy is indicated when the rectal tumor has invaded the wall of the bowel or has spread into adjacent lymph nodes. 5-Fluorouracil or capecitabine are being used concomitantly with radiotherapy to sensitize tumor cells to radiation. In addition, concomitant chemoradiotherapy is indicated when the margins of resection are positive. However, no significant differences in overall survival were reported till now. EORTC 22921 was one randomized trial of 1011 patients that assessed the role of adjuvant 5-FU after preoperative chemoradiation for patients with T3 or T4 resectable tumor. Patients were divided in four arms including preoperative radiotherapy with or without chemotherapy and preoperative radiotherapy with or without chemotherapy followed by adjuvant chemotherapy. The OS for a median follow-up of 10.4 years was similar in the four groups (48.4–52.9%). There were no differences either in DFS rates or in the cumulative incidence of distant metastases [36]. A number of treatment strategies have been recently studied by various clinical trials, yet still no conclusive decisions have been taken. The major aim remains the patient's benefit from a better tumor resection with less side effects, longer survival, and minor recurrence rates.

2.4.4. Surveillance

Survivorship care is a follow-up that takes place after the end of treatment to provide a better disease control and a less recurrence morbidity. A thorough physical examination with a tumor marker CEA should be performed systematically every 3–6 months for 2 years. In case of normal results, the frequency can be reduced to 6 months for an additional 3 years. Radiological imaging including CT scans or MRIs is indicated once a year for a total of 5 years. Colonoscopy is also suggested at an interval of 1 year after treatment and then after 3 and 5 years if results are normal.

2.5. Treatment of stage III CRC

Stage III colon cancer is characterized by tumor of any size (T1–T4) with metastasis to regional lymph nodes. A partial colectomy to remove the involved part of the colon along with adjacent lymph nodes, followed by adjuvant chemotherapy (not beyond 8 weeks of surgery), is considered

the standard of care for this stage. However, in rectal cancer, tumor size (T3–T4, with invasion through intestinal muscular layer) with clinical positive lymph nodes is suggestive for neoadjuvant chemoradiotherapy and followed by adjuvant chemotherapy for a lower risk of recurrence rate. The European Society for Medical Oncology (ESMO) guidelines recommended in 2013 a stratification of the risk factors for disease recurrence of rectal cancer according to the following items identified by pretreatment MRI. These included the tumor invasion depth (T staging), the number of metastatic lymph nodes (N staging), the distance to anus, invasion of mesorectal fascia (MRF), and extramural vascular invasion (EMVI). Four risk groups were stratified (ultralow-, low-, medium-, and high-risk groups). Surgery alone was the choice for the ultralow-risk group, while neoadjuvant chemoradiotherapy with adjuvant chemotherapy was the best choice for the medium- and high-risk groups; the low-risk group showed a beneficial effect of adding chemoradiotherapy or chemotherapy [37]. These findings are compatible with the NCCN guidelines which recommended neoadjuvant chemoradiotherapy and adjuvant chemotherapy for those patients with high risk of local recurrence, including stage II (T3–T4, with tumor invading through the intestinal muscle layer) and stage III (positive lymph nodes) [17].

2.5.1. Choice of chemotherapy

After a wide surgical resection with anastomosis, the standard chemotherapy protocol is approved to be oxaliplatin and 5-FU/folinic acid (FOLFOX4 or FLOX). In the MOSAIC study, the addition of oxaliplatin to 5-FU/LV (FOLFOX) showed a significantly increased DFS at 6 years, with a reduction in the risk of recurrence of 23% compared with the control arm (5-FU/LV), with an OS absolute gain of 4.2%. Similar results were obtained in the NSABP C-07 study, either in DFS at 3 years or in terms of reduction in the risk of recurrence. As a result of these studies, FOLFOX has been adopted adjuvantly on a biweekly basis, for a period of 12 cycles. In case of contradiction to oxaliplatin, 5-FU/LV administered intravenously according to de Gramont, AIO, or Mayo Clinic regimen, or oral fluoropyrimidines (capecitabine) are comparable in benefit. Other drugs such as topoisomerase I inhibitor (irinotecan) or anti-VEGFR agent (bevacizumab) or *KRAS* wild-type drug (cetuximab) did not succeed in adding any advantage either in DFS or in OS in stage III colon cancer [38].

In neoadjuvant rectal treatment, 5-fluorouracil remains the standard chemotherapeutic agent to be administered concomitantly with radiation. In ASCO 2011, NASBP R-04 firstly randomly compared the effect of capecitabine (an oral fluoropyrimidine) and 5-FU in preoperative concurrent chemoradiotherapy of rectal cancer. The results showed neither significant difference in pathological complete response (pCR) rate nor in third and fourth degree of adverse reaction rate [39]. Recently, in Germany, a randomized clinical phase III multicenter non-inferiority study showed no statistical difference of 3 years of DFS and local recurrence rate between capecitabine and 5-FU, concluding that capecitabine can substitute 5-FU as adjuvant or neoadjuvant chemotherapy for locally advanced rectal cancer [40]. The role of oxaliplatin in radiotherapy has been thoroughly examined as in many randomized studies as STAR-01, ACCORD 12/0405, NSABP R-04, and PETACC 6. Unfortunately, neither study succeeded in showing any significant increase of the pCR rate or downstage rate comparing to single drug (5-FU or

capecitabine). In addition, ACCORD 12/0405 reported same OS (88%) in both combined two drugs (capecitabine with oxaliplatin) and single drug (capecitabine) [39, 41]. As a conclusion, single-agent fluoropyrimidine (5-FU or capecitabine) used concomitantly with pelvic radiotherapy remains the standard of care in stage III CRC.

2.5.2. Surveillance

Regular follow-up is highly advised in stage III CRC due to the high rate of recurrence. Detecting early relapse can be performed through a meticulous regular physical checkup with tumor marker CEA every 3 months for the first 2 years. A thoraco-abdomino-pelvic CT scan is required every 6 months for the first 2 years. A colonoscopy is advised in a 6-month period for the first year after treatment. The period of physical examination with CEA can be elongated for a 6-month period for the following 3 years in case of normal results. The CT scan period can be lengthened to 1 year for the following 5 years, and the colonoscopic evaluation can be further extended to once every 3 years in case of normal previous results.

2.6. Treatment of stage IV CRC

Almost 20–30% of the newly diagnosed CRC patients present with distant metastatic disease at the time of initial presentation. And, up to 50% of the early-stage CRC patients will eventually relapse with metastatic disease. Metastasis can occur in different organs and most commonly to the liver (50–60% of the cases). The lungs are less frequent (10–20%) and are more common in rectal than in colon cancer. Other less often places are the peritoneum, ovaries, adrenal glands, bones, and brain. In case of locally recurrent disease or with resectable metastases, the standard of care remains curative surgical intervention. Chemoradiotherapy or chemotherapy alone can also be considered an acceptable approach in case of rendering a tumor resectable. For non-resectable tumors and/or disseminated metastatic disease, systemic chemotherapy stays the main therapeutic approach. Fluoropyrimidines (5-FU and capecitabine) are the mainstay in all protocols used in metastatic colorectal cancer (mCRC). For nearly 40 years (mid-1950 to 1996), 5-FU was the only agent approved for mCRC treatment. Later on, different cytotoxic agents appeared, as the topoisomerase I inhibitor (irinotecan) and the third-generation platinum analog (oxaliplatin), which both led to considerable advances in mCRC treatment along with fluoropyrimidines. Targeted monoclonal antibodies, such as VEGF inhibitor (bevacizumab) and EGFR inhibitor wild-type *KRAS* (cetuximab and panitumumab), opened a new era in the management of mCRC. Many other promising targeted therapies include the anti-VEGF recombinant fusion protein (ziv-aflibercept), the dual targeting VEGFR2-TIE2 tyrosine kinase inhibitor (regorafenib), the human monoclonal antibody (IgG1) anti-VEGFR2 (ramucirumab), the anti-immune checkpoint programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) (nivolumab and pembrolizumab), and the combination of trifluridine, a nucleoside analog, with tipiracil, an inhibitor of the enzyme thymidine phosphorylase (trifluridine/tipiracil) which have been approved for combination treatment of mCRC, in addition to many other new drugs under investigations.

2.6.1. Choice of chemotherapy

Various randomized clinical trials have been designed to determine the efficacy of oxaliplatin versus irinotecan together with 5-FU/LV or capecitabine. The most well-known trial was the GERCOR C97-3 study conducted by Tournigand and coworkers [42] in France which investigated 5-FU/LV (46-hour infusion) and oxaliplatin (FOLFOX6) compared with 5-FU/LV and irinotecan (FOLFIRI) in mCRC. A similar efficacy was observed in both arms with respect to overall response rate (ORR, 56% versus 54%, respectively), median time to tumor progression (8.5 versus 8.1 months), and median OS (20.6 versus 21.5 months). Similar results were obtained by CALGB Cooperative Group (CALGB 80203) and the Hellenic Cooperative Oncology Group in Greece. Based on these results, both FOLFOX and FOLFIRI have been approved for first-line treatment in mCRC. A meta-analysis of six clinical studies was conducted by Guo et al. [43] to investigate the clinical efficacy of the oral capecitabine (Xeloda) plus irinotecan (XELIRI) versus FOLFIRI regimen in the first-line treatment of mCRC. The results showed no significant differences in terms of ORR, PFS, or OS between the two arms. Another important randomized study to compare XELOX non-inferiority with respect to FOLFOX6 in the first-line treatment of mCRC was conducted by Ducreux and coworkers [44]. No differences were observed between both arms in terms of the clinical efficacy endpoints of ORR (42% versus 46%, respectively), PFS (8.8 versus 9.3 months, respectively), and OS (19.9 versus 20.5 months, respectively). Based on these and many other studies, it has been established that both protocols FOLFOX and FOLFIRI can be safely substituted by oral XELOX and XELIRI in terms of clinical efficacy (PFS and OS).

It is has been proven that doublet chemotherapy has superior clinical efficacy over single-agent fluoropyrimidine chemotherapy. However, a new question emerged: is triplet chemotherapy with 5-FU, oxaliplatin, and irinotecan can provide improved clinical efficacy over doublet chemotherapy? To answer this question, the Gruppo Oncologico Nord Ovest (GONO) of Italy conducted the first randomized phase III study to compare 5-FU/LV, oxaliplatin, and irinotecan (FOLFOXIRI) with FOLFIRI in the front-line setting [45]. After a median follow-up of 5 years, the final analysis confirmed the superiority of the FOLFOXIRI regimen over FOLFIRI, in terms of improved ORR, PFS, and median OS [46]. However, there was significantly higher grade 2/grade 3 neurotoxicity (19% versus 0%) and grade 3/grade 4 neutropenia (50% versus 28%) compared with FOLFIRI, the matter that limits the use of this regimen to relatively more fit patient population (ECOG performance status 0–1).

2.6.2. Chemotherapy in association with targeted therapy

In order to understand the role of targeted therapy in treating mCRC, we should first perceive their mode of action on a molecular level. Bevacizumab (Avastin®) is a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting vascular endothelial growth factor A (VEGF-A), which stimulates angiogenesis in a variety of diseases, including cancer [47]. In 2004, bevacizumab has been approved in the United States for use in combination with standard chemotherapy for metastatic colon cancer. The CALGB/SWOG 80405 phase III randomized study compared the potential benefit of cetuximab and bevacizumab added to conventional chemotherapy (FOLFOX or FOLFIRI) [48]. In contrast to the FIRE study that showed identical ORR and PFS, but a 3.7-month improvement in OS toward the cetuximab arm, CALGB/SWOG 80405

study showed no significant difference at all either in PFS (10.4 versus 10.8 months) or in OS (29.9 versus 29.0 months) in patients treated with cetuximab compared with bevacizumab. Recently, Venook and coworkers investigated the potential effect of primary tumor location on the clinical efficacy of patients treated on CALGB/SWOG 80405 study. It was strange to report that there was a significant improvement in OS ($p < .0001$) for patients with left-sided tumors compared with right-sided tumors (33.3 versus 19.4 months). For the bevacizumab arms, the OS was maintained high in both groups (left-sided tumors versus right-sided tumors) and significantly higher for left-sided primary tumors (31.4 versus 24.2 months). However, in the cetuximab arms, the OS in left-sided tumors was 19.3 months (which was 36.0 months) and only 16.7 months for right-sided tumors. These findings highlighted the importance of sidedness as an important predictive marker and in determining response to anti-EGFR antibody in mCRC [49].

Cetuximab (Erbitux®) and panitumumab (Vectibix®) are both monoclonal antibodies that inhibit the epidermal growth factor receptor (EGFR). Both drugs were approved by the FDA to treat mCRC that exhibit *KRAS* wild-type genes in 2009. However, due to high rate of cetuximab resistance (45%), further studies identified the role of *NRAS* and *BRAF V600E* in treatment response [50]. The *KRAS*, *NRAS*, and *BRAF* are oncogenes that encode proteins involved in the mitogen-activated protein kinase (MAPK) signaling pathway, which regulates cell proliferation and survival. Mutations in these genes are found in about 45%, 4%, and 8% of mCRC, respectively [51], and this is responsible for activating excess proteins, whose activation does not require EGFR upstream signaling, leading to negative feedback loops that limit EGFR activation, the fact that limits the role of anti-EGFR drugs. Therefore, only the wild type of *KRAS* and *NRAS* is indicated for the treatment of EGFR inhibitors. Mutation in *BRAF V600E* was also considered as bad indicator in response to EGFR inhibitors and a strong negative prognostic marker in mCRC. Data from the randomized phase III Medical Research Council COIN trial in mCRC showed an OS of 8.8 versus 14.4 versus 20.1 months, respectively, for patients with *BRAF*-mutant, *KRAS* exon 2-mutant, and *KRAS* exon 2 wild type [52]. Moreover, the presence of *BRAF* mutation in mCRC has been associated with big primary tumors (T4), poor histologic differentiation, and peritoneal carcinomatosis [53–55].

Due to the poor prognosis factor of the BRAF V600E-mutated gene, many trials tried to establish a standard treatment for BRAF-mutated mCRC. Vemurafenib is a BRAF enzyme inhibitor, which interrupts the B-Raf/MEK step on the B-Raf/MEK/ERK pathway, in case where BRAF possesses V600E mutation. In 2017, it has been approved by the FDA for the treatment of late-stage melanoma with BRAF V600E-mutated gene. In 2010, a phase I trial for solid tumors including colorectal cancer was launched to study the effect of vemurafenib (PLX4032) on mCRC patients with mutant BRAF. Unfortunately, the results were not as promising as they were in malignant melanoma, with median PFS of 3.7 months [56]. Loupakis and coworkers studied in a retrospective exploratory analysis of a phase II trial the effect of FOLFOXIRI regimen with bevacizumab on BRAF-mutated mCRC patients. Data found PFS and OS of 11.8 and 24.1 months, respectively [57]. Two limitations were reported in the study: the first was that only patients older than 70 were included, or those who fit (ECOG PS 0) 71–75 old patients, and the second was the rarity of BRAF-mutant patients (8% of the population). In TRIBE phase III study, FOLFOXIRI regimen was studied either with bevacizumab or alone as first-line treatment mCRC, and the median OS was 31 versus 25.8 months in favor of the combination.

However, in the mutant BRAF subgroup, the median OS was 13.4 months [58, 59]. According to ASCO recommendations in 2017, FOLFOXIRI with or without bevacizumab should be considered in patients with a BRAF mutation and good performance status.

The programmed death-ligand 1 (PD-L1) with its receptor programmed cell death protein 1 (PD-1) is T-cell surface checkpoint protein that plays a major role in suppressing the immune system, promoting self-tolerance by downregulating T-cell inflammatory activity, and leading to carcinogenesis [60]. In the recently updated 2017 NCCN guideline, two novel anti-PD-1 antibodies, nivolumab (Opdivo®) and pembrolizumab (Keytruda®), have been indicated as treatment options for patients with unresectable MSI-H- or MMR-deficient CRC, although not yet FDA approved for mCRC [17]. This was based on the interim results of two ongoing studies: KEYNOTE-016, a phase II study of pembrolizumab as monotherapy in MSI-H-/MMR-deficient tumors, and CheckMate 142, a study of nivolumab versus nivolumab combination with ipilimumab, another monoclonal antibody, in recurrent or mCRC. This decision has been taken into account due to the impressive durable response in both studies [61, 62].

Ziv-aflibercept (Zaltrap®), a novel anti-VEGF, is a recombinant fusion protein that consists of vascular endothelial growth factor (VEGF)-binding portions from the extracellular domains of human VEGF receptors 1 and 2 fused to the Fc portion of the human immunoglobulin (IgG) 1 [63]. In 2012, it has been approved by the FDA for use in combination with FOLFIRI for the treatment of patients with mCRC that is resistant to or has progressed following an oxaliplatin-containing regimen treatment. A randomized double-blind placebo-controlled global multicenter phase III VELOUR trial randomized two groups: one to receive FOLFIRI with ziv-aflibercept and the other FOLFIRI with placebo. A statistically significant improvement in OS was observed in patients in the FOLFIRI plus ziv-aflibercept group compared with the FOLFIRI plus placebo group [HR 0.82 (95% CI, 0.71–0.94), p = 0.0032, stratified log-rank test]. The median OS was 13.5 versus 12.06 months, and the median PFS was 6.9 versus 4.7 months, respectively, in the ziv-aflibercept group compared with the placebo group [64].

Regorafenib (Stivarga®), a new oral anti-angiogenic drug, is an oral multi-kinase inhibitor which targets angiogenic, stromal, and oncogenic receptor tyrosine kinase (RTK). It inhibits many membrane-bound and intracellular kinases that are involved in normal cellular functions and pathologic processes, mainly the VEGFR2-TIE2 tyrosine kinase receptors. In 2012, it has been approved by FDA for the treatment of mCRC patients which have been previously treated with fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy and with the anti-VEGF therapy bevacizumab and, if KRAS wild type, with an anti-EGFR therapy. The approval was based on the results of an international randomized (2:1), double-blind, placebo-controlled CORRECT trial. The patients were randomized to get either oral regorafenib or placebo. A statistically significant prolongation in overall survival was observed in regorafenib arm [hazard ratio (HR) 0.77 (95% CI 0.64–0.94), p = 0.0102]. The median survival time was 6.4 versus 5 months in favor of the regorafenib group (phase III, 2011; FDA, 2012) [65].

Ramucirumab (Cyramza®) is a fully human monoclonal antibody (IgG1), which works by blocking the binding of VEGF to its receptor VEGFR2, hence preventing the downstream effect of VEGF in angiogenesis. Recently, it has been approved by FDA for use in combination with

FOLFIRI for the treatment of patients with mCRC whose disease has progressed on a first-line regimen containing bevacizumab, oxaliplatin, and fluoropyrimidine [66]. A randomized double-blind multinational trial divided patients into FOLFIRI plus ramucirumab-receiving group and FOLFIRI plus placebo. A statistically significant improvement in OS was observed in patients who received FOLFIRI plus ramucirumab compared with those who received FOLFIRI plus placebo [median overall survival 13.3 versus 11.7 months; HR 0.85 (95% CI 0.73–0.98), p = 0.023, stratified log-rank test]. The DFS was also in favor of ramucirumab arm (5.7 versus 4.5 months) [67].

Trifluridine/tipiracil (TFD/TPI) (Lonsurf®) is a new combination drug approved in 2015 for the treatment of mCRC. It is a combination of two active components: trifluridine, a nucleoside analog, and tipiracil, a thymidine phosphorylase inhibitor, which prevents trifluridine rapid metabolism, hence increasing its bioavailability. In 2015, it has been approved by the FDA for use in patients with mCRC who have been treated with fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy, an anti-vascular endothelial growth factor (VEGF) biological therapy and an anti-epidermal growth factor receptor (EGFR) therapy, if RAS wild type [68]. Based on a pivotal phase III study (RECOURSE) to assess the efficacy and safety of TFD/TPI compared with that of placebo in a large international population, the outcomes were in favor of TFD/TPI arm, in terms of median OS (7.1 versus 5.3 months) and median PFS (2.0 versus 1.7 months) [69].

2.6.3. Disease recurrence

In case of recurrence, surgical option for liver or lung metastases should be considered in the first place followed by adjuvant chemotherapy, albeit others prefer to administer neoadjuvant chemotherapy for 2–3 months before any metastasectomy. In non-resectable tumors and disseminated metastasis, chemotherapy remains the mainstay in treating disease recurrence. It should be based on non-previously used protocols, i.e., if FOLFOX was used in previous treatment modalities, FOLFIRI should be the right option, and if both FOLFOX and FOLFIRI have been used, the choice shifts toward XELOX or XELIRI. Another alternative is to use infusional 5-FU or oral capecitabine as monotherapies. Other possibilities are the use of newly approved drugs as ziv-aflibercept with FOLOIRI, ramucirumab plus FOLFIRI, oral regorafenib, trifluridine/tipiracil, and nivolumab or pembrolizumab in case of MSI-H or dMMR. According to the last NCCN guideline in 2017, adjuvant Stereotactic Body Radiation Therapy (SBRT) should be considered in some localized lung or liver lesions. Moreover, the hepatic arterial infusion (HAI) pump therapy can be used as a substitute to systemic chemotherapy in unresectable CRC liver metastases, where it demonstrated significant tumor response rates [70]. Chemoembolization or embolization via radioactive beads is another way to treat liver metastases through the hepatic artery in chemorefractory colorectal tumors [71]. In case of peritoneal carcinomatosis, a novel strategy has emerged combining cytoreductive peritonectomy with hyperthermic intraperitoneal chemoperfusion (HIPEC), with a median survival of 3 years [72]. Many other options are being studied to be used as palliative treatment in advanced metastatic disease, such as external-beam radiotherapy, photodynamic therapy, cryotherapy, and radiofrequency ablation, in addition to oncothermia and many others under trials to palliate and manage the disease burden (**Table 6**).

Locally recurrent disease, with resectable metastases	Colectomy + metastasectomy	
Locally recurrent disease (T4)	Chemoradiotherapy or chemotherapy alone followed by colectomy	
Non-resectable tumors and/or disseminated metastatic disease	Chemotherapy	FOLFOX or FOLFIRI (substitutable by XELOX or XELIRI)
		FOLFOXIRI (ECOG good performance status and BRAF V600E mutation)
	Targeted therapy*	Bevacizumab (for right-sided tumors)
		bevacizumab or cetuximab, or panitumumab (for left-sided tumors and wild-type *KRAS* and *NRAS* genes)
		Bevacizumab (for left-sided tumors and mutant-type KRAS and NRAS genes)
Disease recurrence		Nivolumab or pembrolizumab as monotherapy (for MSI-H or dMMR)
		FOLFIRI + ziv-aflibercept
		FOLFIRI + ramucirumab
		Regorafenib as monotherapy
		Trifluridine/tipiracil as monotherapy
		Clinical trials

*Targeted therapy should be added to chemotherapy, unless otherwise mentioned.

Table 6. Metastatic CRC treatment algorithm.

2.7. Miscellaneous

2.7.1. Lynch syndrome and familial adenomatous polyposis syndrome

Lynch syndrome, or hereditary nonpolyposis colorectal cancer (HNPCC), is an autosomal dominant disorder that increases the risk of many types of cancer, including endometrial, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, skin, and particularly colon cancer [73]. It is considered the most common hereditary colorectal diseases and accounts for 1–3% of all CRC. It is associated with inherited mutation in the mismatch repair (MMR) genes MLH1, MSH2, MSH6, and PMS2. This defect in MMR genes leads to tumor DNA microsatellite instability (MSI) and promotes carcinogenesis [74]. For this reason MSI profiling with immunohistochemistry testing for DNA mismatch repair has been considered essential in diagnosing Lynch syndrome (LS). The revised Bethesda guidelines have endorsed the testing for MSI, for families at high risk, in any of the following situations in CRC diagnosed in patients <50 years of age, the presence of Lynch-associated tumors, and MSI-H identified in patients <60 years old, identifying Lynch-related tumors in one or more first-degree relative and in patients <50 years of age and identifying Lynch-related tumors in two or more second-degree relatives regardless

of age [75, 76]. The mainstay in the treatment of Lynch syndrome is colectomy. However, due to the risk of developing synchronous or metachronous secondary tumors, subtotal colectomy with ileorectal anastomosis should be considered in young patients [76]. Recently, three kinds of chemotherapy have been investigated for the treatment of LS: 5-FU with leucovorin, oxaliplatin, and irinotecan. Most studies showed no benefit of chemotherapy in such patients, just one small study on stage IV CRC reported one complete response and three partial responses with MSI-H tumors compared to MSI-L/MSS tumors [77]. The use of acetylsalicylic acid (aspirin) as chemoprevention by patients with LS is highly supported to reduce the risk of CRC [78]. The Colorectal Adenoma/Carcinoma Prevention Programme 2 (CAPP2) trial was conducted to study aspirin chemoprevention that has colorectal cancer as the primary endpoint. The initial findings did not show any significant difference in colorectal adenoma or cancer formation up to 4 years. In 2010, after a longer follow-up (56 months), the results showed a significant decrease in the incidence of CRC and LS-related cancers between the aspirin (600 mg) and placebo groups. Prescription of aspirin for people at high risk was recommended, but the optimum dose and duration of treatment remain to be established, hopefully in CAPP3 [79]. The colonoscopic surveillance in Lynch syndrome is recommended from the age of 20–25 years and repeated at 1–2 years of interval.

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder caused by a germline mutation in the adenomatous polyposis coli (APC) gene, on chromosome 5q21, and characterized by the presence of numerous adenomatous polyps in the colon and rectum. It is responsible for about 1% of all CRC cases, and, often, extracolonic manifestations can take place as in Gardner syndrome (sebaceous cysts, epidermoid cysts, fibromas, desmoid tumors, osteomas, dental anomalies and congenital hypertrophy of the retinal pigment epithelium (CHRPE)), Turcot syndrome (brain tumors), gastric and duodenum polyps, soft tissue tumors, and thyroid cancers. FAP can be subdivided into classical FAP, attenuated FAP (AFAP), and gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) [80]. Clinical diagnosis can be based on the number of polyposis, where more than 100 adenomas can be counted in case of FAP, from 10 to 99 in case of AFAP, and gastric polyps restricted to the body and fundus of the stomach (gastric fundic gland polyposis) in case of GAPPS [81]. Identification of a heterozygous germline pathogenic variant in APC should be confirmed by a molecular genetic testing for a definitive diagnosis. Proctocolectomy with ileal pouch-anal anastomosis (IPAA) is recommended in case of diffuse spreading out of the polyps with severe familial phenotype presence. Total colectomy with ileorectal anastomosis (IRA) is advised in case of scarce adenomas with a mild familial phenotype presence. In AFAP, endoscopic polypectomy can be considered in case of reduced polyposis number. In GAPPS, gastrectomy is recommended since gastric carcinoma is detected in 13% of GAPPS. Regular yearly endoscopic surveillance should be taken into account to detect any disease recurrence. In families with classic FAP, endoscopic evaluation should begin at age of 12–14 years and be continued lifelong in mutation carriers. Regular physical examination and screening via CT scans or MRI for extracolonic manifestations should also start early in life or as soon as colorectal polyposis is diagnosed [82].

MUTYH-associated polyposis (MAP) is another inheritable form of FAP that is caused by autosomal recessive mutations of the MUTYH gene [83]. It accounts for about 10–20% of all polyposis patients [2]. Clinically, in MAP patients, between 20 and 99 adenomas should be present upon endoscopy [84]; however, a molecular genetic testing is necessary to differentiate between APC

and MUTYH mutations [85]. In case of reduce polyposis number, endoscopic polypectomy can be sufficient. In case of polyp dissemination all around the colic frame, IPAA is the treatment of choice, and if the rectum is intact, IRA can be used to conserve it [85, 86]. Regular annual checkup by endoscopy should be maintained in all families presenting MAP disorder [87].

Author details

Hamid Elia Daaboul and Mirvat El-Sibai*

*Address all correspondence to: mirvat.elsibai@lau.edu.lb

Department of Natural Sciences, School of Arts and Sciences, Lebanese American University, Byblos, Lebanon

References

[1] Haggar FA, Boushey RP. Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors. Clinics in Colon and Rectal Surgery. 2009;**22**(04):191-197

[2] Nielsen M, et al. Genotype–phenotype correlations in 19 Dutch cases with APC gene deletions and a literature review. European Journal of Human Genetics. 2007;**15**(10):1034-1042

[3] Johnson CM, et al. Meta-analyses of colorectal cancer risk factors. Cancer Causes & Control. 2013;**24**(6):1207-1222

[4] Aarons CB, Shanmugan S, Bleier JI. Management of malignant colon polyps: Current status and controversies. World Journal of Gastroenterology: WJG. 2014;**20**(43):16178

[5] Gordon PH, Nivatvongs S. Principles and Practice of Surgery for the Colon, Rectum, and Anus. NW, USA: CRC Press; 2007

[6] Kashida H, Kudo S-E. Early colorectal cancer: Concept, diagnosis, and management. International Journal of Clinical Oncology. 2006;**11**(1):1-8

[7] Ramirez M, et al. Management of the malignant polyp. Clinics in Colon and Rectal Surgery. 2008;**21**(04):286-290

[8] Chandrasekhara V, Ginsberg GG. Endoscopic mucosal resection: Not your father's polypectomy anymore. Gastroenterology. 2011;**141**(1):42-49

[9] Takahashi T, et al. Borderline cases between benignancy and malignancy of the duodenum diagnosed successfully by endoscopic submucosal dissection. Scandinavian Journal of Gastroenterology. 2009;**44**(11):1377-1383

[10] Repici A, et al. Endoscopic mucosal resection for early colorectal neoplasia: Pathologic basis, procedures, and outcomes. Diseases of the Colon & Rectum. 2009;**52**(8):1502-1515

[11] Williams J, et al. Management of the malignant colorectal polyp: ACPGBI position statement. Colorectal Disease. 2013;**15**(s2):1-38

[12] Rex DK, et al. Guidelines for colonoscopy surveillance after cancer resection: A consensus update by the American Cancer Society and US Multi-Society Task Force on colorectal cancer. CA: A Cancer Journal for Clinicians. 2006;**56**(3):160-167

[13] Castells A, et al. Clinical practice guideline. Prevention of colorectal cancer. 2009 update. Asociación Española de Gastroenterología. Gastroenterologia y Hepatologia. 2009;**32**(10): 717 e1

[14] Alabi AA, et al. Preoperative serum vascular endothelial growth factor-a is a marker for subsequent recurrence in colorectal cancer patients. Diseases of the Colon & Rectum. 2009; **52**(5):993-999

[15] Bujanda L, et al. Malignant colorectal polyps. World Journal of Gastroenterology: WJG. 2010;**16**(25):3103

[16] Carrara A, et al. Analysis of risk factors for lymph nodal involvement in early stages of rectal cancer: When can local excision be considered an appropriate treatment? Systematic review and meta-analysis of the literature. International Journal of Surgical Oncology. 2012;**2012**

[17] Benson AB, et al. Colon Cancer, version 1.2017, NCCN clinical practice guidelines in oncology. Journal of the National Comprehensive Cancer Network. 2017;**15**(3):370-398

[18] Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010;**138**(6):2073-2087 e3

[19] Dotan E, Cohen SJ. Challenges in the management of stage II colon cancer. Seminars in Oncology. 2011

[20] Gryfe R, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. New England Journal of Medicine. 2000;**342**(2):69-77

[21] Watanabe T, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. New England Journal of Medicine. 2001;**344**(16):1196-1206

[22] Pitari G, et al. The paracrine hormone hypothesis of colorectal cancer. Clinical Pharmacology & Therapeutics. 2007;**82**(4):441-447

[23] Hyslop T, Waldman SA. Guanylyl cyclase C as a biomarker in colorectal cancer. Biomarkers in Medicine. 2013;**7**(1):159-167

[24] Hartman D, et al. Mutant allele-specific imbalance modulates prognostic impact of KRAS mutations in colorectal adenocarcinoma and is associated with worse overall survival. International Journal of Cancer. 2012;**131**(8):1810-1817

[25] Group QC, Adjuvant chemotherapy versus observation in patients with colorectal cancer: A randomised study. The Lancet, 2007. **370**(9604): p. 2020-2029

[26] Hutchins G, et al. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. Journal of Clinical Oncology. 2011;**29**(10):1261-1270

[27] Russo A, et al. The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of p53 mutation: Influence of tumor site, type of mutation, and adjuvant treatment. Journal of Clinical Oncology. 2005;**23**(30):7518-7528

[28] Iacopetta B. TP53 mutation in colorectal cancer. Human Mutation. 2003;**21**(3):271-276

[29] Grady WM, Markowitz SD. Genetic and epigenetic alterations in colon cancer. Annual Review of Genomics and Human Genetics. 2002;**3**(1):101-128

[30] Xu Y, Pasche B. TGF-β signaling alterations and susceptibility to colorectal cancer. Human Molecular Genetics. 2007;**16**(R1):R14-R20

[31] Calascibetta A, et al. Analysis of the thymidylate synthase gene structure in colorectal cancer patients and its possible relation with the 5-fluorouracil drug response. Journal of Nucleic Acids. 2010;**2010**

[32] Yothers G, et al. Oxaliplatin as adjuvant therapy for colon cancer: Updated results of NSABP C-07 trial, including survival and subset analyses. Journal of Clinical Oncology. 2011;**29**(28):3768-3774

[33] Popat S, Hubner R, Houlston R. Systematic review of microsatellite instability and colorectal cancer prognosis. Journal of Clinical Oncology. 2005;**23**(3):609-618

[34] Jen J, et al. Allelic loss of chromosome 18q and prognosis in colorectal cancer. New England Journal of Medicine. 1994;**331**(4):213-221

[35] Bertagnolli M, et al. Presence of 18q loss of heterozygosity (LOH) and disease-free and overall survival in stage II colon cancer: CALGB protocol 9581. Journal of Clinical Oncology. 2009;**27**(15_suppl):4012-4012

[36] Bosset J-F, et al. Fluorouracil-based adjuvant chemotherapy after preoperative chemoradiotherapy in rectal cancer: Long-term results of the EORTC 22921 randomised study. The Lancet Oncology. 2014;**15**(2):184-190

[37] Van Cutsem E, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2014;**25**(suppl_3):iii1-iii9

[38] Labianca R, et al. Primary colon cancer: ESMO Clinical Practice Guidelines for diagnosis, adjuvant treatment and follow-up. Annals of Oncology. 2010;**21**(suppl_5):v70-v77

[39] Roh M, et al. The impact of capecitabine and oxaliplatin in the preoperative multimodality treatment in patients with carcinoma of the rectum: NSABP R-04. Journal of Clinical Oncology. 2011;**29**(15_suppl):3503-3503

[40] Hofheinz R-D, et al. Chemoradiotherapy with capecitabine versus fluorouracil for locally advanced rectal cancer: A randomised, multicentre, non-inferiority, phase 3 trial. The Lancet Oncology. 2012;**13**(6):579-588

[41] Rödel C, et al. Preoperative chemoradiotherapy and postoperative chemotherapy with fluorouracil and oxaliplatin versus fluorouracil alone in locally advanced rectal cancer: Initial results of the German CAO/ARO/AIO-04 randomised phase 3 trial. The Lancet Oncology. 2012;**13**(7):679-687

[42] Tournigand C, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: A randomized GERCOR study. Journal of Clinical Oncology. 2004;**22**(2): 229-237

[43] Guo Y, et al. Capecitabine plus irinotecan versus 5-FU/leucovorin plus irinotecan in the treatment of colorectal cancer: A meta-analysis. Clinical Colorectal Cancer. 2014;**13**(2): 110-118

[44] Ducreux M, et al. Capecitabine plus oxaliplatin (XELOX) versus 5-fluorouracil/leucovorin plus oxaliplatin (FOLFOX-6) as first-line treatment for metastatic colorectal cancer. International Journal of Cancer. 2011;**128**(3):682-690

[45] Falcone A, et al. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: The Gruppo Oncologico Nord Ovest. Journal of Clinical Oncology. 2007;**25**(13):1670-1676

[46] Masi G, et al. Randomized trial of two induction chemotherapy regimens in metastatic colorectal cancer: An updated analysis. Journal of the National Cancer Institute. 2010;**103**(1): 21-30

[47] Los M, Roodhart JM, Voest EE. Target practice: Lessons from phase III trials with bevacizumab and vatalanib in the treatment of advanced colorectal cancer. The Oncologist. 2007;**12**(4):443-450

[48] Venook AP, et al. CALGB/SWOG 80405: Phase III Trial of Irinotecan/5-FU/Leucovorin (FOLFIRI) or Oxaliplatin/5-FU/Leucovorin (mFOLFOX6) with Bevacizumab (BV) or Cetuximab (CET) for Patients (Pts) with KRAS Wild-Type (Wt) Untreated Metastatic Adenocarcinoma of the Colon or Rectum (MCRC). American Society of Clinical Oncology; 2014

[49] Venook AP, et al. Impact of Primary (1°) Tumor Location on Overall Survival (OS) and Progression-Free Survival (PFS) in Patients (Pts) with Metastatic Colorectal Cancer (mCRC): Analysis of CALGB/SWOG 80405 (Alliance). American Society of Clinical Oncology; 2016

[50] Hsu H-C, et al. Mutations of KRAS/NRAS/BRAF predict cetuximab resistance in metastatic colorectal cancer patients. Oncotarget. 2016;**7**(16):22257

[51] Douillard J-Y, et al. Panitumumab–FOLFOX4 treatment and RAS mutations in colorectal cancer. New England Journal of Medicine. 2013;**369**(11):1023-1034

[52] Maughan TS, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: Results of the randomised phase 3 MRC COIN trial. The Lancet. 2011;**377**(9783):2103-2114

[53] Tran B, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer. 2011;**117**(20):4623-4632

[54] Yaeger R, et al. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. Cancer. 2014;**120**(15):2316-2324

[55] Atreya CE, et al. Differential radiographic appearance of BRAF V600E–mutant metastatic colorectal cancer in patients matched by primary tumor location. Journal of the National Comprehensive Cancer Network. 2016;**14**(12):1536-1543

[56] Kopetz S, et al. PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors. Journal of Clinical Oncology. 2010;**28**(15_suppl):3534-3534

[57] Loupakis F, et al. FOLFOXIRI plus bevacizumab as first-line treatment in BRAF mutant metastatic colorectal cancer. European Journal of Cancer. 2014;**50**(1):57-63

[58] Loupakis F, et al. FOLFOXIRI plus Bevacizumab (Bev) Versus FOLFIRI plus Bev as First-Line Treatment of Metastatic Colorectal Cancer (MCRC). Results of the phase III randomized TRIBE trial. Alexandria VA, USA: American Society of Clinical Oncology; 2013

[59] Falcone A, et al. FOLFOXIRI/Bevacizumab (Bev) Versus FOLFIRI/Bev as First-Line Treatment in Unresectable Metastatic Colorectal Cancer (mCRC) Patients (Pts). Results of the phase III TRIBE trial by GONO group. Alexandria VA, USA: American Society of Clinical Oncology; 2013

[60] Wang X, et al. PD-L1 expression in human cancers and its association with clinical outcomes. OncoTargets and Therapy. 2016;**9**:5023

[61] Le DT, et al. PD-1 blockade in tumors with mismatch-repair deficiency. New England Journal of Medicine. 2015;**372**(26):2509-2520

[62] Overman MJ, et al. Nivolumab in Patients with DNA Mismatch Repair Deficient/Microsatellite Instability High Metastatic Colorectal Cancer: Update from CheckMate 142. Alexandria VA, USA: American Society of Clinical Oncology; 2017

[63] Mullard A. 2012 FDA drug approvals. Nature Reviews. Drug Discovery. 2013;**12**(2):87

[64] Van Cutsem E, et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. Journal of Clinical Oncology. 2012;**30**(28):3499-3506

[65] Yoshino T, et al. Randomized phase III trial of regorafenib in metastatic colorectal cancer: Analysis of the CORRECT Japanese and non-Japanese subpopulations. Investigational New Drugs. 2015;**33**(3):740-750

[66] Verdaguer H, Tabernero J, Macarulla T. Ramucirumab in metastatic colorectal cancer: Evidence to date and place in therapy. Therapeutic Advances in Medical Oncology. 2016; **8**(3):230-242

[67] Tabernero J, et al. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): A randomised, double-blind, multicentre, phase 3 study. The Lancet Oncology. 2015;**16**(5):499-508

[68] Raedler LA. Lonsurf (Trifluridine plus Tipiracil): A new oral treatment approved for patients with metastatic colorectal cancer. American Health & Drug Benefits. 2016;**9**(Spec Feature):97

[69] Kish T, Uppal P. Trifluridine/tipiracil (lonsurf) for the treatment of metastatic colorectal cancer. Pharmacy and Therapeutics. 2016;**41**(5):314

[70] Ko Y, Karanicolas P. Hepatic arterial infusion pump chemotherapy for colorectal liver metastases: An old technology in a new era. Current Oncology. 2014;**21**(1):e116

[71] Fiorentini G, et al. Chemoembolization in colorectal liver metastases: The rebirth. Anticancer Research. 2014;**34**(2):575-584

[72] Ceelen W. Current management of peritoneal carcinomatosis from colorectal cancer. Minerva Chirurgica. 2013;**68**(1):77-86

[73] Kastrinos F, et al. Risk of pancreatic cancer in families with lynch syndrome. JAMA. 2009;**302**(16):1790-1795

[74] Buecher B, et al. Role of microsatellite instability in the management of colorectal cancers. Digestive and Liver Disease. 2013;**45**(6):441-449

[75] Umar A, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (lynch syndrome) and microsatellite instability. Journal of the National Cancer Institute. 2004;**96**(4):261-268

[76] Vasen HF et al. Guidelines for the clinical management of lynch syndrome (hereditary non-polyposis cancer). Journal of Medical Genetics. 2007;**44**(6):353-362

[77] Fallik D, et al. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. Cancer Research. 2003;**63**(18):5738-5744

[78] Ait Ouakrim D, et al. Aspirin, ibuprofen, and the risk for colorectal cancer in Lynch Syndrome. JNCI: Journal of the National Cancer Institute. 2015;**107**(9)

[79] Burn J, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: An analysis from the CAPP2 randomised controlled trial. The Lancet. 2012; **378**(9809):2081-2087

[80] Galiatsatos P, Foulkes WD. Familial adenomatous polyposis. The American Journal of Gastroenterology. 2006;**101**(2):385

[81] Jasperson KW, Patel SG, Ahnen DJ. APC-Associated Polyposis Conditions. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mefford HC, Stephens K, Amemiya A, Ledbetter N, editors. SourceGeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017

[82] Provenzale D, et al. Genetic/familial high-risk assessment: Colorectal version 1.2016, NCCN clinical practice guidelines in oncology. Journal of the National Comprehensive Cancer Network. 2016;**14**(8):1010-1030

[83] Al-Tassan N, et al. Inherited variants of MYH associated with somatic G: C [arrow right] T: A mutations in colorectal tumors. Nature Genetics. 2002;**30**(2):227

[84] Nielsen M, et al. MUTYH-associated polyposis (MAP). Critical Reviews in Oncology/ Hematology. 2011;**79**(1):1-16

[85] Sieber OM, et al. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. New England Journal of Medicine. 2003;**348**(9):791-799

[86] Bolocan A, et al. Map syndrome (MYH associated polyposis) colorectal cancer, etiopathological connections. Journal of Medicine and Life. 2011;**4**(1):109

[87] Syngal S, et al. ACG clinical guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. The American Journal of Gastroenterology. 2015;**110**(2):223

Chapter 4

Acquired and Intrinsic Resistance to Colorectal Cancer Treatment

Romina Briffa, Simon P. Langdon,
Godfrey Grech and David J. Harrison

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70781

Abstract

First line therapy for colorectal cancer (CRC) is usually fluoropyrimidine monotherapy and oxaliplatin, or irinotecan-based therapy. Additionally, targeted therapies such as bevacizumab, aflibercept, ramucirumab, regorafenib, cetuximab and panitumumab are indicated in combination with chemotherapy in metastatic CRC. Resistance of CRC to treatment is the principal rationale for treatment failure. Resistance can be intrinsic (primary resistance) or acquired (secondary resistance). Here, we discuss the classical model of resistance, which focuses primarily on mechanisms involving alterations in drug metabolism, increased drug efflux, secondary mutations in drug targets, inactivation of apoptotic pathways, p53 and DNA damage repair. Other resistance mechanisms, including the Warburg effect, cancer stem cells, intra-tumor heterogeneity and pharmacoepigenomic mechanisms will also be discussed. We conclude the chapter with a systems medicine approach to predict response to treatment for the discovery and validation of predictive biomarkers that are urgently needed.

Keywords: colorectal cancer, chemotherapy, targeted therapy, intrinsic resistance, acquired resistance, predictive biomarkers

1. Introduction

The mainstay of colorectal cancer (CRC) treatment is curative surgery, although in some cases patients are administered neo-adjuvant therapy. Surgery is usually followed by adjuvant therapy in patients presenting with Stage III and Stage IV disease. Additionally, adjuvant therapy is sometimes administered to high risk stratified Stage II patients. Adjuvant treatment for stage III CRC patients consists of chemotherapy including 5-fluorouracil (5-FU), oxaliplatin and

capecitabine—usually administered combination therapy [1]. Patients having advanced CRC are frequently treated with targeted therapy in combination with chemotherapy, or as a single agent, and more recently with immunotherapy (**Table 1**). The rationale for using combination therapy is to avoid treatment resistance, promote a synergistic effect and reduce potential toxicity (refer to **Table 2**).

Therapeutic agent	Class	Colorectal cancer indications	Date of first launch worldwide [1–6]
5-Fluorouracil (5-FU)	Antimetabolite, pyrimidine analogue	Used as a single agent or in combination	1962
Epirubicin	Cytotoxic antibiotics	Used as a single agent or in combination	1984
Irinotecan	Topoisomerase I inhibitor	1. Indicated for the treatment of mCRC: • in combination with 5-FU and folinic acid in chemotherapy naïve patients • as a single agent in patients who have failed a 5-FU-based regimen 2. In combination with cetuximab is indicated for the treatment of patients with EGFR-expressing KRAS wild-type mCRC, who had not received prior treatment for metastatic disease or after failure of irinotecan-including cytotoxic therapy 3. In combination with 5-FU, folinic acid and bevacizumab is indicated for first-line treatment of patients with mCRC 4. In combination with capecitabine with or without bevacizumab is indicated for first-line treatment of patients with mCRC	1994
Oxaliplatin	Platinum derivative, alkylating agent	Oxaliplatin in combination with 5-FU and folinic acid is indicated for: 1. adjuvant treatment of stage III colon cancer after complete resection of primary tumor 2. treatment of mCRC	1996
Raltitrexed	Antimetabolite	Palliative treatment of advanced colorectal cancer where 5-FU and folinic acid-based regimens are either not tolerated or inappropriate	1996
Capecitabine	Antimetabolite	1. Used as a single agent or in combination 2. Used for the adjuvant treatment of stage III colon cancer patients 3. Used in mCRC	1998
Cetuximab	Monoclonal antibody, EGFR inhibitor	Indicated for the treatment of patients with EGFR-expressing, RAS wt mCRC: • in combination with irinotecan-based chemotherapy • in first-line in combination with FOLFOX • as a single agent in patients who have failed oxaliplatin- and irinotecan-based therapy and who are intolerant to irinotecan	2003

Therapeutic agent	Class	Colorectal cancer indications	Date of first launch worldwide [1–6]
Bevacizumab	Monoclonal antibody, VEGF inhibitor	In combination with fluoropyrimidine-based chemotherapy is indicated for treatment of mCRC	2004
Panitumumab	Monoclonal antibody, EGFR inhibitor	Indicated for the treatment of wt *RAS* mCRC: 1. first-line in combination with FOLFOX or FOLFIRI 2. second-line in combination with FOLFIRI for patients who have received first-line fluoropyrimidine-based chemotherapy (excluding irinotecan) 3. monotherapy after failure of fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens	2006
Regorafenib	Angiogenesis inhibitor, tyrosine kinase inhibitor	mCRC patients who have been previously treated with, or are not considered candidates for available therapies	2013
Aflibercept	Angiogenesis inhibitor, tyrosine kinase inhibitor	In combination with FOLFIRI is indicated in mCRC that is resistant to or has progressed after an oxaliplatin-containing regimen	2013
Trifluridine/ tipiracil hydrochloride	Antimetabolite	Treatment of mCRC patients who have been previously treated with, or are not considered candidates for available therapies	2015
Pembrolizumab	Anti-PD1 immunotherapy	Unresectable or metastatic, MSI-H or dMMR CRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan	2017

Table 1. Colorectal cancer drugs currently on the market.

Combination regimens	Therapeutic agents
deGramont/modified de Gramont	5-Fluorouracil, folinic acid
FOLFIRINOX	Folinic acid, 5-fluorouracil, irinotecan, oxaliplatin
FOLFIRI	Folinic acid, 5-fluorouracil, irinotecan
FOLFOX	Folinic acid, 5-fluorouracil, oxaliplatin
XELOX	Oxaliplatin, capecitabine
FOLFIRI + cetuximab	Folinic acid, 5-fluorouracil, irinotecan, cetuximab
FOLFOX + cetuximab	Folinic acid, 5-fluorouracil, oxaliplatin, cetuximab
FOLFIRI + panitumumab	Folinic acid, 5-fluorouracil, irinotecan, panitumumab
FOLFOX + panitumumab	Folinic acid, 5-fluorouracil, oxaliplatin, panitumumab
FOLFIRI + aflibercept	Folinic acid, 5-fluorouracil, irinotecan, aflibercept
FOLFIRI + bevacizumab	Folinic acid, 5-fluorouracil, irinotecan, bevacizumab

Table 2. Drug combinations to treat colorectal cancer.

Patients' responses to treatment are limited, and in fact less than one-third of patients respond to 5-fluorouracil as a single agent [2, 3]. However, when used in combination, for instance, with oxaliplatin-based therapy, 50% response rate is obtained [4]. Resistance to 5-fluorouracil can be due to loss of SMAD4 [5], thymidylate synthase (TYMS) amplification [6], defective mismatch repair (MMR) genes [7], high level expression of thymidylate synthase (TS) [8], increased DPD activity [9], microsatellite instability [9], modulation of the Bcl2 family members [10], cell cycle perturbation [11], decreased ATP synthase [12] and adaptation to oxidative stress [13]. General mechanisms attributed to oxaliplatin resistance include cellular transport, detoxification, DNA repair, cell death, epigenetic alteration and NF-κβ signaling pathway [14].

Although, the use of cetuximab and panitumumab in combination with other agents is very effective, they are not sufficiently potent as single agents and are reported to work in only around 10% of cases [15]. Over the last decade, a number of papers on anti-epidermal growth factor receptor (EGFR) resistance mechanisms have been published [16–18]. Resistance mechanisms attributed to EGFR resistance include, but are not limited to, low EGFR gene copy number, low expression of AREG and EREG, EGFR S492R mutation, RAS mutation, BRAF V600E mutation, PIK3CA exon 20 mutation, PTEN loss, STAT3 phosphorylation, activated IGF1R, MET amplification, HER2 amplification, altered VEGF/VEGFR and EMT [16, 19].

Unfortunately, the lack of predictive markers would allow clinicians to select patients who are most likely to benefit from a specific therapy remains a challenge. A recent review by Wu et al. reported that in the context of metastatic cancer, approximately 90% of treatment failure is due to multi-drug resistance [20]. Currently, the only markers that predict potential toxicity to 5-FU treatment are DPD deficiency, DPYD mutation, UGT1A1 and high TS expression [21]. Moreover, the only marker that predicts lack of response to 5-FU is mismatch repair deficiency (dMMR), while pembrolizumab, dMMR predicts increase in response [7]. With respect to anti-*EGFR* treatment, *KRAS*, *NRAS* and *BRAF* mutations are the only biological markers that predict lack of response and hence pose a contraindication to treatment administration [21].

Both intrinsic resistance, which is characterized by cancer cells having only a slight or no response to treatment from the beginning, and acquired resistance that is described as, initially, having a clinical response, ensued by development of resistance will be discussed [22]. Several studies that discuss resistance to specific chemotherapeutic agents have been published and therefore we will be solely referring to salient resistance mechanisms to CRC therapies.

2. The classical model of resistance

2.1. Drug metabolism

Chemotherapeutic agents are extensively metabolized by Phase I, Phase II and Phase III enzymes. Phase I enzymes, which are mostly involved in chemical modification, include the heme protein cytochrome superfamily CYP450, which is sub-divided into 74 gene families and is involved in oxidation reactions. CYP3A is involved in irinotecan metabolism while CYP2A6, CYP2C8 and CYP1A2 are involved in tegafur activation [23].

Increased expression of dihydropyrimidine dehydrogenase (DYPD) or thymidine phosphorylase (TP) is correlated with resistance to 5-FU chemotherapy [24]. On the other hand, in a cohort of 177 CRC patients, there was a correlation between high TP expression and a better survival rate in the doxifluridine arm ($p = 0.025$) [25]. TP has also been reported to increase in both hypoxic and hypoglycemic environments, which will be discussed later [26]. Furthermore, polymorphic changes in DYPD account for life-threatening adverse effects in patients treated with 5-FU or its derivatives [27]. On the other hand, patients having a low expression of DYPD cannot metabolize 5-FU efficiently [28]. Decreased orotate phosphoribosyl transferase (OPRT) expression in gastric cancer is associated with resistance to 5-FU [29].

Phase II enzymes are involved in conjugation and include glutathione (GSH), glutathione-S-transferase (GSTs), uridine diphosphate glucuronosyltransferases (UGT) and NADH quinone oxidases (NQO). One of the oxaliplatin resistance mechanisms entails elevation of glutathione mediated by γ-glutamyl transpeptidase [30]. Additionally, GSTπ1 is associated with oxaliplatin and cisplatin resistance mechanisms [31]. SN-38, the active metabolite of irinotecan, is inactivated by way of glucuronidation by UGT [32]. UGT1A1 is one of the main genes involved in glucuronidation and is reported as being highly polymorphic; subsequently patients having UGT1A1*28 polymorphisms tend to suffer from increased risks of toxicity as reported in a cohort of colorectal cancer patients [33]. Both CYP450 and GSTs have been implicated in the metabolism of chemotherapeutic agents, but their predictive value is still uncertain [32].

Members of the ATP-binding cassette (ABC) superfamily are involved in Phase III drug metabolism [34] and to date 49 ABC transporters have been documented in humans [35]. The role of ABC transporters is to use energy from ATP hydrolysis to move their substrates across biological membranes and against concentration gradients, thereby limiting cellular accumulation of their substrates [36]. ABC members include P-glycoprotein (MDR1/ABCB1), breast cancer resistance protein (BCRP/ABCG2) and transporters of the multidrug resistance-associated protein (MRP/ABCC) family like the multi-drug resistance protein 5 (ABCC5), which bestows resistance to 5 FU via transporting the monophosphate metabolites in colorectal and breast cancers [37]. MDR1 is found to be highly expressed in the epithelial cells of the colon, overexpressed in a number of tumors, and has been associated with treatment failure [38]. At least 12 ABC transporters from 4 ABC sub-families have been shown to have a role in *in vitro* drug resistance (reviewed in Ref. [39]). MRP5 has been reported to confer cross-resistance to a number of anti-cancer agents including 5-FU, oxaliplatin and a number of antifolates [37]. The authors postulated that resistance might be instigated via drug efflux mechanisms which interfere with 5-FU's ability to impede both DNA and RNA synthesis. P-glycoprotein is a drug efflux pump and exerts its mode of action by lowering the intracellular concentration of a number of drugs, which subsequently leads to increased drug resistance [40]. BCRP is also involved in irinotecan efflux and is reported to be overexpressed in colon cancer, subsequently increasing chemoresistance.

2.2. Drug targets

5-FU naive CRC patients exhibiting high expression of TS and disturbed folate pools are intrinsically resistant to 5-FU [9]. A meta-analysis of over 3000 pooled CRC cases by Popat and

colleagues concluded that the variation of TS expression in CRC patients could explain inter-individual variation in clinical outcome, and patients with low TS expression treated with 5-FU had better overall survival [41]. CRC patients who are chemoresponsive to 5-FU have lower TS enzymatic activity compared to those patients who fail to respond [42]. Furthermore, the low availability of 5,10-methylenetetrahydrofolate and its polyglutamates also contributed to intrinsic resistance [43]. An indirect resistance mechanism reported in hepatocellular carcinoma cells is the induction of the expression of the transcription factor Late SV40 Factor (LSF) that regulates TS expression, by way of the astrocyte elevated gene-1 (AEG-1) [44].

Additionally, TS mRNA increases in a number of patients treated with 5-FU, resulting in acquired resistance [45]. In a review by Holohan et al., the authors explained further that 5-FU can post-transcriptionally upregulate the TS expression as a result of the inhibition of a negative feedback loop where the substrate free TS binds to and inhibits the translation of thymidylate synthase mRNA [38].

Watson and colleagues reported that patients with TYMS amplification treated with adjuvant chemotherapy had a median overall survival of 18 months shorter when compared to patients with low or normal TYMS copy number [46]. A total of 113 mCRC patients were enrolled in this study (62 exposed and 51 unexposed to 5-FU prior to resection) and the investigators concluded that TYMS copy number gain was associated with patients treated with 5-FU-based neoadjuvant treatment [46].

Guo and colleagues have demonstrated that a possible mechanism of acquired 5-FU resistance can be due to disruption in cell cycle. Using two 5-FU resistant and two sensitive cell lines, Guo and colleagues showed that the protein expression of CDK2 (total and phosphorylated threonine 160), Cyclin D3, and Cyclin A was significantly decreased in the 5-FU resistant cell lines. On the other hand, $p21^{WAF1}$ expression was modestly increased in both resistant cell lines [11]. The authors postulated that the G1 and S phase delay in the 5-FU resistant cell lines occurs because of Cyclin E—CDK2 complex deficiency. Additionally, the Cyclin A—CDK2 complex is also deficient and may assist in bringing about a delay in the S phase of 5-FU resistance cell lines [11]. Guo and colleagues speculated that the slowing down of the cell cycle might interfere with the active 5-FU metabolites being incorporated into DNA and also allows the cells to repair the DNA damage [11].

Montagut and colleagues confirmed one mechanism of acquired resistance to cetuximab, where they showed that an acquired EGFR ectodomain mutation (S492R) prevented the effective binding of cetuximab to the receptor [47]. On the other hand, overexpression of EGFR in CRC has been poorly correlated with response to anti-EGFR therapy [48]. One of the determinants of poor response is because KRAS mutant patients have a constantly activated KRAS, irrelevant to the phosphorylation status of EGFR. Fluorescence *in situ* hybridisation (FISH) analysis was carried out on a cohort of 58 mCRC patients treated with panitumumab and it was observed that patients that did not exhibit an EGFR copy number gain or chromosome 7 polysomy or amplification were associated with treatment failure ($p = 0.0009$, $p = 0.0007$) [49]. Another possible mechanism of resistance using an *in vitro* model postulated that increased Src family kinases activity leads to lengthened EGFR activity, increased EGFR-modulated HER3 activity, and activation of the PI3K/AKT pathway [50].

A study by Lievre and colleagues on a cohort of 30 mCRC patients reported a highly significant association between non-response to cetuximab and mutant KRAS (n = 19, p = 0.0003) [51]. This association was further confirmed by other larger studies [52, 53]. A study by Misale and colleagues unprecedentedly described that a significant number of wild-type KRAS CRC patients, who are initially responsive to anti-EGFR therapies, acquire resistance due to *de novo* KRAS mutations resulting from continuing mutagenesis [54]. Another somatic mutation, associated with treatment resistance in CRC is PIK3CA [55], is mutated in 25–32% of CRC patients [56].

2.3. DNA damage repair

Mismatch repair deficiency (dMMR) can occur because of both sporadic and hereditary CRC. In the autosomal dominant hereditary non-polyposis colon cancer (HNPCC), which is also referred to as Lynch Syndrome, dMMR arises primarily due to inactivating germline mutations in either MLH1, MSH2, PMS2, or MSH6 [57]. Furthermore, loss of function of the remaining allele can occur via various mechanisms, namely loss of heterozygosity, mutations, gene conversion, and also promoter methylation [58]. On the other hand, epigenetic hypermethylation of MLH1 accounts for the majority of sporadic dMMR in CRC [59]. A recent study by Ye and colleagues concluded that miR-1290 promotes 5-FU resistance by directly targeting hMSH2 [60].

An *in vitro* study on CRC cell lines showed that MMR-proficient cell lines were more sensitive to the therapeutic doses of 5-FU (5–10 μM) compared to MMR-deficient cell lines [61]. Furthermore, patients who are high microsatellite instable (MSI-H) do not show any survival advantage when administered 5-FU-based chemotherapies [62]. The scientific literature not only alludes to the fact that dMMR tumor cells have a distinct response to standard chemotherapies, but also to many emerging therapies for CRC [58]. In an *in vitro* study, Tajima and colleagues showed that resistance of dMMR cancer cells to 5-FU can arise due to the incorporation of 5-FU metabolites in DNA [63].

Mechanisms attributed to resistance to oxaliplatin include increased DNA repair, impaired DNA adduct formation, over-expression of copper transporters (increased levels of ATP7B correlated with poor outcome in CRC patients) [64], enhanced drug detoxification, and increased tolerance to DNA damage. While NER and recombination repair mechanisms do not distinguish between cisplatin and oxaliplatin adducts, mismatch repair, damage-recognition proteins, and translesion DNA polymerases do distinguish between the two [65].

Increased excision repair cross complementation group 1 (ERCC1) mRNA expression correlates with resistance to oxaliplatin [66]. A polymorphism (Gln mutant allele) in X-ray repair cross complementation group 1 (XRCC1), which is involved in single strand break, adduct formation, and base excision repair, was associated with treatment resistance in a cohort of 61 patients treated with 5-FU and oxaliplatin [67].

2.4. p53

An *in vitro* study on the NCI-60 panel investigated the relationship between a group of p53 mutant and p53 wild-type cell lines and chemosensitivity to 123 drugs used in cancer treatment.

One of the findings was that the median GI50 for p53 mutant cell lines treated with 5-FU was sixfold higher than the GI50 of p53 wild-type cell lines [68].

The TP53 Colorectal Cancer International Collaborative Study published a study consisting of a patient cohort of 3583 samples from 25 different research groups in 17 countries. One of the aims of this study was to investigate whether there was a prognostic impact of TP53 mutations and treatment subgroups. In 1334 Dukes' C patients (792 wild-type TP53 and 542 mutant TP53), the wild-type TP53 patients treated with chemotherapy showed significantly better survival in the proximal and rectal tumor groups ($p = 0.006$ and $p = <0.001$, respectively) and a trend towards statistical significance ($p = 0.022$) was observed for the distal tumor group [69]. In the mutant TP53 group, patients receiving chemotherapy had better survival only in the proximal colon group ($p = <0.001$), with the authors concluding that TP53 mutation had no predictive value within Dukes' C patients treated by surgery alone or surgery and chemotherapy [69]. The authors advised caution in interpreting these observations, since the chemotherapy treatment was not always 5-FU based. When the 5-FU-based regimens were grouped together, the authors reported that chemotherapy can have an impact on survival based on TP53 mutational status and tumor sites [69]. Furthermore, this study showed that wild-type TP53 rectal patients received a significant survival benefit from 5-FU-based chemotherapy, irrespective of whether or not radiotherapy was received [69].

In 2008, Ahmed and colleagues carried out a study on 41 Dukes' C CRC patients that had a curative resection of the primary tumor and were administered 5-FU adjuvant treatment. The p53 mutation status was confirmed by gene sequencing and the study concluded that there was significant advantage for the wild type p53 patients in the time to develop metastasis and overall survival within the two groups receiving 5-FU adjuvant treatment [70].

2.5. Apoptosis

Failure of cells to undergo apoptosis may affect treatment efficacy [71]. Apoptosis can occur via two main signaling pathways: extrinsic (receptor-mediated pathway) and/or intrinsic (mitochondrial-mediated pathway [72]. Although a number of studies have reported the involvement of extrinsic pathway in treatment resistance, namely CD95 (Fas), it has been shown that under certain situations most chemotherapy-treated cells undergo intrinsic apoptosis [71, 73].

By way of an *in vitro* model, Gourdier and colleagues demonstrated that acquired resistance to oxaliplatin can occur via a defect in the intrinsic apoptosis pathway [74]. Furthermore, expression of cleaved caspase-3 and Bax was lost in the 68-fold oxaliplatin-trained resistant cell line, while the expression of Bcl-2, Bak, and Bcl-X_L remained unaltered, suggesting that they are not involved in the acquired resistance mechanism to oxaliplatin [74]. Transcription factor NFκβ is known to be constitutively activated in colorectal cancers and although it has been associated with pro-apoptotic function, it is not always the case [75]. 5-FU has been shown to induce NFκβ expression via IKKβ and consequently chemoresistance in colorectal cell lines [76]. Apart from the fact that oxaliplatin, like 5-FU, can cause NFκβ constitutive activation, this activation imparts chemoresistance via c-FLIP and Mcl-1 [77].

http://dx.doi.org/10.5772/intechopen.70781

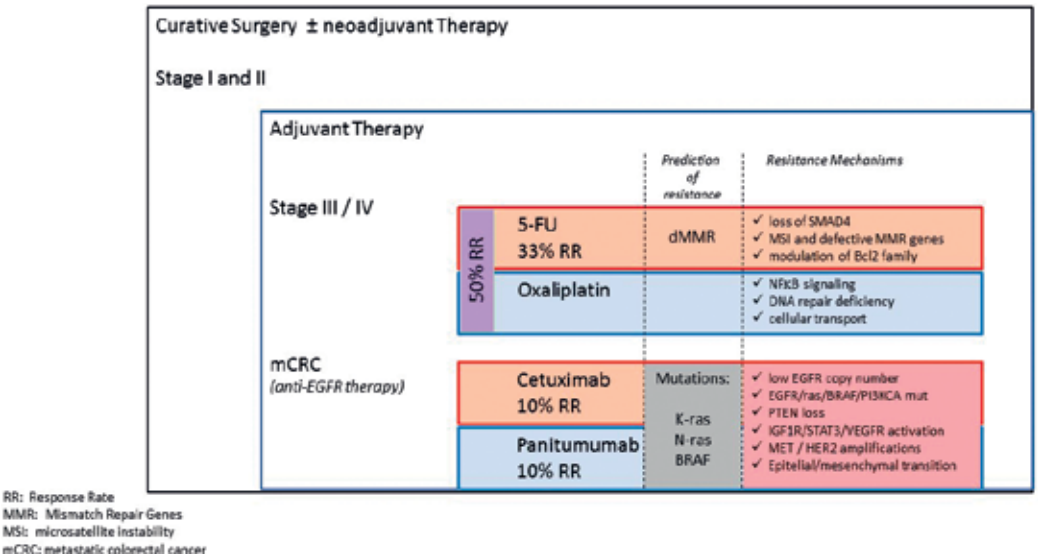

Figure 1. Treatment for colorectal cancer patients according to the stage at diagnosis.

A study identified double stranded RNA dependent protein kinase (PKR), as a key molecule in inducing apoptosis in colon cancer cells—irrelevant of the p53 status [78]. The authors proceeded by demonstrating that PKR knockdown cells responded poorly to treatment with 5-FU. The importance of integrins and apoptosis in chemoresistance is being investigated by a number of groups. A study by Liu and colleagues focussed on the involvement of the β6-integrin-ERK-MAP kinase pathway in conferring chemoresistance to 5-FU in colon cancer lines (**Figure 1**) [79].

3. Novel resistance mechanisms

3.1. Warburg effect

Over the past decade, the significance of the Warburg effect in the field of oncology has gained momentum and a number of original research papers [80, 81] and reviews have been published on this topic [82]. The Warburg effect, also referred to as the glycolytic phenotype, is singularized by an increased rate of aerobic glycolysis together with irreversible injury to mitochondrial oxidative phosphorylation [83] and is favored by the majority of tumors [84]. Subsequently, Tong and colleagues showed that aerobic glycolysis is involved in cancer cell proliferation and tumorigenesis in a model of HCT116 colorectal cell lines [85]. A number of mechanisms that affect increased glycolysis, and therefore contribute to the Warburg effect, include mitochondrial defects, adaptation to hypoxic conditions, oncogenic signals, and altered metabolic enzymes [86]. A number of these mechanisms occur via hypoxia inducible factor-1 (HIF-1) [87].

A putative major player in the Warburg effect and cancer is the uncoupling protein coding-2 (UCP2) [88]. UCP2 is located in the mitochondrial inner membrane and its main function is that of a mitochondrial transporter protein, that creates proton leaks across the inner mitochondrial membrane, ergo uncoupling oxidative phosphorylation from ATP synthesis [89]. Furthermore, UCP2 might act as a negative regulator of ROS production [90].

Horimoto et al. postulated that UCP2 is involved in colon tumor adaptation and is correlated with neoplastic changes [91]. UCP2 gene expression and protein expression was assessed on a small cohort of 10 patients, where a paired normal and tumor sample for each patient was processed. Gene expression results demonstrated an average of 3.88 ± 0.85-fold difference in UCP2 mRNA expression between the tumor (T) and peritumoral (P) paired samples. The same ratio was found for UCP2 protein expression and furthermore a strong linear correlation between T/P ratio of UCP mRNA and protein expression ($r = 0.91$, $p = 0.0015$) was confirmed. Additionally, immunohistochemistry (IHC) for UCP2 was carried out on a cohort of 9 hyperplastic polyps, 17 adenomas, and 107 adenocarcinoma and positive scores were 11.1, 58.8, and 86%, respectively.

A comparable study was undertaken on a larger cohort of colon cancer patients and it yielded the same results and correlations in addition to association of UCP2 expression and metastasis [88]. Altered colon cancer metabolism, as confirmed through measurement of UCP2 expression in these studies, can also contribute to resistance to cancer therapies. An *in vitro* study investigated the rate of cell death caused by 5-FU, with respect to different metabolic rates, as quantified by the bioenergetic signature [92]. This study demonstrated the bioenergetic signature directly correlates with the apoptotic response to treatment with 5-FU [92].

Tumor adaptation to hypoxic and acidic microenvironments strongly selects for tumor cells that are resistant to chemo- and radiotherapy [93]. The slowing down of cell cycling induces a decreased rate of cell division, thereby decreasing chemotherapy activity [93]. Furthermore, hypoxia dysregulates several DNA damage response pathways and prevents effective functioning of proteins involved in homologous recombination (HR), non-homologous end joining (NHEJ), and the mismatch repair (MMR) pathways, thereby driving genetic instability [94]. The cascade of events triggered by chronic hypoxia may also bring about amplification of multidrug resistant gene ABCB1 via induction of chromosomal fragile sites [94].

An *in vitro* study on colon carcinoma cell lines demonstrated that low oxygen concentration resulted in decreased protein expression of Bid, Bad, and Bax [87]. A further series of experiments illustrated that all three CRC cell lines studied expressed a functional HIF-1 pathway and the authors showed that in a hypoxic environment Bid down-regulation occurs via HIF-1, while down-regulation of Bax and Bad occurs independently of HIF-1 function [87]. Additionally, under anoxic conditions, SW480, HCT116, and HT29 were resistant to etoposide treatment and SW480 was also resistant to oxaliplatin. Further investigation by Erler and colleagues demonstrated that down regulation of Bid and/or Bax contributed to etoposide resistance in this model [87]. An important observation from this study was that Bak was least responsive to hypoxia and thereby it might be crucial for drug-induced apoptosis [87].

The PI3K/Akt signaling pathway enhances aerobic glycolysis, and dual PI3K/mTOR inhibitors can influence the cancer cell metabolic programme [95]. Oncogenic mutations involving

this pathway, MAPK, and Src pathways have been shown to increase HIF-1 expression in both hypoxic and normoxic conditions [96]. Inhibiting HIF1 decreases proliferation, influences anaerobic glycolysis, encourages apoptosis, and reduces resistance to chemo- and radiotherapy [93].

Moderate evidence in the literature demonstrates that 18q LOH/SMAD4 loss has potential for it being used as a marker to predict response to 5-FU-based therapies [97]. Papageorgis and colleagues demonstrated that a SMAD4 defect suppresses hypoxia-induced cell death, induces aerobic glycolysis, and promotes 5-FU resistance in the HCT116 cell line model [98]. Furthermore, the authors observed a physical interaction between SMAD4 and HIF1α and postulated that the acquired chemoresistance in 18q-deficient CRC may be explained by Smad4 negatively regulating HIF1α-induced GLUT1 expression and the rate of aerobic glycolysis [98]. Other oncogenes/tumor suppressor genes known to be involved in the stimulation of glycolytic energy include Ras, c-myc, Src, and p53 [99].

Downregulation of pyruvate kinase M2 (PKM2) is also known to promote the Warburg effect metabolic phenotype and tumorigenesis [80]. Additionally, PKM2 is a HIF-1 target gene and concurrently a co-activator of HIF-1 [93]. A study by Tamada and colleagues on a number of cancer cell lines, which also comprised of CRC cell lines HCT116 p53 wild-type and HCT116 p53 null, demonstrated CD44-regulated glycolysis in p53 deficient cells via interaction with PKM2 [100]. Furthermore, the authors speculated that CD44 functions as a scaffold between a tyrosine kinase and PKM2 near the cell membrane, ergo down-regulating the activity of PKM2 [100]. By means of a set of elegant experiments, the authors showed evidence that CD44 silencing in p53 deficient cell lines sensitized the cells to cisplatin, 5-FU, and adriamycin in normoxia, and that CD44 silencing of p53 wild-type cells under hypoxic conditions increased sensitivity to these three chemotherapeutic agents [100].

The relationship of hypoxia and resistance to both radio- and chemotherapy has been explored for the last decade and several mechanisms have been postulated. As evidenced by a number of studies referred above, the Warburg effect is an adaptive mechanism used by solid tumors to overcome stress caused by hypoxia and also contributes to resistance to both chemotherapy and targeted inhibitors.

3.2. Clonal evolution

Another contributor to therapy failure is the innate Darwinian aspect of cancer [101]. In a review of clonal evolution, Greaves and Maley describe the complexity of cancer and the selective pressure for resistant cells to thrive when treated with chemotherapeutic agents. Similarly, adaptive microenvironmental mechanisms such as hypoxia and acidosis lead to both phenotypic and genotypic heterogeneity [102]. This evolution not only affects the genomic instability of the tumor but also contributes towards resistance to therapy, including targeted therapies [102].

A retrospective study analyzing circulating tumor DNA from a cohort of 28 mCRC patients suggested that development of resistance to panitumumab can occur in metastatic lesions, having a sub-clone encompassing just 1 of 42 mutations associated with resistance to panitumumab. Subsequently, the time for recurrence is basically the time taken for that sub-clone to

repopulate the lesion [103]. Furthermore, the authors concluded that resistance mutation in KRAS and other genes were likely to be present prior to starting panitumumab therapy [103].

A number of mechanisms contributing to acquired resistance to 5-FU-based therapies include alteration of the drug's specific target, drug inactivation, influx and efflux of drugs in the cells, drug-induced damage, and evasion of apoptosis [104]. In an attempt to comprehend these complex mechanisms, Tentes et al. investigated 5-FU acquired resistance in the SW620 cell line model [105]. A significant finding reported in this study consisted of the maintenance of a 5-FU resistant phenotype, albeit by culturing the trained cell line in drug-free media for 15 weeks. The authors concluded that the resistant clones may have acquired an altered genetic background and unique gene expression patterns due to long-term exposure to 5-FU, and that this scenario might explain relapses caused by residual disease of chemo-resistant cells. Besides, overlapping mechanisms of resistance to 5-FU could be observed in the trained resistant cell line [105].

3.3. Intra-tumor heterogeneity

A published study evidenced that intra-tumor heterogeneity is a considerable hurdle to both predictive and prognostic biomarker development [106]. One of the principal results in this study highlighted that 63–69% of all somatic mutations are not detectable across every tumor area, hence confirming that one biopsy is not representative of the whole tumor [106]. Intra-tumor heterogeneity is one of the main challenges to patients being successfully treated and can also contribute to patients having relapses [107].

Chromosomal instability (CIN) is associated with both intrinsic- and acquired-drug resistance and also involved in intra-tumor heterogeneity [108]. A number of hypotheses surrounding CIN, Darwinian selection, and intra-tumoural heterogeneity are currently being investigated. Evidence has been obtained to indicate that cells having a high degree of chromosomal instability are more predisposed to exhibit intrinsic resistance [108].

Lee and colleagues conducted a study on a panel of 27 CRC cell lines (18 of which were CIN^+) and demonstrated that CIN^+ cell lines were significantly more intrinsically resistant to the inhibitors used (Kolmogorov-Smirnov test $p < 0.0001$) and, even at similar proliferation rates, CIN^+ cell lines were more resistant to treatment when compared to CIN^- (one sided Wilcoxon-Mann-Whitney test, $p = 0.049$) [55]. Furthermore, according to previous reports, patients treated with 5-FU-based therapy who exhibited CIN^+ did not obtain as much benefit from the treatments, when compared to patients having diploid CRC [109]. This acquired multidrug resistance has been attributed to cell heterogeneity due to multiple chromosomal re-assortments in these aneuploid cells [55]. One of the hypotheses that Lee and colleagues discussed is that there is a distinct CIN^+ survival phenotype that triggers an endurance to ongoing chromosomal rearrangements which is also related to drug resistance [55].

Phenotypic heterogeneity arises from both genetic and non-genetic influences [110]. Non-genetic influences can emerge from phenotypic plasticity and differentiation of cancer stem cells [107]. The cellular phenotype is affected by several factors namely, stochastic fluctuations (noise), genotypes, microenvironment, and the gene regulatory network [110]. In their

review, Marusyk and colleagues remark that even though genetic heterogeneity is not likely to contribute considerably to phenotypic heterogeneity, it still supports tumor evolution during tumorigenesis and treatment resistance [110]. Phenotypic heterogeneity manifests as phenotypic diverse subpopulations of subpopulation of tumor cells, histologic alterations, different patterns of disease progression, prognosis, diagnosis, and also responses to therapy [111]. This necessitates further investigations on therapeutic resistance of CRC with respect to phenotypic heterogeneity.

3.4. Pharmacoepigenomics

Epigenetic modifications are implicated in the progression of chemoresistance [112]. They bring about changes in gene expression that are autonomous of changes in DNA sequence and persevere over numerous cell divisions. In contrast to genetic modifications, epigenetic transformations are reversible [113]. As a result, the field of pharmacoepigenomics is now gaining more popularity. One of the main mechanisms of action of chemotherapeutic agents is by inducing DNA damage which subsequently leads to either DNA repair, apoptosis, or cell-cycle arrest [114]. A number of genes implicated in these biological processes in cancer cells are epigenetically regulated [115]. Drug resistance has been associated with hypermethylation of promoter regions of pro-apoptotic genes, hypomethylation of drug efflux promoters, and also modified promoter methylation patterns of DNA repair genes [116]. Furthermore, global histone modification patterns may also be involved in drug resistance [117].

Sugita and colleagues conducted a study on a cohort of 80 gastric cancer patients and investigated the relationship between methylation of BNIP3 and DAPK with respect to response to 5-FU-based therapy. This study confirmed a relationship between poor response rate and methylation of one or both genes when compared to patients that did not have methylation ($p = 0.003$) [118]. Furthermore, a study on 112 primary colorectal patients substantiated that BNIP3 is methylated in CRC patients, with approximately 58% of the cohort exhibiting methylation [119]. Additionally, 30 patients having BNIP3 methylation were non-responsive to irinotecan therapy [119]. An *in vitro* study demonstrated that cells having a methylated $p16^{Ink4A}$ were more resistant to irinotecan-induced cell cycle arrest [120] . Cheetham and colleagues presented support that hypermethylation of the SPARC promoter is frequently found in both CRC tumor and cell lines when compared to normal colon ($p = 0.03$) [121]. A previous *in vitro* study by the same group showed that mRNA and protein expression of SPARC were low in chemoresistant tumors and thereby the authors concluded that hypermethylation of the SPARC promoter might be a potential mechanism of low SPARC expression, leading to resistance to therapy [121, 122].

Dynamic chromatin modification can also influence resistance to treatment and a publication by Sharma and colleagues illustrated this resistance mechanism. “Drug-tolerant persisters” were detected while studying the acute response of human cell lines with respect to different treatments. These cells remained viable in conditions where other cells failed to thrive, and since they were encountered at a higher frequency than expected, the authors associated this observation to epigenetic regulation [123]. Following several elegant experiments, the authors concluded that this transiently acquired drug resistant phenotype is capable of arising *de novo*

and requires the histone demethylase KDM5A/RBP2/Jarid1A. This particular histone demethylase secures a metastable chromatin state which contributes towards the ability of cells to tolerate drug exposure. Additionally, this chromatin state is dependent on IGF-1R signaling, which has also been associated with drug resistance in a number of other studies [124, 125].

3.5. Additional mechanisms

Resistance to the newest drug, trifluridine was attributed to decreased changes in expression of mRNA and miRNA located on chromosome 9. This could have been due to either genome deletion or LOH of let-7d-5p, a miRNA inversely associated to trifluridine-induced proliferative effects; hence low expression would lead to decreased effectiveness [126] .

Resistance-promoting adaptive responses include the (1) epithelial-mesenchymal transition (EMT) which is associated with invasive capacity, increased motility and related to chemotherapy, and targeted therapy resistance [38]; (2) Oncogenic bypass and pathway redundancy, also referred to as compensatory signaling pathway via crosstalk mechanisms is involved in acquired resistance to cetuximab. With EGFR deregulated, HER2, HER3, cMET, MAPK, and Akt are subsequently switched on and as a result, acquired resistance can ensue since some RTKs share signaling pathways involved in proliferation and survival [127]. (3) Activation of pro-survival signaling: ADAM17, known to be deregulated in CRC, is also known to be activated with chemotherapy and has been implicated with growth factor shedding, growth factor receptor activation, and drug resistance [128].

Another downstream resistance mechanism is autophagy. In CRC, BRAF V600E induces autophagic properties. Recently, Goulielmaki and colleagues reported that PI3K/AKT/MTOR inhibitors induce autophagic tumor properties, whereas RAF/MEK/ERK signaling inhibitors reduce expression of autophagic markers. They showed that pre-treatment of autophagy inhibitor 3-MA followed by its combination with BRAFV600E targeting drug PLX4720 can synergistically sensitize resistant colorectal tumors [129].

Another recent review highlighted the involvement of telomerase in drug resistance in cancer [130]. The main telomerase-related mechanism highlighted in this review included hTERT translocation, hTERT and cell resistance to stress, G-quadruplex inhibitors specific, telomerase inhibition and the mechanism by which telomerase helps cancer cells resistance to DNA damage/apoptosis. Recent literature also implicates the mammalian vault complexes in drug resistance [130].

4. Conclusion

During the last decade, a number of groups have started taking a systems medicine approach to better understand treatment resistance in colorectal cancer. As described by the Coordinating Action Systems Medicine, this approach comprises the iterative and reciprocal feedback between clinical investigations and practice with computational, statistical and mathematical multi-scale analysis and modeling of pathogenic mechanisms, disease progression and remission, disease spread and cure, treatment responses and

adverse events, as well as disease prevention both at the epidemiological and individual patient level [131].

We are already witnessing a number of success stories and by integrating data, especially with respect to understanding mechanisms of resistance, we are now moving away from clinical trials directed to specific tumors towards umbrella trials (multiple molecular targets in a single tumor) and basket trials (single molecular abnormality across multiple cancer types). This evolution can be clearly seen in CRC, where we started with one gene, one drug approach, and moved towards a multi-gene, multi-drug approach and currently we are at a multi-molecular, multi-drug approach [132].

This systems medicine approach is helping to accelerate bench to bedside developments and an example is the EXACT trial, where treatment-refractory cancer patients are administered an individualized treatment concept based on prospective biomarkers assessed in a real-time biopsy [133].

All of the above has had a major impact on the clinic and as we can now witness, we have achieved a better patient stratification, earlier and more sensitive diagnostics and drug repurposing. Nonetheless, it is important that we continue working to overcome the major challenges we are still facing. These include, but are not limited to morphologic and molecular heterogeneity of cancer, treatment resistance, drug addiction, and other challenges such as standardization of methods, infrastructure, and cost and big data. Hence, it will be important to have appropriate biomarkers to inform clinicians on administering the most effective treatment to the individual patient at the right time.

Acknowledgements

RB is supported by a REACH HIGH Scholars Programme – Post-Doctoral Grants. The grant is part-financed by the European Union, Operational Programme II — Cohesion Policy 2014–2020 investing in human capital to create more opportunities and promote the wellbeing of society – European Social Fund.

Author details

Romina Briffa[1,2]*, Simon P. Langdon[3], Godfrey Grech[2] and David J. Harrison[1]

*Address all correspondence to: rb228@st-andrews.ac.uk

1 School of Medicine, University of St Andrews, Fife, United Kingdom

2 Department of Pathology, Faculty of Medicine and Surgery, University of Malta, Msida, Malta

3 Cancer Research UK Edinburgh Centre and Division of Pathology Laboratory, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom

References

[1] Bastos DA, Ribeiro SC, de Freitas D, Hoff PM. Combination therapy in high-risk stage II or stage III colon cancer: Current practice and future prospects. Therapeutic Advances in Medical Oncology 2010 Jul;**2**(4):261-272

[2] Weidlich S, Walsh K, Crowther D, Burczynski ME, Feuerstein G, Carey FA, et al. Pyrosequencing-based methods reveal marked inter-individual differences in oncogene mutation burden in human colorectal tumours. British Journal of Cancer. 2011 Jul 12;**105**(2):246-254

[3] Rô Me Viguier J, Rie Boige V, Miquel C, Pocard M, Giraudeau B, Sabourin J-C, et al. ERCC1 codon 118 polymorphism is a predictive factor for the tumor response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. Clinical Cancer Research. 2005;**11**(17):6212-6217

[4] Virag P, Fischer-Fodor E, Perde-Schrepler M, Brie I, Tatomir C, Balacescu L, et al. Oxaliplatin induces different cellular and molecular chemoresistance patterns in colorectal cancer cell lines of identical origins. BMC Genomics. 2013;**14**(1):480

[5] Zhang B, Zhang B, Chen X, Bae S, Singh K, Washington MK, et al. Loss of Smad4 in colorectal cancer induces resistance to 5-fluorouracil through activating Akt pathway. British Journal of Cancer. 2014 Feb 18;**110**(4):946-957

[6] Watson RG, Muhale F, Thorne LB, Yu J, O'Neil BH, Hoskins JM, et al. Amplification of thymidylate synthetase in metastatic colorectal cancer patients pretreated with 5-fluorouracil-based chemotherapy. European Journal of Cancer. 2017 Jun 15;**46**(18):3358-3364

[7] Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. Journal of Clinical Oncology. 2010 Jul 10;**28**(20):3219-3226

[8] van Triest B, Pinedo HM, van Hensbergen Y, Smid K, Telleman F, Schoenmakers PS, et al. Thymidylate synthase level as the main predictive parameter for sensitivity to 5-fluorouracil, but not for folate-based thymidylate synthase inhibitors, in 13 nonselected colon cancer cell lines. Clinical Cancer Research. 1999 Mar;**5**(3):643-654

[9] Zhang N, Yin Y, S-J X, Chen W-S. 5-Fluorouracil: Mechanisms of resistance and reversal strategies. Molecules. 2008 Aug 5;**13**(8):1551-1569

[10] Sasaki S, Watanabe T, Kobunai T, Konishi T, Nagase H, Sugimoto Y, et al. hRFI overexpressed in HCT116 cells modulates Bcl-2 family proteins when treated with 5-fluorouracil. Oncology Reports. 2006 May;**15**(5):1293-1298

[11] Guo X, Goessl E, Jin G, Collie-Duguid ESR, Cassidy J, Wang W, et al. Cell cycle perturbation and acquired 5-fluorouracil chemoresistance. Anticancer Research. 2008;**28**(1A):9-14

[12] Shin Y-K, Yoo BC, Chang HJ, Jeon E, Hong S-H, Jung M-S, et al. Down-regulation of mitochondrial F1F0-ATP synthase in human colon cancer cells with induced 5-fluorouracil resistance. Cancer Research. 2005 Apr 15;**65**(8):3162-3170

[13] Hwang IT, Chung YM, Kim JJ, Chung JS, Kim BS, Kim HJ, et al. Drug resistance to 5-FU linked to reactive oxygen species modulator 1. Biochemical and Biophysical Research Communications. 2007 Jul 27;**359**(2):304-310

[14] Martinez-Balibrea E, Martínez-Cardús A, Ginés A, Ruiz de Porras V, Moutinho C, Layos L, et al. Tumor-related molecular mechanisms of oxaliplatin resistance. Molecular Cancer Therapeutics. 2015;**14**(8):1767-1776

[15] Van Emburgh BO, Sartore-Bianchi A, Di Nicolantonio F, Siena S, Bardelli A. Acquired resistance to EGFR-targeted therapies in colorectal cancer. Molecular Oncology. 2014 Sep;**8**(6):1084-1094

[16] Zhao B, Wang L, Qiu H, Zhang M, Sun L, Peng P, et al. Mechanisms of resistance to anti-EGFR therapy in colorectal cancer. Oncotarget. 2017 Jan 17;**8**(3):3980-4000

[17] Misale S, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to anti-EGFR therapy in colorectal cancer: From heterogeneity to convergent evolution. Cancer Discovery. 2014;**4**(11):1269-1280

[18] Dienstmann R, Salazar R, Tabernero J. Overcoming resistance to anti-EGFR therapy in colorectal cancer. American Society of Clinical Oncology Educational Book. 2015;**35**: e149-e156

[19] Sartore-Bianchi A, Loupakis F, Argilés G, Prager GW. Challenging chemoresistant metastatic colorectal cancer: Therapeutic strategies from the clinic and from the laboratory. Annals of Oncology. 2016 Aug;**27**(8):1456-1466

[20] Wu G, Wilson G, George J, Liddle C, Hebbard L. Overcoming treatment resistance in cancer: Current understanding and tactics. Cancer Letters. 2017;**387**:69-76

[21] Al-Hajeili M, Shields AF, Hwang JJ, Wadlow RC, Marshall JL. Molecular testing to optimize and personalize decision making in the management of colorectal cancer. Oncology (Williston Park, N.Y.). 2017;**31**(4):301-312

[22] Martinez-Rivera M, Siddik ZH. Resistance and gain-of-resistance phenotypes in cancers harboring wild-type p53. Biochemical Pharmacology. 2012 Apr 15;**83**(8):1049-1062

[23] Purnapatre K, Khattar SK, Saini KS. Cytochrome P450s in the development of target-based anticancer drugs. Cancer Letters. 2008 Jan 18;**259**(1):1-15

[24] Muhale FA, Wetmore BA, Thomas RS, McLeod HL. Systems pharmacology assessment of the 5-fluorouracil pathway. Pharmacogenomics. 2011 Mar;**12**(3):341-350

[25] Hasegawa S, Seike K, Koda K, Takiguchi N, Oda K, Hasegawa R, et al. Thymidine phosphorylase expression and efficacy of adjuvant doxifluridine in advanced colorectal cancer patients. Oncology Reports. 2005 Apr;**13**(4):621-626

[26] Griffiths L, Dachs GU, Bicknell R, Harris AL, Stratford IJ. The influence of oxygen tension and pH on the expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast tumor cells grown in vitro and in vivo. Cancer Research. 1997 Feb 15;**57**(4):570-572

[27] Diasio RB, Beavers TL, Carpenter JT. Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. The Journal of Clinical Investigation. 1988 Jan;**81**(1, 1):47-51

[28] Dervieux T, Meshkin B, Neri B. Pharmacogenetic testing: Proofs of principle and pharmacoeconomic implications. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis. 2005 Jun;**573**(1-2):180-194

[29] Tsutani Y, Yoshida K, Sanada Y, Wada Y, Konishi K, Fukushima M, et al. Decreased orotate phosphoribosyltransferase activity produces 5-fluorouracil resistance in a human gastric cancer cell line. Oncology Reports. 2008 Dec;**20**(6):1545-1551

[30] Hector S, Bolanowska-Higdon W, Zdanowicz J, Hitt S, Pendyala L. In vitro studies on the mechanisms of oxaliplatin resistance. Cancer Chemotherapy and Pharmacology. 2001 Nov 21;**48**(5):398-406

[31] Stoehlmacher J, Park DJ, Zhang W, Groshen S, Tsao-Wei DD, MC Y, et al. Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. Journal of the National Cancer Institute. 2002 Jun 19;**94**(12):936-942

[32] Imyanitov EN, Moiseyenko VM. Molecular-based choice of cancer therapy: Realities and expectations. Clinica Chimica Acta. 2007 Apr;**379**(1-2):1-13

[33] Marcuello E, Altés A, Menoyo A, Del Rio E, Gómez-Pardo M, Baiget M. UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer. British Journal of Cancer. 2004 Aug 16;**91**(4):678-682

[34] Lee C, Raffaghello L, Longo VD. Starvation, detoxification, and multidrug resistance in cancer therapy. Drug Resistance Updates. 2012;**15**(1-2):114-122

[35] Vasiliou V, Vasiliou K, Nebert DW. Human ATP-binding cassette (ABC) transporter family. Human Genomics. 2009 Apr;**3**(3):281-290

[36] DeGorter MK, Xia CQ, Yang JJ, Kim RB. Drug transporters in drug efficacy and toxicity. Annual Review of Pharmacology and Toxicology. 2012 Feb 10;**52**(1):249-273

[37] Pratt S, Shepard RL, Kandasamy RA, Johnston PA, Perry Iii W, Dantzig AH. The multidrug resistance protein 5 (ABCC5) confers resistance to 5-fluorouracil and transports its monophosphorylated metabolites. Molecular Cancer Therapeutics. May 1 2005;**4**(5): 855-863

[38] Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: An evolving paradigm. Nature Reviews. Cancer. 2013 Oct;**13**(10):714-726

[39] Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. Nature Reviews. Drug Discovery. 2006 Mar 1;**5**(3):219-234

[40] Abraham J, Earl HM, Pharoah PD, Caldas C. Pharmacogenetics of cancer chemotherapy. Biochimica et Biophysica Acta. 2006 Dec;**1766**(2):168-183

[41] Popat S, Matakidou A, Houlston RS. Thymidylate synthase expression and prognosis in colorectal cancer: A systematic review and meta-analysis. Journal of Clinical Oncology. 2004 Feb 1;**22**(3):529-536

[42] Etienne M-C, Chazal M, Laurent-Puig P, Magné N, Rosty C, Formento J-L, et al. Prognostic value of tumoral thymidylate synthase and p53 in metastatic colorectal cancer patients receiving fluorouracil-based chemotherapy: Phenotypic and genotypic analyses. Journal of Clinical Oncology. 2002 Jun 15;**20**(12):2832-2843

[43] Aschele C, Sobrero A, Faderan MA, Bertino JR. Novel mechanism(s) of resistance to 5-fluorouracil in human colon cancer (HCT-8) sublines following exposure to two different clinically relevant dose schedules. Cancer Research. 1992 Apr 1;**52**(7):1855-1864

[44] Yoo BK, Gredler R, Vozhilla N, Su Z-Z, Chen D, Forcier T, et al. Identification of genes conferring resistance to 5-fluorouracil. Proceedings of the National Academy of Sciences. 2009 Aug 4;**106**(31):12938-12943

[45] Libra M, Navolanic PM, Talamini R, Cecchin E, Sartor F, Tumolo S, et al. Thymidylate synthetase mRNA levels are increased in liver metastases of colorectal cancer patients resistant to fluoropyrimidine-based chemotherapy. BMC Cancer. 2004 Mar 25;**4**:11

[46] Watson RG, Muhale F, Thorne LB, Yu J, O?Neil BH, Hoskins JM, et al. Amplification of thymidylate synthetase in metastatic colorectal cancer patients pretreated with 5-fluorouracil-based chemotherapy. European Journal of Cancer. 2010 Dec;**46**(18):3358-3364

[47] Montagut C, Dalmases A, Bellosillo B, Crespo M, Pairet S, Iglesias M, et al. Identification of a mutation in the extracellular domain of the epidermal growth factor receptor conferring cetuximab resistance in colorectal cancer. Nature Medicine. 2012 Jan 22;**18**(2): 221-223

[48] Ibrahim AEK, Arends MJ. Molecular typing of colorectal cancer: Applications in diagnosis and treatment. Diagnostic Histopathology. 2012 Feb;**18**(2):70-80

[49] Sartore-Bianchi A, Moroni M, Veronese S, Carnaghi C, Bajetta E, Luppi G, et al. Epidermal growth factor receptor gene copy number and clinical outcome of metastatic colorectal cancer treated with panitumumab. Journal of Clinical Oncology. 2007 Aug 1;**25**(22):3238-3245

[50] Wheeler DL, Iida M, Kruser TJ, Nechrebecki MM, Dunn EF, Armstrong EA, et al. Epidermal growth factor receptor cooperates with Src family kinases in acquired resistance to cetuximab. Cancer Biology & Therapy. 2009 Apr;**8**(8):696-703

[51] Lievre A, Bachet J-B, Le Corre D, Boige V, Landi B, Emile J-F, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Research. 2006 Apr 15;**66**(8):3992-3995

[52] Di Fiore F, Blanchard F, Charbonnier F, Le Pessot F, Lamy A, Galais MP, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. British Journal of Cancer. 2007 Apr 23;**96**(8):1166-1169

[53] De Roock W, Piessevaux H, De Schutter J, Janssens M, De Hertogh G, Personeni N, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Annals of Oncology. 2008 Mar;**19**(3):508-515

[54] Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature. 2012 Jun 13;**486**(7404):532-536

[55] Lee AJX, Endesfelder D, Rowan AJ, Walther A, Birkbak NJ, Futreal PA, et al. Chromosomal instability confers intrinsic multidrug resistance. Cancer Research. 2011 Mar 1; **71**(5):1858-1870

[56] Samuels Y, Velculescu VE. Oncogenic mutations of PIK3CA in human cancers. Cell Cycle. 2004 Oct 12;**3**(10):1221-1224

[57] Arnold CN, Goel A, Boland CR. Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines. International Journal of Cancer. 2003 Aug 10;**106**(1):66-73

[58] Hewish M, Lord CJ, Martin SA, Cunningham D, Ashworth A. Mismatch repair deficient colorectal cancer in the era of personalized treatment. Nature Reviews. Clinical Oncology. 2010 Apr 23;**7**(4):197-208

[59] Veigl ML, Kasturi L, Olechnowicz J, Ma AH, Lutterbaugh JD, Periyasamy S, et al. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. Proceedings of the National Academy of Sciences of the United States of America. 1998 Jul 21;**95**(15):8698-8702

[60] Ye L, Jiang T, Shao H, Zhong L, Wang Z, Liu Y, et al. miR-1290 Is a biomarker in DNA-mismatch-repair-deficient colon cancer and promotes resistance to 5-fluorouracil by directly targeting hMSH2. Molecular Therapy. Nucleic Acids. 2017;**7**:453-464

[61] Carethers JM, Chauhan DP, Fink D, Nebel S, Bresalier RS, Howell SB, et al. Mismatch repair proficiency and in vitro response to 5-fluorouracil. Gastroenterology. 1999 Jul;**117**(1):123-131

[62] Carethers JM, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. Gastroenterology. 2004 Feb;**126**(2):394-401

[63] Tajima A, Hess MT, Cabrera BL, Kolodner RD, Carethers JM. The mismatch repair complex hMutS alpha recognizes 5-fluorouracil-modified DNA: Implications for chemosensitivity and resistance. Gastroenterology. 2004 Dec;**127**(6):1678-1684

[64] Martinez-Cardús A, Martinez-Balibrea E, Bandrés E, Malumbres R, Ginés A, Manzano JL, et al. Pharmacogenomic approach for the identification of novel determinants of acquired resistance to oxaliplatin in colorectal cancer. Molecular Cancer Therapeutics. 2009;**8**(1):194-202

[65] Chaney SG, Campbell SL, Bassett E, Wu Y. Recognition and processing of cisplatin- and oxaliplatin-DNA adducts. Critical Reviews in Oncology/Hematology. 2005 Jan;**53**(1):3-11

[66] Shirota Y, Stoehlmacher J, Brabender J, Xiong Y-P, Uetake H, Danenberg KD, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. Journal of Clinical Oncology. 2001 Dec 1;**19**(23):4298-4304

[67] Stoehlmacher J, Ghaderi V, Iobal S, Groshen S, Tsao-Wei D, Park D, et al. A polymorphism of the XRCC1 gene predicts for response to platinum based treatment in advanced colorectal cancer. Anticancer Research. 2001;**21**(4B):3075-3079

[68] O'Connor PM, Jackman J, Bae I, Myers TG, Fan S, Mutoh M, et al. Characterization of the p53 tumor suppressor pathway in cell lines of the National Cancer Institute anticancer drug screen and correlations with the growth-inhibitory potency of 123 anticancer agents. Cancer Research. 1997 Oct 1;**57**(19):4285-4300

[69] Russo A, Bazan V, Iacopetta B, Kerr D, Soussi T, Gebbia N, et al. The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of *p53* mutation: Influence of tumor site, type of mutation, and adjuvant treatment. Journal of Clinical Oncology. 2005 Oct 20;**23**(30):7518-7528

[70] Ahmed IAM, Kelly SB, Anderson JJ, Angus B, Challen C, Lunec J. The predictive value of p53 and p33 (ING1b) in patients with Dukes'C colorectal cancer. Colorectal Disease. 2008 May;**10**(4):344-351

[71] Fulda S, Debatin K-M. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene. 2006 Aug 7;**25**(34):4798-4811

[72] Indran IR, Tufo G, Pervaiz S, Brenner C. Recent advances in apoptosis, mitochondria and drug resistance in cancer cells. Biochimica et Biophysica Acta (BBA) – Bioenergetics. 2011 Jun;**1807**(6):735-745

[73] Yang SY, Sales KM, Fuller B, Seifalian AM, Winslet MC. Apoptosis and colorectal cancer: Implications for therapy. Trends in Molecular Medicine. 2009 May;**15**(5):225-233

[74] Gourdier I, Del Rio M, Crabbé L, Candeil L, Copois V, Ychou M, et al. Drug specific resistance to oxaliplatin is associated with apoptosis defect in a cellular model of colon carcinoma. FEBS Letters. 2002 Oct 9;**529**(2-3):232-236

[75] Li F, Sethi G. Targeting transcription factor NF-κB to overcome chemoresistance and radioresistance in cancer therapy. Biochimica et Biophysica Acta (BBA) – Reviews on Cancer. 2010 Apr;**1805**(2):167-180

[76] Fukuyama R, Ng KP, Cicek M, Kelleher C, Niculaita R, Casey G, et al. Role of IKK and oscillatory NFκB kinetics in MMP-9 gene expression and chemoresistance to 5-fluorouracil in RKO colorectal cancer cells. Molecular Carcinogenesis. 2007 May;**46**(5):402-413

[77] Jani TS, DeVecchio J, Mazumdar T, Agyeman A, Houghton JA. Inhibition of NF-κB signaling by quinacrine is cytotoxic to human colon carcinoma cell lines and is synergistic

in combination with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) or oxaliplatin. The Journal of Biological Chemistry. 2010 Jun 18;**285**(25):19162-19172

[78] Garcia MA, Carrasco E, Aguilera M, Alvarez P, Rivas C, Campos JM, et al. The chemotherapeutic drug 5-fluorouracil promotes PKR-mediated apoptosis in a p53- independent manner in colon and breast cancer cells. Ulasov I, editor. PLoS One 2011 Aug 24;**6**(8):e23887

[79] Liu S, Wang J, Niu W, Liu E, Wang J, Peng C, et al. The β6-integrin-ERK/MAP kinase pathway contributes to chemo resistance in colon cancer. Cancer Letters. 2013 Jan 28;**328**(2): 325-334

[80] Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature. 2008 Mar 13;**452**(7184):230-233

[81] Derdak Z, Mark NM, Beldi G, Robson SC, Wands JR, Baffy G. The mitochondrial uncoupling protein-2 promotes chemoresistance in cancer cells. Cancer Research. 2008 Apr 15;**68**(8):2813-2819

[82] Bensinger SJ, Christofk HR. New aspects of the Warburg effect in cancer cell biology. Seminars in Cell & Developmental Biology. 2012 Jun;**23**(4):352-361

[83] Ayyasamy V, Owens KM, Desouki MM, Liang P, Bakin A, Thangaraj K, et al. Cellular model of Warburg effect identifies tumor promoting function of UCP2 in breast cancer and its suppression by genipin. Lewin A, editor. PLoS One 2011 Sep 15;**6**(9):e24792

[84] Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. The Journal of General Physiology. 1927 Mar 7;**8**(6):519-530

[85] Tong X, Zhao F, Mancuso A, Gruber JJ, Thompson CB. The glucose-responsive transcription factor ChREBP contributes to glucose-dependent anabolic synthesis and cell proliferation. Proceedings of the National Academy of Sciences. 2009 Dec 22;**106**(51): 21660-21665

[86] Pelicano H, Martin DS, R-H X, Huang P. Glycolysis inhibition for anticancer treatment. Oncogene. 2006 Aug 7;**25**(34):4633-4646

[87] Erler JT, Cawthorne CJ, Williams KJ, Koritzinsky M, Wouters BG, Wilson C, et al. Hypoxia-mediated down-regulation of Bid and Bax in tumors occurs via hypoxia-inducible factor 1-dependent and -independent mechanisms and contributes to drug resistance. Molecular and Cellular Biology. 2004 Apr;**24**(7):2875-2889

[88] Kuai X-Y, Ji Z-Y, Zhang H-J. Mitochondrial uncoupling protein 2 expression in colon cancer and its clinical significance. World Journal of Gastroenterology. 2010 Dec 7;**16**(45): 5773-5778

[89] Rousset S, Mozo J, Dujardin G, Emre Y, Masscheleyn S, Ricquier D, et al. UCP2 is a mitochondrial transporter with an unusual very short half-life. FEBS Letters. 2007 Feb 6;**581**(3):479-482

[90] Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, et al. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nature Genetics. 2000 Dec 1;**26**(4):435-439

[91] Horimoto M, Resnick MB, Konkin TA, Routhier J, Wands JR, Baffy G. Expression of uncoupling protein-2 in human colon cancer. Clinical Cancer Research. 2004 Sep 15;**10**(18): 6203-6207

[92] Sánchez-Aragó M, Cuezva JM. The bioenergetic signature of isogenic colon cancer cells predicts the cell death response to treatment with 3-bromopyruvate, iodoacetate or 5-fluorouracil. Journal of Translational Medicine. 2011 Feb 8;**9**:19

[93] Ward C, Langdon SP, Mullen P, Harris AL, Harrison DJ, Supuran CT, et al. New strategies for targeting the hypoxic tumour microenvironment in breast cancer. Cancer Treatment Reviews. 2013 Apr;**39**(2):171-179

[94] Bristow RG, Hill RP. Hypoxia and metabolism: Hypoxia, DNA repair and genetic instability. Nature Reviews. Cancer. 2008 Mar;**8**(3):180-192

[95] Zhang J, Roberts TM, Shivdasani RA. Targeting PI3K signaling as a therapeutic approach for colorectal cancer. Gastroenterology. 2011 Jul;**141**(1):50-61

[96] Semenza GL. Targeting HIF-1 for cancer therapy. Nature Reviews. Cancer. 2003 Oct; **3**(10):721-732

[97] Pritchard CC, Grady WM. Colorectal cancer molecular biology moves into clinical practice. Gut. 2011 Jan 1;**60**(1):116-129

[98] Papageorgis P, Cheng K, Ozturk S, Gong Y, Lambert AW, Abdolmaleky HM, et al. Smad4 inactivation promotes malignancy and drug resistance of colon cancer. Cancer Research. 2011 Feb 1;**71**(3):998-1008

[99] Sosa V, Moliné T, Somoza R, Paciucci R, Kondoh H, LLeonart ME. Oxidative stress and cancer: An overview. Ageing Research Reviews. 2013 Jan;**12**(1):376-390

[100] Tamada M, Nagano O, Tateyama S, Ohmura M, Yae T, Ishimoto T, et al. Modulation of glucose metabolism by CD44 contributes to antioxidant status and drug resistance in cancer cells. Cancer Research. 2012 Mar 15;**72**(6):1438-1448

[101] Greaves M, Maley CC. Clonal evolution in cancer. Nature. 2012 Jan 18;**481**(7381):306-313

[102] Gillies RJ, Verduzco D, Gatenby RA. Evolutionary dynamics of carcinogenesis and why targeted therapy does not work. Nature Reviews. Cancer. 2012 Jun 14;**12**(7):487-493

[103] Diaz Jr LA, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012 Jun 13;**486**(7404):537-540

[104] Longley DB, Allen WL, Johnston PG. Drug resistance, predictive markers and pharmacogenomics in colorectal cancer. Biochimica et Biophysica Acta (BBA) – Reviews on Cancer. 2006 Dec;**1766**(2):184-196

[105] Tentes IK, Schmidt WM, Krupitza G, Steger GG, Mikulits W, Kortsaris A, et al. Long-term persistence of acquired resistance to 5-fluorouracil in the colon cancer cell line SW620. Experimental Cell Research. 2010 Nov;**316**(19):3172-3181

[106] Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. The New England Journal of Medicine. 2012 Mar 8;**366**(10):883-892

[107] Marusyk A, Polyak K. Tumor heterogeneity: Causes and consequences. Biochimica et Biophysica Acta (BBA) – Reviews on Cancer. 2010 Jan;**1805**(1):105-117

[108] McGranahan N, Burrell RA, Endesfelder D, Novelli MR, Swanton C. Cancer chromosomal instability: Therapeutic and diagnostic challenges. EMBO Reports. 2012 May 18;**13**(6):528-538

[109] Barratt PL, Seymour MT, Stenning SP, Georgiades I, Walker C, Birbeck K, et al. DNA markers predicting benefit from adjuvant fluorouracil in patients with colon cancer: A molecular study. Lancet (London, England). 2002 Nov 2;**360**(9343):1381-1391

[110] Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: A looking glass for cancer? Nature Reviews. Cancer. 2012 Apr 19;**12**(5):323-334

[111] Urbach D, Lupien M, Karagas MR, Moore JH. Cancer heterogeneity: Origins and implications for genetic association studies. Trends in Genetics. 2012 Nov;**28**(11):538-543

[112] Crea F, Nobili S, Paolicchi E, Perrone G, Napoli C, Landini I, et al. Epigenetics and chemoresistance in colorectal cancer: An opportunity for treatment tailoring and novel therapeutic strategies. Drug Resistance Updates. 2011 Dec;**14**(6):280-296

[113] Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. Nature Biotechnology. 2010 Oct 13;**28**(10):1069-1078

[114] Jeggo PA, L?brich M. Contribution of DNA repair and cell cycle checkpoint arrest to the maintenance of genomic stability. DNA Repair (Amst). 2006 Sep 8;**5**(9-10):1192-1198

[115] Lahtz C, Pfeifer GP. Epigenetic changes of DNA repair genes in cancer. Journal of Molecular Cell Biology. 2011 Feb;**3**(1):51-58

[116] Wilting RH, Dannenberg J-H. Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance. Drug Resistance Updates. 2012 Feb;**15**(1-2):21-38

[117] Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. Nature Reviews. Genetics. 2006 Jan 1;**7**(1):21-33

[118] Sugita H, Iida S, Inokuchi M, Kato K, Ishiguro M, Ishikawa T, et al. Methylation of BNIP3 and DAPK indicates lower response to chemotherapy and poor prognosis in gastric cancer. Oncology Reports. 2011 Feb 1;**25**(2):513-518

[119] Shimizu S, Iida S, Ishiguro M, Uetake H, Ishikawa T, Takagi Y, et al. Methylated BNIP3 gene in colorectal cancer prognosis. Oncology Letters. 2010 Sep 1;**1**(5):865-872

[120] Crea F, Giovannetti E, Cortesi F, Mey V, Nannizzi S, Gallegos Ruiz MI, et al. Epigenetic mechanisms of irinotecan sensitivity in colorectal cancer cell lines. Molecular Cancer Therapeutics. 2009 Jul 1;**8**(7):1964-1973

[121] Cheetham S, Tang MJ, Mesak F, Kennecke H, Owen D, Tai IT. SPARC promoter hypermethylation in colorectal cancers can be reversed by 5-Aza-2′deoxycytidine to increase

SPARC expression and improve therapy response. British Journal of Cancer. 2008 Jun 3;**98**(11):1810-1819

[122] Tai IT, Dai M, Owen DA, Chen LB. Genome-wide expression analysis of therapy-resistant tumors reveals SPARC as a novel target for cancer therapy. The Journal of Clinical Investigation. 2005 Jun;**115**(6):1492-1502

[123] Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell. 2010 Apr 2;**141**(1):69-80

[124] Spitzner M, Emons G, Kramer F, Gaedcke J, Rave-Frank M, Scharf J-G, et al. A gene expression signature for chemoradiosensitivity of colorectal cancer cells. International Journal of Radiation Oncology. 2010 Nov 15;**78**(4):1184-1192

[125] Dallas NA, Xia L, Fan F, Gray MJ, Gaur P, van Buren G, et al. Chemoresistant colorectal cancer cells, the cancer stem cell phenotype, and increased sensitivity to insulin-like growth factor-I receptor inhibition. Cancer Research. March 1 2009;**69**(5):1951-1957

[126] Tsunekuni K, Konno M, Asai A, Koseki J, Kobunai T, Takechi T, et al. MicroRNA profiles involved in trifluridine resistance. Oncotarget. 2017;**8**:53017-53027

[127] Wheeler DL, Huang S, Kruser TJ, Nechrebecki MM, Armstrong EA, Benavente S, et al. Mechanisms of acquired resistance to cetuximab: Role of HER (ErbB) family members. Oncogene. 2008 Jun 26;**27**(28):3944-3956

[128] Kyula JN, Van Schaeybroeck S, Doherty J, Fenning CS, Longley DB, Johnston PG. Chemotherapy-induced activation of ADAM-17: A novel mechanism of drug resistance in colorectal cancer. Clinical Cancer Research. 2010 Jul 1;**16**(13):3378-3389

[129] Goulielmaki M, Koustas E, Moysidou E, Vlassi M, Sasazuki T, Shirasawa S, et al. BRAF associated autophagy exploitation: BRAF and autophagy inhibitors synergise to efficiently overcome resistance of BRAF mutant colorectal cancer cells. Oncotarget. 2016 Feb 22;**7**(8):9188-9221

[130] Lipinska N, Romaniuk A, Paszel-Jaworska A, Toton E, Kopczynski P, Rubis B. Telomerase and drug resistance in cancer. Cellular and Molecular Life Sciences. 2017 Jun;**16**:1-12

[131] CASYM Europe. CASyM and the Road to Systems Medicine [Internet]. Available from: https://www.casym.eu/casym-systems-medicine [Accessed Jul 21, 2017]

[132] Dienstmann R, Vermeulen L, Guinney J, Kopetz S, Tejpar S, Tabernero J. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. Nature Reviews. Cancer. 2017 Jan 4;**17**(2):79-92

[133] Prager G, Mader RM, Wrba F, Zielinski C. The EXACT trial: An individualized treatment protocol for solid tumors. Journal of Clinical Oncology. 2014 May 20;**32**(15 Suppl):e14002-e14002

Chapter 5

The Prognostic Significance of the Expression Change of EGFR during Neoadjuvant Chemoradiotherapy in Patients with Rectal Carcinoma

Igor Richter

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73275

Abstract

Aim of the study: the aim of this retrospective study was to determine the prognostic impact of epidermal growth factor receptor (EGFR) expression changes during neoadjuvant chemoradiotherapy in patients with locally advanced rectal cancer.

Material and methods: fifty patients with locally advanced rectal cancer were evaluated. All the patients were administered the total dose of 44 Gy. Capecitabine was concomitantly administered in the dose 825 mg/m^2 in two daily oral administrations. Surgery was indicated 4–8 weeks from the chemoradiotherapy completion. EGFR expression in the pretreatment biopsies and in the resected specimens was assessed with immunohistochemistry.

Results: all 50 patients received radiotherapy without interruption up to the total planned dose. The median disease-free survival was 64.9 months, and median overall survival was 76.4 months. Increased EGFR expression was found in 12 patients (26.1%). A statistically significant shorter overall survival ($p < 0.0001$) and disease-free survival ($p < 0.0001$) were found in patients with increased expression of EGFR compared with patients where no increase in the expression of EGFR during neoadjuvant chemoradiotherapy was observed.

Conclusions: the overexpression of EGFR during neoadjuvant chemoradiotherapy for locally advanced rectal adenocarcinoma is associated with significant shorter overall survival and disease-free survival.

Keywords: rectal cancer, radiotherapy, chemotherapy, neoadjuvant treatment, targeted treatment

1. Introduction

Colorectal cancer is one of the most common cancers in developed countries. The incidence of rectal adenocarcinoma represents approximately 30% of all colorectal cancers. The rectal adenocarcinoma typically develops distant metastasis, and the local relapses in presacral area could be identified in 50% of the cases in clinical stage III [1]. The incidence of local relapses could be reduced by radiotherapy. Meta-analysis of 22 clinical studies demonstrated that neoadjuvant or adjuvant radiotherapy significantly reduced the local relapse incidence compared to surgery alone [2]. A neoadjuvant chemoradiotherapy followed by total mesorectal excision is the current standard of the treatment in patients with locally advanced rectal adenocarcinoma. Neoadjuvant chemoradiation has shown a lower incidence of local recurrence and better toxicity profile compared to adjuvant therapy, but no survival benefit was shown [2]. The combination of radiotherapy with 5-flurouracil (5-FU) or capecitabine has demonstrated a higher number of pathological complete remissions and lower incidence of local relapses compared to the treatment with radiotherapy alone [3]. The main prognostic factors of rectal adenocarcinoma are clinical stage, radicality of surgery, pretreatment concentration of CEA, tumor grade, angioinvasion, and mucinous histology. Epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), oncoprotein p53, and survivin were studied as the potential new biomarkers for rectal adenocarcinoma. The aim of this retrospective study was to determine the prognostic impact of EGFR expression changes during neoadjuvant chemoradiotherapy in patients with locally advanced rectal adenocarcima.

1.1. Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR/HER1/erbB-1) is a 170-kDa transmembrane glycoprotein. EGFR belongs to the ErbB family of the tyrosine kinase receptors [4]. More than 10 ligands are known to bind to the EGFR, including epidermal growth factor (EGF), amphiregulin, epiregulin, neuregulin, transforming growth factor alpha (TGF-α), betacellulin, and Heparin-binding EGF-like growth factor (HB-EGF) [5]. EGFR could be activated by the ionizing radiation, too. Ligand binding results in homodimerization of two EGFR molecules or heterodimerization of an EGFR molecule with another member of the ErbB family. After dimerization and internalization, autophosphorylation of the intracellular tyrosine kinase domain occurs, which activates different intracellular transduction pathways. The results are cell proliferation, acceleration of cell repopulation, and apoptosis inhibition. These related transduction pathways include Ras/Raf/MAPK, PI3K/AKT, JAK/STAT, or PLC/PKC. The major signaling route is the Ras/Raf/MAPK pathway that results in cell proliferation. The PI3K/AKT pathway activates PI3K/AKT, causing apoptosis inhibition [6]. EGFR could be directly translocated to the cell nucleus with a direct activation of transcription factors [7]. EGFR is very important for the reparation of normal epidermal cells. The most important mechanism of the increased activity of EGFR is its overexpression in cancer cells. The other mechanisms include increased production of EGFR ligands,

http://dx.doi.org/10.5772/intechopen.73275

activation mutation of EGFR receptor, and loss of intracellular regulation mechanisms or EGFR1 gene amplification. The activation of EGFR on cell surface leads to progression of cell cycle, cell proliferation, angiogenesis, and apoptosis inhibition. It is also associated with more aggressive properties of cancer cells and resistance to the radiotherapy or chemotherapy [8, 9]. The overexpression of EGFR is responsible for the increased motility of the cancer cells [10].

1.2. EGFR and his role in radiotherapy

The reparation, redistribution, repopulation, and reoxygenation are the basic mechanisms of interaction between radiation and cells. EGFR is important for reparation of the damage cells caused by radiation. EGFR is directly translocated to the cell nucleus with a direct activation of transcription factors and results in cells reparation [7]. Similarly, activation of EGFR by radiation results in the activation of Ras/Raf/MAPK pathways with increased expression of DNA reparation genes (Rad51, ATM, XRCC1) [11]. EGFR has influence on the redistribution of cells after radiation. It was found that EGFR inhibitors could cause the redistribution of the cell cycle by G1 phase blockade [12]. Radiobiological studies confirmed the critical role of EGFR in cytoprotective and pro-proliferative response of tumor cells after irradiation. The increased EGFR expression after radiotherapy is related to accelerated repopulation of cancer cells [13]. Increased tumor repopulation during radiotherapy leads to recovery of clonogenic tumor cells, thereby causing counterproductivity to radiation therapy alone [14].

1.2.1. The prognostic significant of EGFR expression in rectal cancer

The overexpression of EGFR is observed in 50–60% of rectal carcinoma and is associated with worse prognosis [15–17]. Azria evaluated the prognostic significance of EGFR expression in pretreatment biopsy on 77 patients with rectal cancer treated by neoadjuvant radiotherapy. The expression of EGFR was observed in 56% patients. In median follow-up of 36 months, it was observed that significantly high number of the local recurrences occurred in patients with overexpression of EGFR above 25% in multivariate analysis (HR 7.18; $p = 0.037$) [18]. Another study evaluated 92 patients with locally advanced rectal carcinoma treated by neoadjuvant chemoradiotherapy. The EGFR expression was observed in 71% of the patients. The patients with overexpression of EGFR had significantly shorter overall survival ($p = 0.013$), significantly shorter disease-free survival ($p = 0.002$) and significantly shorter survival without distance metastases ($p = 0.003$) compared with patients without EGFR expression [19]. Giralt in his study presented a total of 87 patients treated for the locally advanced rectal cancer by neoadjuvant treatment. EGFR overexpression was observed in 52 cases (60% of patients). The patients with overexpression of EGFR had significantly less pathological complete response ($p = 0.006$), shorter DFS compared to patients without EGFR overexpression ($p = 0.003$) [20].

1.2.2. EGFR inhibitors

The two dominant EGFR inhibition strategies under clinical investigation are used. One group of EGFR inhibitors are small molecules called tyrosine kinase inhibitors (TKI): gefitinib and

erlotinib are used in treatment in patients with non-small lung cancer as a palliative treatment. The other possibility of EGFR inhibition is adoption of monoclonal antibodies that bind to extracellular domain of EGFR. Cetuximab and panitumumab are the most commonly used inhibitors in metastatic colorectal cancer. Cetuximab is a chimeric mouse anti-EGFR monoclonal antibody that first received US Food and Drug Administration approval in 2004 for the treatment of irinotecan-refractory colorectal cancer [21]. Panitumumab is a fully human anti-EGFR antibody. With the development of molecular biology, it was found that an important predictive factor for anti-EGFR monoclonal antibodies is the status of K-RAS gene [22]. K-RAS belongs to the RAS genes family. The other members of RAS genes family are N-RAS and H-RAS genes. The products of RAS genes are regulatory proteins that regulate pathways after EGFR activation. Mutation of K-RAS gene is observed in 30–50% cases of colorectal cancer. Cetuximab and panitumumab were evaluated in treating patients with metastatic colorectal cancer with FOLFOX and FOLFIRI regimen. The best results were observed in the group of patients with wild type of RAS genes.

1.2.3. The combination of neoadjuvant chemoradiotherapy and anti-EGFR treatment

Monoclonal anti-EGFR antibodies have shown efficiency in the treatment of metastatic colorectal cancer. Neoadjuvant treatment of rectal cancer has been the topic of several clinical studies of I/II phases evaluating the benefits of monoclonal antibodies against EGFR combined with chemotherapy. The chemotherapy regimens include 5-FU, capecitabine, oxaliplatine, or irinotecan. The doses of radiation were in the range of 45–50.4 Gy. The primary point was to use the number of pathologically completed responses as the predictor of longer disease-free survival (DFS) and overall survival (OS) [23–26]. More DFS and OS dates are observed in treatment of cetuximab than panitumumab. Eleven clinical studies showed average number of pCR in only 10.7% (range 0–25%) of cases [27–37]. On the other hand, the percentage of pCR in separate chemoradiotherapy was 13.5% in 3157 patients in meta-analysis of clinical studies II/III phase [38]. The occurrence of toxicity grade III/IV was described in 30% in combination of neoadjuvant chemotherapy and cetuximab. The most common side effect was diarrhea, while leucopenia, anemia, and elevation of liver transaminases were infrequently observed. The acneiformic rash was observed in 87% of cases but predominantly in grade I/II. Hypersensitization reactions after infusional application of cetuximab were observed in 5–10% of cases. Panitumumab was evaluated in neoadjuvant treatment of rectal cancer with chemotherapy and radiotherapy in clinical study II phase. A total of 60 patients were treated. The percentage of pCR was 21 [39]. It seems that the results of combination cetuximab and chemotherapy in metastatic colorectal cancer or combination of cetuximab and radiotherapy in locally advanced squamous cell carcinoma of the head and neck could not be interpolated to the neoadjuvant treatment of locally advanced rectal cancer [21, 22, 40]. Some studies evaluated the prognostic significant of K-RAS mutation status in neoadjuvant treatment of rectal cancer. A study mentioned above evaluated the influence of panitumumab in neoadjuvant treatment of rectal cancer and failed to demonstrate the prognostic significance of K-RAS gene mutation state and response rate [39]. An EXPERT study evaluated 161 patients with locally advanced rectal cancer. The treatment combined neoadjuvant chemoradiotherapy (potentiated by capecitabine) and CAPOX regimen before and after chemoradiotherapy for both study arms. Cetuximab has been adopted

http://dx.doi.org/10.5772/intechopen.73275

in one arm in all phases of treatment. The patients with wild-type K-RAS treated with cetuximab have shown longer OS (HR 0.27; p = 0.034) compared to patients treated without cetuximab [41]. The following study demonstrated a higher percentage of pCR (37 vs. 11%) in patients in wild-type K-RAS compared to those with K-RAS mutations. In that study, a total of 39 patients with locally rectal cancer were treated with neoadjuvant chemoradiotherapy and cetuximab. K-RAS mutation status was observed in 6 patients, and the other 30 patients had wild-type K-RAS gene [42]. On the other hand, some studies have shown that no prognostic influence of mutation status of K-RAS gene on patients was observed [33, 43]. An interesting fact is the lower incidence of K-RAS mutation status in rectal carcinoma (12–30%) compared to colon carcinoma [43, 44]. The results of studies evaluating the influence of anti-EGFR antibodies with the combination of neoadjuvant chemoradiotherapy for rectal cancer are not satisfactory [27–37]. More options of how to better individualize the treatment of patients with EGFR inhibitors are under investigation. One of them is the research about the dynamics of EGFR expression during the neoadjuvant chemoradiotherapy, which is the focus of our study.

2. Materials and methods

A total of 50 patients with locally advanced rectal cancer were evaluated in our study. The median age was 61.4 years (range 40–78 years). TNM stage II was described in 28 and TNM stage III in 22 patients. The anatomical localization was as follows: 24 patients lower rectum (<5 cm from the anal verge), 24 patients middle rectum (>5–10 cm), and 2 patients upper rectum (above 10 cm from the anal verge). All patients had a histologically verified adenocarcinoma in a pretreatment biopsy: 3 patients grade I, 38 patients grade II, and 9 patients grade III. Pretreatment concentration of CEA was evaluated in 29 patients. Median CEA level was 3.2 (0.5–377) μg/L. Eleven patients had the elevation of CEA.

2.1. Treatments

Neoadjuvant treatment consisted of external beam radiation and chemotherapy. The source of radiation was a linear accelerator Elekta Precise or Elekta Synergy (Elekta, Sweden). The photon energy was 15 MeV. Patients were treated in supine position with full bladder (**Figure 1**). The localization was held on RTG simulator with AP projection. Then patients absolved the planning CT with reconstruction of slices of thickness 5 mm. The contouring of targeted volumes and organs at risk was performed by planning system PrecisPlan 2.15 (**Figure 2**). Patients were irradiated by 3D conformal radiotherapy technique or IMRT using segmented fields (**Figure 3**). A total dose of 44 Gy in 22 fractions (single dose 2 Gy) was administered. The target volume consisted of rectum with tumor, mesorectum, and pelvic regional lymph nodes. All patients were treated by using one targeted volume. The organs at risk were bladder and bowel sac. The verification was performed once a week with the help of cone-beam CT or portal image. Capecitabine was concomitantly administered with a dosage of 825 mg/m^2 twice daily by oral administrations for the whole duration of radiotherapy, including weekends. Surgery was performed 4–8 weeks after the end of chemoradiotherapy.

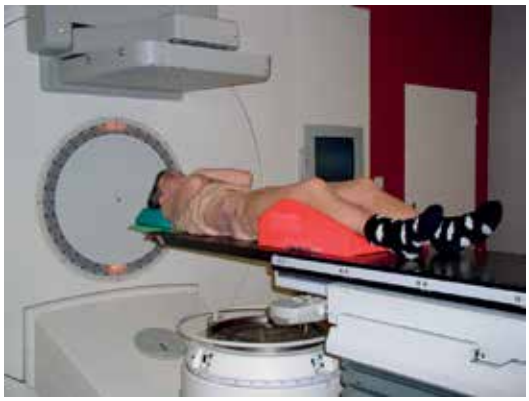

Figure 1. Supine position in irradiated patient in our department of oncology.

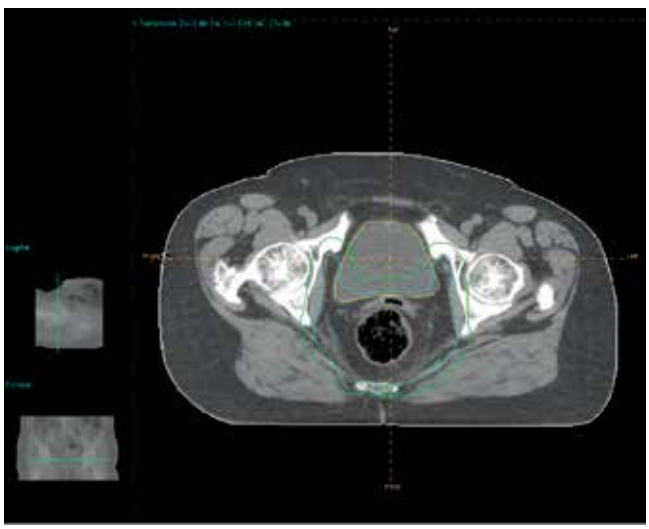

Figure 2. Targeted volumes in radiotherapy of rectal cancer.

2.2. Immunohistochemical determination of EGFR

Routinely fixed, paraffin-embedded blocks of pretreatment biopsies and resected specimens were cut in 3 μm sections. Slides were deparaffinized with xylene and rehydrated and subsequently treated with proteinase K for antigen retrieval. Endogenous peroxidase activity was blocked with peroxidase block solution with 3% hydrogen peroxide. Sections were incubated in complete medium for 30 min at room temperature with EGFR pharmDx monoclonal mouse anti-human IgG1 antibody (EGFR pharmDx™, DakoCytomation, Denmark). A labeled polymer-HRP was then applied and incubated for 30 min. DAB+ substrate-chromogen solution was used for visualization after 10 min incubation, after which slides were counterstained with hematoxylin. As a control for EGFR expression, EGFR pharmDx control slides containing section of two pelleted, formalin-fixed, paraffin-embedded human cell lines were used: one representing a moderate level

http://dx.doi.org/10.5772/intechopen.73275

of EGFR protein expression and the other no EGFR expression. Specimens were examined under a light microscope. All slides were assessed for EGFR expression by a trained pathologist who was blinded for tumor response data. The evaluation was semiquantitative as the color intensity of at least 1% of tumor cells was assessed as follows: 0 = none, 1+ = mild, 2+ = moderate, 3+ = strong (**Figure 4**).

2.3. Statistical analysis methods

Disease-free survival (DFS) and overall survival (OS) were counted from the date of the start therapy and analyzed using the Kaplan–Meier method. Relationship between the level of

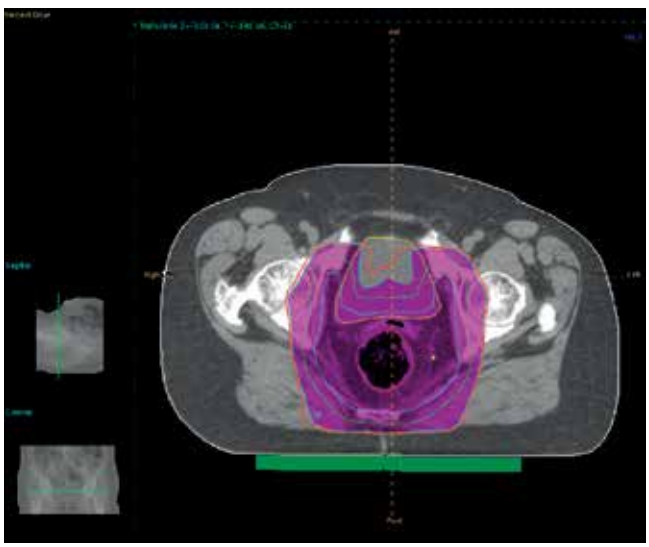

Figure 3. Isodose plane of the radiotherapy of rectal cancer.

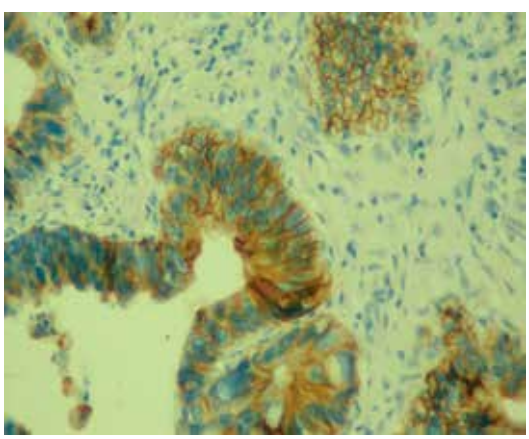

Figure 4. EGFR expression score 3+. Magnification 200x.

EGFR expression and clinical/histopathological characteristic were analyzed using the chi-squared test. Fisher exact test was used on a four-field table when the number of cases was fewer than 10. The prognostic significance of EGFR expression in biopsies and resected specimens and prognostic significance of increased EGFR expression during neoadjuvant chemoradiotherapy on treatment outcomes was assessed by the log-rank test. Multivariate analysis was performed using the Cox regression. We considered $p < 0.05$ to be statistically significant. All statistical analyses were performed using the NCSS 9 statistical software program (NCSS, USA).

3. Results

All 50 patients received radiotherapy without interruption up to the total planned dose. No patient died during the treatment. Concomitant chemotherapy was discontinued prematurely in 4 patients because of hematologic and gastrointestinal toxicity. No patient was hospitalized because of acute treatment toxicity. Non-hematological toxicity evaluation did not achieve grade III or grade IV. The most common types of toxicity were gastrointestinal complaints observed in 44 patients, of them 16 have had nausea and vomiting grade I or II. Hematological toxicity in general was expressed in 25 patients. Anemia grade I was found in 9 patients, grade II in 10 patients, grade III in 1 patient. Grade I leukopenia was found in 11 cases and grade II in 2 patients. One patient has had a grade II thrombocytopenia.

Surgery was conducted in all the patients following 4–8 weeks from neoadjuvant chemoradiotherapy completion. The median time between chemoradiotherapy completion and surgery was 44 days (6.3 weeks). In 30 patients, sphincter-saving surgery was performed, and 20 patients underwent amputation of the rectum. No patient was assessed by the surgeon and found inoperable. R0 resection was performed in 47 patients, and microscopically positive margin was described by a pathologist in 3 patients. No patient was left surgically macroscopic residue. According to the pathological TNM classification, 14 patients were postoperatively in the first clinical stage, 24 patients in the second clinical stage, and 8 patients in the third clinical stage. In four patients, pCR was achieved. Downstaging was described in 30 patients. Progression was reported in four patients. At the date of analysis, median follow-up was 51.3 months.

3.1. Overall survival

To the date of analysis, 21 patients died, and 29 were alive. The median of OS was 76.4 months (95% CI: 57.3–76.9). The 3-year OS evaluated in all patients was 92% (**Figure 5**).

3.2. Disease-free survival

At the time of assessment, recurrence occurred in 25 patients, while the other 25 patients had no signs of recurrence. A local recurrence was found in 8 patients, and generalization of disease was reported in 17 patients. The most common sites of metastases were the liver (8 patients) and lungs (7 patients). One patient suffered from brain metastases, and metastatic involvement of

http://dx.doi.org/10.5772/intechopen.73275

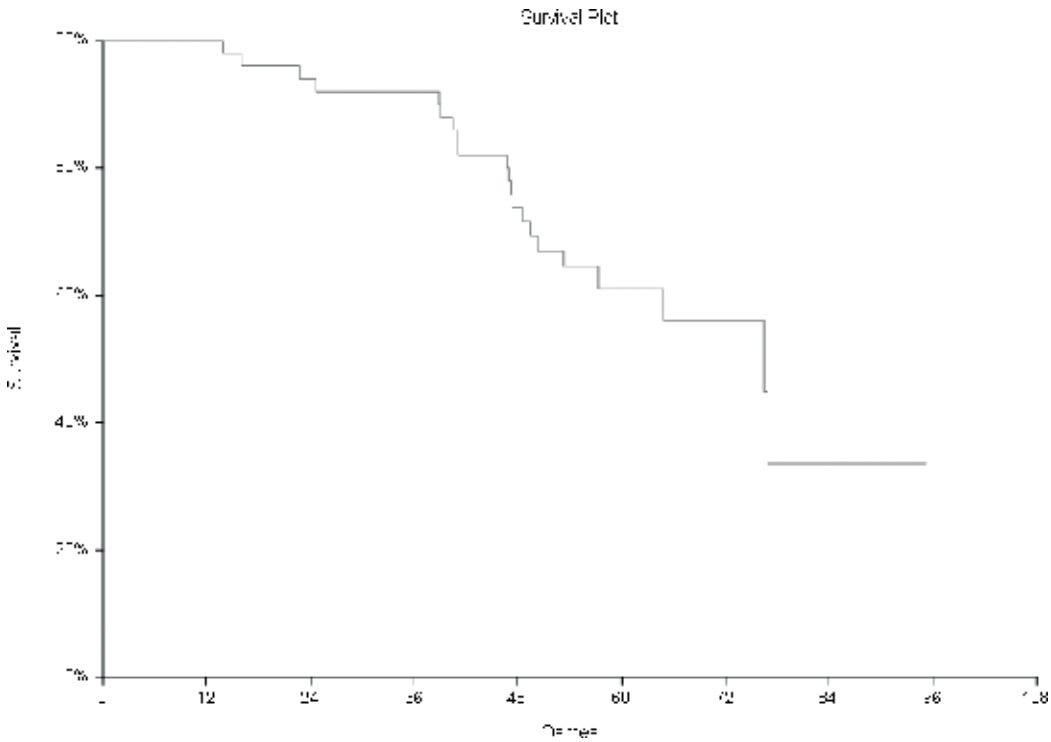

Figure 5. Overall survival (in months) in patients treated by chemoradiotherapy for rectal cancer.

retroperitoneal lymph nodes was found in another patient. The median of DFS was 64.9 months (95% CI 26.4–67.8). The 3-year DFS evaluated in all patients was 56% (**Figure 6**).

3.3. EGFR expression

EGFR expression was examined by both endobiopsy and surgical resection after neoadjuvant chemoradiotherapy. Endobiopsy EGFR was examined in all 50 patients. EGFR 1+ was observed in 18 patients, EGFR 2+ in 5 patients, and EGFR 3+ in 5 patients. Overall, EGFR expression was detected in 28 patients, while 22 patients were not detected with EGFR expression in endobiopsy. EGFR expression was examined and evaluated in 46 patients in the resection. In four patients, EGFR expression was not examined in resection because pCR after neoadjuvant chemoradiotherapy had been achieved. EGFR 1+ was found in 8 patients, EGFR 2+ in 11 patients, and EGFR 3+ in 4 patients. Overall, EGFR expression was detected in 23 patients. In 23 patients, no expression of EGFR was detected in the resection samples. Forty-six patients were enrolled into the evaluation of EGFR expression changes. In four patients, no change expression of EGFR was detected because it had achieved pCR after neoadjuvant chemoradiotherapy. Increased EGFR expression was found in 12 patients. In 34 patients, no increased expression of EGFR was observed (23 patients without any change of EGFR expression, 11 patients with a decrease of EGFR expression).

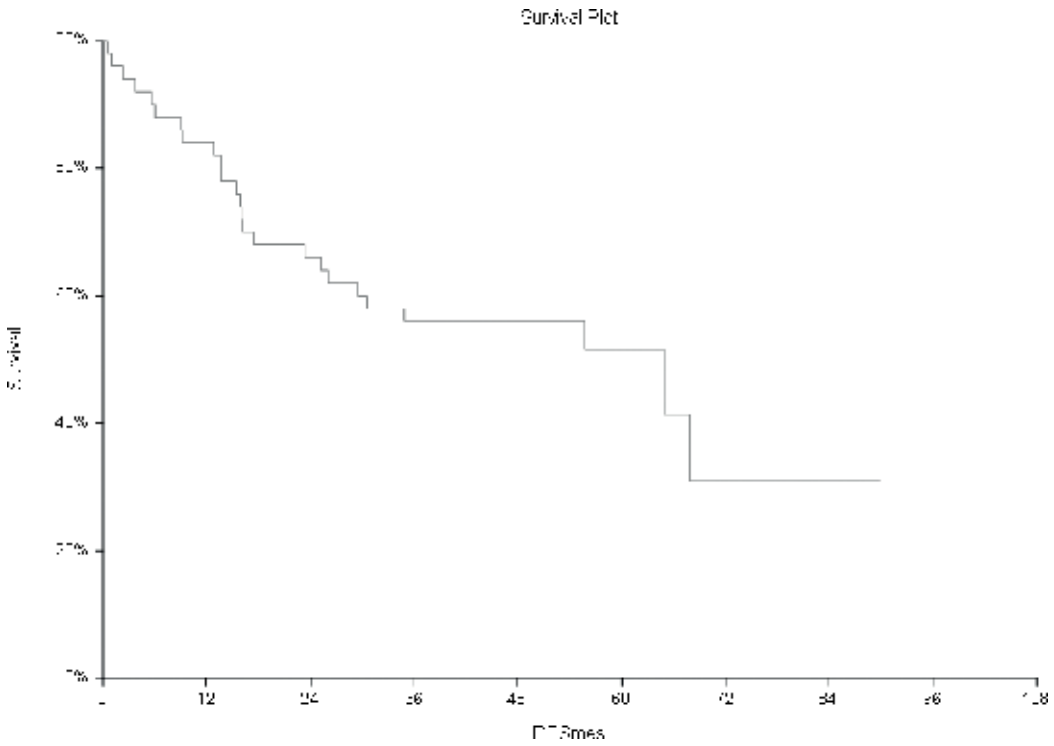

Figure 6. Disease-free survival (in months) in patients treated by chemoradiotherapy for rectal cancer.

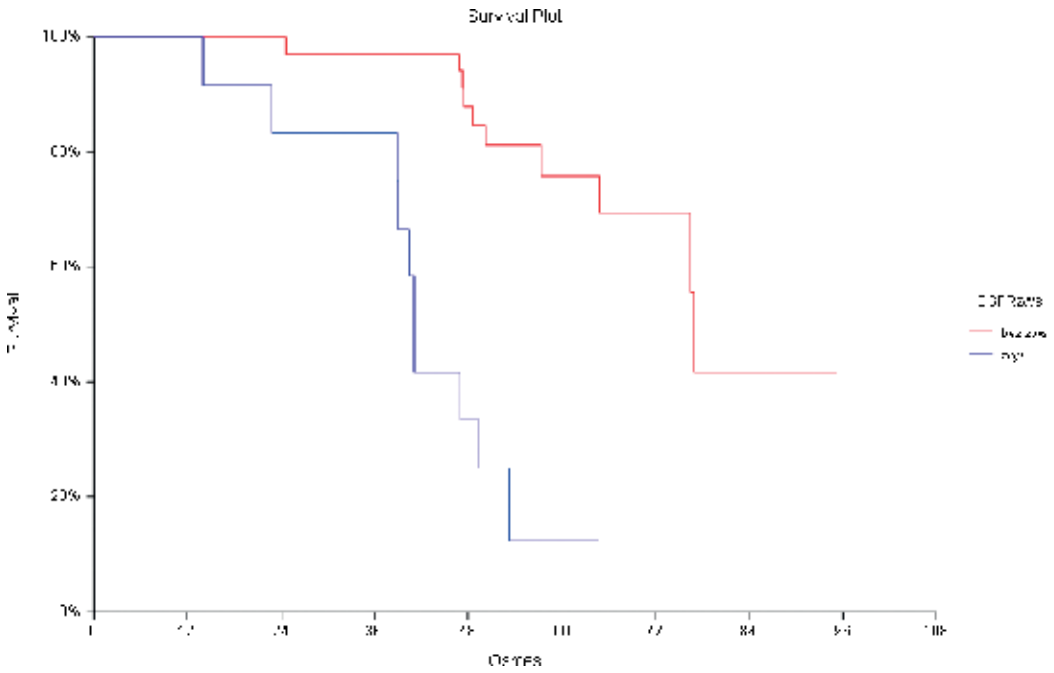

Figure 7. Overall survival (in months) in patients with increased EGFR expression (blue line) and patients without increased EGFR expression (red line) after chemoradiotherapy.

http://dx.doi.org/10.5772/intechopen.73275

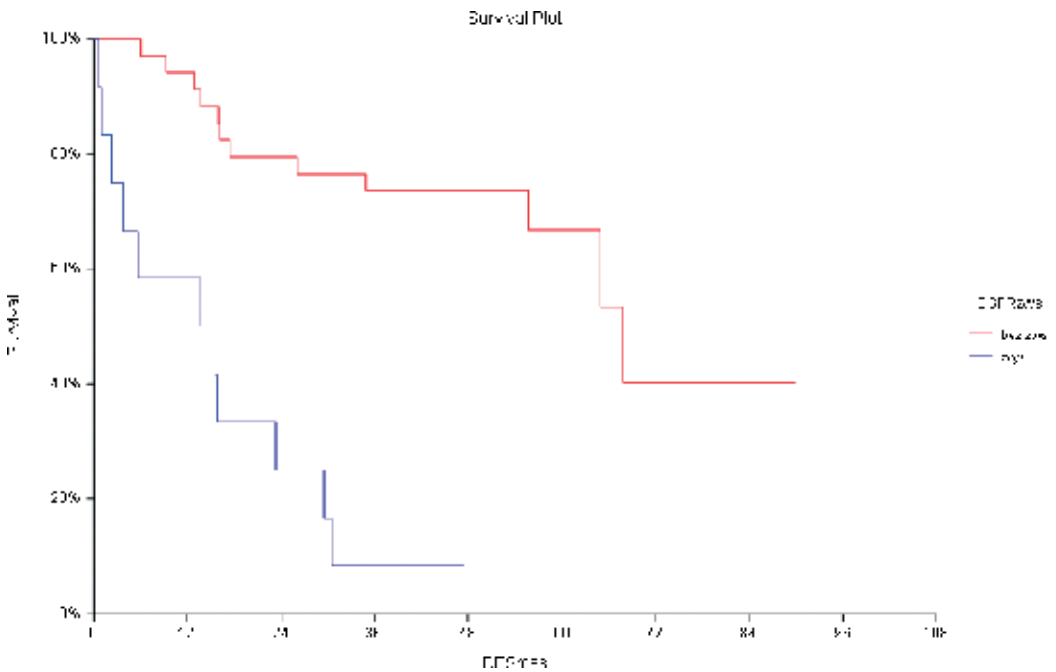

Figure 8. Disease-free survival (in months) in patients with increased EGFR expression (blue line) and patients without increased EGFR expression (red line) after chemoradiotherapy.

The increase of EGFR expression during neoadjuvant treatment had significant impact on OS and DFS. The median of OS in patients with increase of EGFR expression was 41.1 months (95% CI 39.1–47.0). Median of OS in patients without increase of EGFR expression was 76.9 months (95% CI 76.4–76.9). Log-rank: $p < 0.001$ (**Figure 7**). The median of DFS in patients with increase of EGFR expression was 13.7 months (95% CI 3.8–15.8). Median of DFS in patients without increase of EGFR expression was 67.2 months (95% CI 55.7–67.8). Log-rank: $p < 0.001$ (**Figure 8**).

4. Discussion

The results of the present study demonstrated significantly inferior DFS and OS in patients with tumors that had increased EGFR expression after neoadjuvant chemoradiotherapy. The increased EGFR expression after radiotherapy is related to accelerated repopulation of cancer cells [13]. Increased tumor repopulation during radiotherapy leads to recovery of clonogenic tumor cells, thereby causing counterproductivity to radiation therapy alone [14]. The repopulation of clonogenic tumor cells is therefore undesirable phenomenon in treatment using the radiation. We demonstrated increased expression of EGFR in 12 patients, that is, 26.1% of all evaluated patients. In 2012, a retrospective study was conducted in 53 patients with locally advanced rectal cancer treated by neoadjuvant chemoradiotherapy. The aim of the study was similar to our study. During chemoradiotherapy, 14 patients (26%) had an increase in EGFR expression. Patients with increased EGFR expression during treatment had

significantly shorter DFS (HR 3.02, 95% CI 1.15–7.98, p = 0.003) and OS (HR 2.86, 95% CI 1.10–7.40, p = 0.005) than patients with either no change or decreased EGFR expression. In this study, patients were treated with radiotherapy (total dose 50.4 Gy) and chemotherapy (continual administration of 5-FU) [45]. Both studies demonstrated the prognostic influence of change of EGFR expression on DFS and OS in two different groups of patients treated in two different cancer centers. EGFR was evaluated in different pathology laboratories. In the group of 53 patients, radiotherapy was potentiated by continuous 5-FU and in our group by capecitabine. In both studies, the prognostic significance of EGFR dynamics was confirmed. Therefore, they cannot be considered to be simple coincidence but a proven link. In 2014, we published the comparison of both studies with actual follow-up. A total of 103 patients were evaluated. In patients without increasing EGFR expression, there was significantly longer DFS (HR 3.51, 95% CI 1.62–7.61, p < 0.0001) and OS (HR 3.40, 95% CI 1.64–7.04, p < 0.0001, OBR) compared with patients with increase of EGFR. The patients with increase of expression of EGFR had significantly shorter 5-year DFS (20.9 vs. 63.3%, p < 0.0001) and OS (23.3 vs. 68.8%, p < 0.0001) compared with patients with either no change or decreased EGFR expression (**Figures 9** and **10**) [46].

The overexpression of EGFR is observed in 50–60% of rectal carcinoma and is associated with worse prognosis [15–17]. Some studies demonstrated the prognostic influence of EGFR expression on outcomes [18–20]. The most frequent approach of EGFR determination is immunohistochemical (IHC) reaction. This approach was used in most studies. The advantages of IHC determination are simplicity, rapidity of execution, and conservation of tissues morphology.

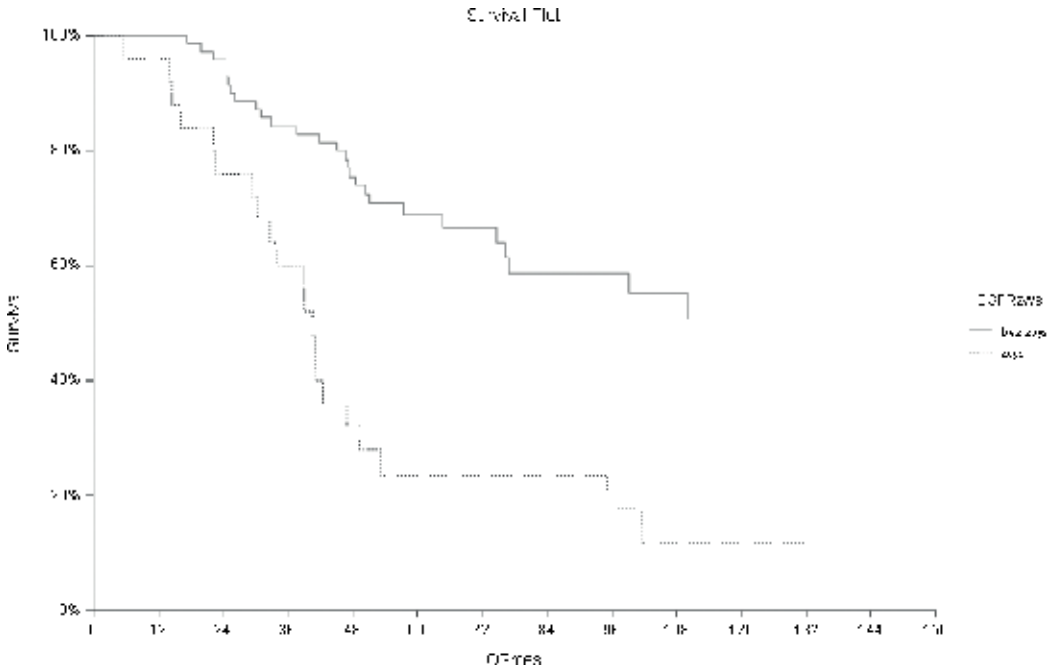

Figure 9. Overall survival (in months) in patients with increased EGFR expression (dotted line) and patients without increased EGFR expression (full line) after chemoradiotherapy.

http://dx.doi.org/10.5772/intechopen.73275

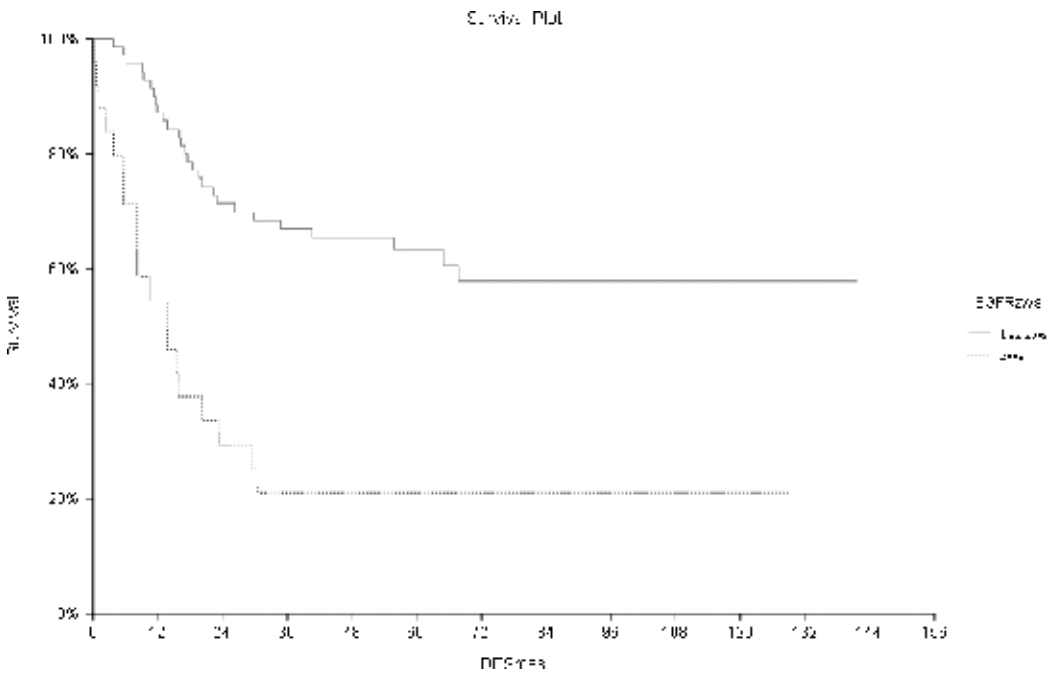

Figure 10. Disease-free survival (in months) in patients with increased EGFR expression (dotted line) and patients without increased EGFR expression (full line) after chemoradiotherapy.

The disadvantages are subjective interpretation by pathologist and existence of more scoring systems for determining EGFR expression. The evaluation of EGFR expression is based on percent range, color intensity, or its combination [21]. Neoadjuvant treatment of rectal cancer has been the topic of several clinical studies I/II phases evaluating the benefits of monoclonal antibodies against EGFR combined with chemotherapy. Eleven clinical studies showed average number of pCR only 10.7% (range 0–25%) of cases [27–37]. The explanation of this will need further understanding of the interaction between radiotherapy, EGFR inhibitors, and cytostatics. Initial studies of this topic showed that prolonged exposure of head and neck cancer cells to EGF could increase the effects of radiation [47, 48]. The reason of radiosensitivity was probably through EGF-induced EGFR degradation. Another early study demonstrated that anti-EGFR antibodies increased radiation-induced apoptosis [49]. Other studies showed the inverse correlation between EGFR expression and response to radiotherapy [50–52]. This relationship between EGFR expression and lower response to radiotherapy was confirmed in human head and neck cancer [53]. EGF is known to induce cyclin D1 expression, a protein that is required for progression from the G1 to S phase. Studies of EGFR signaling inhibition have demonstrated proliferation inhibition of cells in G1 phase [12]. EGFR inhibitors commonly produce cytostatic effects rather than cytotoxicity [54, 55]. The interest of new approach combining the EGFR inhibitors and radiation was generated by the experimental studies that demonstrated of radiation-induced EGFR activity in vitro. Confluent cells in culture treated with ionizing radiation rapidly show increased levels of phosphorylated EGFR [56–59]. The result is cellular proliferation and DNA-damage repair capability. The phenomenon is known as accelerated repopulation. Cetuximab

inhibits this radiation-activated of DNAPK, as well as EGFR nuclear import, DNA repair, and radiation survival [60]. Various preclinical studies demonstrated that EGFR inhibitors increased radiosensitivity in both in vitro and in vivo [10–12, 61, 62]. The most important role is the interaction between chemotherapy and EGFR inhibitors. Nyati discussed in his review whether the results of the combination of neoadjuvant chemoradiotherapy with EGFR inhibitors could be seen in the suboptimal sequence of administered treatment that might lead to an antagonistic rather than a potentiating effect [63]. Administration of EGFR inhibitors before the cytostatic scan arrested the cell cycle in the G1 phase, which can affect the attenuation of the effects of subsequently administered cytostatics, with an impact on other phases of the cell cycle. It is cytostatics used for the treatment of colorectal cancer (5-FU, capecitabine) that have the most highlighted effect on the cell cycle in the S/G2/M phases of the cell cycle [29]. This would lead to the hypothesis that giving chemotherapy before an EGFR inhibitor would be more effective than reverse schedule. A study has evaluated the optimum sequencing for the combination of gemcitabine and gefitinib. It demonstrated that gemcitabine followed by gefitinib was superior to the opposite drug order [64]. Other studies showed similar results with better effect of sequention cytostatics–EGFR inhibitors than vice versa [65, 66]. It is not clear why the sequence of cytostatics before EGFR inhibitor is crucial in the case of cytotoxic agents that are not necessarily S-phase specific. Another mechanism of interaction between cytostatics and EGFR inhibitors is modulation of the EGFR-induced pathway. EGFR phosphorylation occurs in response to various cytotoxic drugs, including oxaliplatine, 5-FU, and irinotecan [67]. The phosphorylation of EGFR by oxaliplatine or 5-FU treatment alone correlates with the inhibition of cell viability and cell growth by gefitinib [67]. EGFR phosphorylation can lead to the EGFR degradation and dead cells, under condition of prolonged cellular stress. The reason is the persistent deoxyribonucleotide pool depletion. The EGFR degradation is dependent on the activation of the proteosome [68]. The other mechanism of synergy between chemotherapy and EGFR inhibitors is through the inhibition of DNA repair. Cytostatics induce various types of DNA damage (strand breaks, DNA adducts, inter- and intra-strand crosslink). The repair of cisplatine induces DNA inter-strand crosslink inhibited by gefitinib [69, 70].

The results of the combination of neoadjuvant treatment and EGFR inhibitors were not successful [27–37]. Similarly, EGFR inhibitors did not demonstrate better outcomes in adjuvant treatment of colorectal cancer. The phase III clinical study evaluated a total of 2686 patients with colorectal cancer treated with the combination of FOLFOX and cetuximab or FOLFOX alone. The primary aim was to evaluate the overall survival. The addition of cetuximab did not demonstrate longer survival compare to chemotherapy alone in median follow-up of 28 months [71]. The other studies evaluating the importance of neoadjuvant or adjuvant treatment with EGFR inhibitors in rectal adenocarcinoma should be performed in future. The study of change of EGFR expression during neoadjuvant chemoradiotherapy is to better individualize the treatment. Our study would be to define the population of patients with increases of EGFR expression after neoadjuvant chemoradiotherapy. In this group of patients (a total about 25% of studied patients), we assumed that phenomenon acceleration repopulation is applied. This phenomenon is observed in a smaller number of cases than in patients with squamous cell head and neck cancer. The patients with the increased EGFR expression would benefit from additional anti-EGFR therapy after surgery. Future prospective study could adopt not only immunohistochemistry ex vivo as in our

study but also whole body immunochemistry in vivo by using PET/EGFR. PET detection of EGFR would facilitate the evaluation of EGFR expression not only after but also during the course of neoadjuvant chemoradiotherapy [72].

In our study, we described local relapse in eight patients who represented 16% of all the patients. The CAO/ARO/AIO-94 study comparing neoadjuvant to adjuvant chemoradiotherapy presented local relapse in 7.1% of patients. The reason is that total mesorectal excision was not used in some of the patients. The significance of TME was conclusively demonstrated in clinical studies [73, 74]. The TME surgical standard treatment of rectal carcinoma was defined. We further observed distant metastases in 17 patients who represented 34% of the patients. The cause is a fact of the early subclinical systemic dissemination in the time of diagnosis [75]. This hypothesis confirmed the results of clinical studies with approximately 30% incidence of distance metastases [76–78].

In our study, the patients relatively tolerated the treatment well. We did not demonstrate the death during the neoadjuvant chemoradiotherapy. In four patients, we stopped capecitabine administration due to the hematological toxicity. The most common type of toxicity was a gastrointestinal toxicity. This fact is caused by radiation to the pelvic area and adverse events of capecitabine. In addition, the symptoms could be caused by the presence of tumor. The rectal carcinoma presents along with hemorrhage, tenesmus, pelvic pain, and diarrhea. These symptoms dominated in patients treated with chemoradiotherapy.

Acknowledgements

The authors would like to thank Bayer Czech Republic for their support and for making the publication of this chapter possible.

Author details

Igor Richter

Address all correspondence to: igor.richter@seznam.cz

Department of Oncology, Regional Hospital Liberec, Liberec, Czech Republic

References

[1] Gray R, Hills R, Stowe R, et al. Adjuvant radiotherapy for rectal cancer: A systemic overview of 8507 patients from 22 randomised trials. Lancet. 2001;**358**:1291-1304

[2] Sauer R, Becker H, Hohenberger W, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. The New England Journal of Medicine. 2004;**351**:1731-1740

[3] Bosset JF, Collette L, Calais G, et al. Chemotherapy with preoperative radiotherapy in rectal cancer. EORTC radiotherapy group trial 22921. The New England Journal of Medicine. 2006;**355**:1114-1123 Erratum in: N Engl J Med. 2007;**357**:728

[4] Willett CG, Duda DG, Czito BG, et al. Targeted therapy in rectal cancer. Oncology. 2007; **21**:1055-1065

[5] Yarden Y. The EGFR family and its ligands in human cancer: Signalling mechanism and therapeutic opportunities. European Journal of Cancer. 2001;**37**:S3-S8

[6] Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nature Reviews. Molecular Cell Biology. 2001;**2**:127-137

[7] Lin SY, Makino K, Xia W, et al. Nuclear localization of EGF receptor and its potential new role as a transcription factor. Nature Cell Biology. 2001;**3**:802-808

[8] Akimoto T, Hunter NR, Buchmiller L, et al. Inverse relationship between epidermal growth factor expression and radiocurability of murine carcinomas. Clinical Cancer Research. 1999; **5**:2884-2890

[9] Liang K, Ang KK, Milas L, et al. The epidermal growth factor receptor mediates radioresistance. International Journal of Radiation Biology Physics. 2003;**57**:246-254

[10] Verbeek BS, Andriaansen-Slot SS, Vroom TM, et al. Overexpression of EGFR and c-erbB2 causes enhanced cell migration in human breast cancer cells and NIH3T3 fibroblasts. FEBS Letters. 1998;**425**:145-150

[11] Bandyopathy D, Mandal M, Adam L, et al. Physical interaction between epidermal growth factor receptor and DNA-dependent protein kinase in mammalian cells. The Journal of Biological Chemistry. 1998;**273**:1568-1573

[12] Chinnaiyan P, Huang S, Vallabhaneni G, et al. Mechanism of enhanced radiation response following epidermal growth factor receptor signaling inhibition by erlotinib (Tarceva). Cancer Research. 2005;**65**:3328-3335

[13] Withers HR, Taylor JM, Maciejewski B, et al. The hazard of accelerated tumour clonogen repopulation during radiotherapy. Acta Oncologica. 1988;**27**:131-146

[14] Begg AC. Prediction of repopulation rates and radiosensitivity in human tumours. International Journal of Radiation Biology. 1994;**65**:103-108

[15] Steele RJ, Kelly P, Ellul B, et al. Epidermal growth factor receptor expression in colorectal cancer. British Journal of Surgery. 1990;**77**:1352-1354

[16] Mayer A, Takimoto M, Fritz E, et al. The prognostic significance of proliferating cell nuclear antigen, epidermal growth factor receptor, and MDR gene expression in colorectal cancer. Cancer. 1993;**71**:2454-2460

[17] Khorana AA, Ryan CK, Cox C, et al. Vascular enndothelial growth factor, CD68, and epidermal growth factor receptor expression and survival in patients with stage II and stage III colon carcinoma: A role for the host response in prognosis. Cancer. 2003;**97**: 960-968

[18] Azria D, Bibeau F, Barbier N, et al. Prognostic impact of epidermal growth factor receptor (EGFR) expression on loco-regional recurrence after preoperative radiotherapy in rectal cancer. BMC Cancer. 2005;**5**:62

[19] Shengjin L, Jae-Sung K, Jin-man K, et al. Epidermal growth factor receptor as a prognostic factor in locally advanced rectal-cancer patiens treated with preoperative chemoradiation. International Journal of Radiation Oncology, Biology, Physics. 2006;**65**:705-712

[20] Giralt J, de las Heras M, Cerezo L, et al. The expression of epidermal growth factor receptor results in a worse prognosis for patients with rectal cancer treated with preoperative radiotherapy. Radiotherapy and Oncology 2005;**74**:101-108

[21] Cunningham D, Humblet Y, Siena S et al. Cetuximab monotherapy and cetuximab plus irinotekan in irinotekan-refractory metastatic colorectal cancer. New England Journal of Medicine. 2004;**351**:337-345

[22] Finocchiaro G, Capuzzo F, Janne PA, et al. EGFR HER2, and K-ras as predictive factors for cetuximab sensitivity in colorectal cancer. Proceedings of American Society of Clinical Oncology. 2007;**25**:168s Abstract 4021

[23] Rödel C, Martus P, Papadoupolos T, et al. Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. Journal of Clinical Oncology. 2005;**23**:8688-8696

[24] Janjan NA, Crane C, Feig BW, et al. Improved overall survival among responders to preoperative chemoradiation for locally advanced rectal cancer. American Journal of Clinical Oncology. 2001;**24**:107-112

[25] Janjan NA, Abbruzzese J, Pazdur R, et al. Prognostic implications of response to preoperative infusional chemoradiation in locally advanced rectal cancer. Radiotherapy and Oncology. 1999;**51**:153-160

[26] Maas M, Nelemans PJ, Valentini V, et al. Long-term outcome in patients with a pathological complete response after chemoradiation for rectal cancer: A pooled analysis of indivudual patient data. The Lancet Oncology. 2010;**11**:835-844

[27] Chung KY, Minsky B, Schrag D, et al. Phase I trial of preoperative cetuximab with concurrent continuous infusion 5-fluorouracil and pelvic radiation in patients with local-regionally advanced rectal cancer. Journal of Clinical Oncoloy. 2006;**24**(18 suppl):Abstract 3560

[28] Machiels JP, Sempoux C, Scalliet P, et al. Phase I/II study of preoperative cetuximab, capecitabine, and external beam radiotherapy in patients with rectal cancer. Annals of Oncology. 2007;**18**:738-744

[29] Rödel C, Arnold D, Hipp M et al. Phase I-II trial of cetuximab, capecitabine, oxaliplatin, and radiotherapy as preoperative treatment in rectal cancer. International Journal of Radiation Oncology, Biology, Physics. 2008;**70**:1081-1086

[30] Hofheinz RD, Horisberger K, Woernle C et al. Phase I trial of cetuximab in combination with capecitabine, weekly irinotecan, and radiotherapy as neoadjuvant therapy for rectal cancer. International Journal of Radiation Oncology, Biology, Physics. 2006;**66**:1384-1390

[31] Horisberger K, Treschl A, Mai S et al. Cetuximab in combination with capecitabine, irinotecan, and radiotherapy for patients with locally advanced rectal cancer: Results of a phase II MARGIT trial. International Journal of Radiation Oncology, Biology, Physics 2009;**74**:1487-1493

[32] Bertolini F, Chiara S, Bengala C et al. Neoadjuvant treatment with single-agent cetuximab followed by 5-FU, cetuximab, and pelvic radiotherapy: A phase II study in locally advanced rectal cancer. International Journal of Radiation Oncology, Biology, Physics. 2009;**73**:466-472

[33] Hong YS, Kim DY, Lee KS, et al. Phase II study of preoperative chemoradiation (CRT) with cetuximab, irinotecan and capecitabine in patiens with locally advanced resectable rectal cancer. Journal of Clinical Oncology. 2007;**25**:18S:174s (abstrakt 4045)

[34] Cabebe EC, Kuo T, Koong M, et al. Phase I trial of preoperative cetuximab in combination with oxaliplatine, capecitabine, and radiation therapy for locally advanced rectal cancer. Journal of Clinical Oncology. 2008;**26**(suppl) abstrakt 15019

[35] Eisterer WM, De Vries A, Oefner D, et al. Neoadjuvant chemoradiation therapy with capecitabine plus cetuximab and external beam radiotherapy in locally advanced rectal cancer (LARC) ABCSG trial R03. Journal of Clinical Oncology. 2009;**27**:15S(part I of II) 195s (abstract 4109)

[36] Velenik V, Ocvirk J, Oblak I, et al. Neoadjuvant cetuximab, capecitabine, and radiotherapy (RT) in locally advanced resectable rectal cancer: Results of a phase II trial. Journal of Clinical Oncology. 2009;**27**:(abstrakt e15029)

[37] Kim SY, Hong YS, Kim DY, et al. Preoperative chemoradiation with cetuximab, irinotecan, and capecitabine in patients with locally advanced resectable rectal cancer: A multicenter phase II study. International Journal of Radiation Oncology, Biology, Physics. 2011;**81**:677-683

[38] Hartley A, Ho KF, McConkey C, et al. Pathological complete response following preoperative chemoradiotherapy in rectal cancer: Analysis of phase II/III trials. The British Journal of Radiology. 2005;**78**:934-938

[39] Pinto C, Di Fabio F, Maiello E, et al. Phase II study of panitumumab, oxaliplatin, 5-fluorouracil, and concurrent radiotherapy as preoperative treatment in high-risk locally advanced rectal cancer patients (StarPan/STAR-02 study). Annals of Oncology. 2011;**22**: 2424-2430

[40] Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamos-cell carcinoma of head and neck. The New England Journal of Medicine. 2006;**354**:567-578

[41] Dewdney A, Cunningham D, Tabernero J, et al. Multicenter randomized phase II clinical trial comparing neoadjuvant oxaliplatin, capecitabine, and preoperative radiotherapy with or without cetuximab followed by total mesorectal excision in patients with high-risk rectal cancer (EXPERT-C). Journal of Clinical Oncology. 2012;**30**:1620-1627

[42] Bengala C, Bettelli S, Bertolini F, et al. Epidermal growth factor receptor gene copy number, K-ras station and pathological response to preoperative cetuximab, 5-FU and radiation therapy in locally advanced rectal cancer. Annals of Oncology. 2009;**20**:469-474

[43] Luna-Pérez P, Segura J, Alvarado I et al. Specific c-K-ras gene station as a tumour-response marker in locally advanced rectal cancer trated with preoperative chemoradiotherapy. Annals of Surgical Oncology. 2000;**7**:727-731

[44] Hirvikoski P, Auvinen A, Cummings B, et al. K-ras and p53 mutations and overexpressions as prognostic factors in fiale rectal carcinoma. Anticancer Research. 1999;**19**:685-691

[45] Dvořák J, Sitorová V, Ryška A, et al. The prognostic significance of changes of tumour epidermal growth factor receptor expression after neoadjuvant chemoradiation in patiens with rectal adenocarcinoma. Strahlentherapie und Onkologie. 2012;**10**:145-147

[46] Richter I, Dvořák J, Blüml A, Čermáková E, Bartoš J, Urbanec M, Sitorová V, Ryška A, Sirák I, Buka D, Ferko A, Melichar B, Petera J. Vliv předoperační chemoradioterapie na změnu exprese epidermálního růstového faktoru u pacientů léčených předoperační chemoradioterapií pro lokálně pokročilý karcinom rekta. Klinická Onkologie. 2014;**27**: 361-366

[47] Kwok TT, Sutherland RM. Enhacement of sensitivity of human squamous carcinoma cells to radiation by epidermal growth factor receptor. Journal of National Cancer Institute. 1989;**81**:1020-1024

[48] Bonner JA, Maihle NJ, Folven BR, et al. The interaction of epidermal growth factor and radiation in human head and neck squamous cell carcinoma cell lines with vastly different radiosensitivities. International Journal of Radiation Oncology, Biology, Physics. 1994; **29**:243-247

[49] Balaban N, Moni J, Shannon M et al. The eefect of ionizing radiation on signal transduction: Antibodies to EGF receptor sensitize A431 cells to radiation. Biochimica et Biophysica Acta. 1996;**1314**:147-156

[50] Sheridan MT, O'Dwyer T, Seymour CB, et al. Potential indicators of radiosenzitivity in squamous cell carcinoma of hte head and neck. Radiation Oncology Investigation. 1997;**5**: 180-186

[51] Akimoto T, Hunter NR, Buchmiller L, et al. Inverse relationship between epidermal growth factor receptor expression and radiocurability of murine carcinomas. Clinical Cancer Research. 1999;**5**:2884-2890

[52] Milas L, Fan Z, Andratschke NH, et al. Epidermal growth factor receptor and tumour response to radiation: In vivo preclinical studies. International Journla of Radiation Oncology Biology Physics. 2004;**58**:966-971

[53] Ang KK, Berkey BA, Tu X, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patiens with advanced head and neck carcinoma. Cancer Research. 2002;**62**:7350-7356

[54] Peng D, Fan Z, Lu Y, et al. Anti-epidermal growth factor receptor monoclonal antibody 225 upregulates p27KIP1 and induces G1 arrest in prostatic cancer cell line DU 145. Cancer Research. 1996;**56**:3666-3669

[55] Di Gennaro E, Barbarino M, Bruzzese F, et al. Critical role of both p27KIP1 and p21CIP1/WAF1 in the antiproliferative effect of ZH 1839 (Iressa), an epidermal growth factor receptor tyrosin kinase inhibitor, in head and neck squamous carcinoma cells. Journal of Cellular Physiology. 2003;**195**:139-150

[56] Schmitd-Ullrich RK, Valerie K, Foglman PB, et al. Radiation-induced autophosphorylation of epidermal growth factor receptor in human malignat mammay and squamous epithelial cells. Radiation Research. 1996;**145**:81-85

[57] Schmitd-Ullrich RK, Mikkelsen RB, Dent P, et al. Radiation-induced prolifaration of the human A431 squamous carcinoma cell is dependent on EGFR tyrosine phosphorylation. Oncogene. 1997;**15**:1191-1197

[58] Dent P, Reardon DB, Park JS, et al. Radiation-induced release of transforming growth factor receptor and mitogen-activated protein kinase pathway in carcinoma cells, leading to increased proliferation and protection from radiation-induced cell dead. Molecular Biology of the Cell. 1999;**10**:2493-2506

[59] Contessa JN, Hampton J, Lammerking G, et al. Ionizing radiation activates Erb-B receptor dependent Akt and p70 S6 kinase signalling in carcinoma cells. Oncogene. 2002;**21**:4032-4041

[60] Dittmann K, Mayer C, Fehrenbacher B, et al. Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. The Journal of Biological Chemistry. 2005;**280**:31182-31189

[61] Milas L, Mason K, Hunter N et al. In vivo enhancement of tumour radioresponse by C225 antiepidermal growth factor receptor antibody. Clinical Cancer Research. 2000;**6**:701-708

[62] Nyati MK, Maheshwari D, Hanasoqe S, et al. Radiosensitization by pan ErbB inhibitor CI-1033 in vitro and in vivo. Clinical Cancer Research. 2004;**10**:691-700

[63] Nyati MK, Morgan MA, Feng FY, et al. Integration of EGFR inhibitors with radiochemotherapy. Nature Reviews. Cancer. 2006;**6**:876-885

[64] Chun PY, Feng FY, Scheuer AM, et al. Synergistic effects of gemcitabine and gefitinib in the treatment of head and neck carcinoma. Cancer Research. 2006;**66**:981-988

[65] Azzariti A, Xu JM, Porcelli L, et al. The schedule-dependent enhanced cytotoxic activity of 7-ethyl-10-hydroxy.Camptothecin (SN-38) in combination with Gefitinib (Iresa, ZD 1839). Biochemical Pharmacology. 2004;**68**:135-144

[66] Xu J, Azzariti A, Severino M, et al. Characterization of sequence-dependent synergy between ZD 1839 (Iressa) and oxaliplatine. Biochemical Pharmacology. 2003;**66**:551-563

[67] Van Schaeybroeck S, Karaiskou-McCaul A, Kelly D, et al. Epidermal growth factor receptor activity determines response of colorectal cancer cells to gefitinib alone and in combination with chemotherapy. Clinical Cancer Research. 2005;**11**:7480-7489

[68] Feng FY. EGFR degradation: A novel mechanism of gemcitanine-induces cell dech: in head and neck cancer cell lines. In: 14th SPORE investagators workshop 154, Baltimore 2006

[69] Friedmann B, Caplin M, Hartley JA, et al. Modulation of DNA repair in vitra after treatment with chemotherapeutic agents by the epidermal growth factor receptor inhibitor gefitinib (ZD 1839). Clinical Cancer Research. 2004;**10**:6476-6486

[70] Friedmann B, Caplin M, Savic B, et al. Interaction of the epidermal growth factor receptor and the DNA-dependent protein kinase pathway following gefitinib treatment. Molecular Cancer Therapeutics. 2006;**5**:209-218

[71] Alberts SR, Sargent DJ, Nair S, et al. Effect of oxaliplatine, fluorouracil, and leucovorine with or without cetuximab on survival among patiens with resected stage III colon cancer: A randomised trial. JAMA. 2012;**307**:1383-1393

[72] Mishani E, Abourbeh G. Cancer molecular imaging: Radionuclide-based biomarkers of the epidermal growth factor receptor (EGFR). Current Topics in Medicinal Chemistry. 2007;**7**:1755-1772

[73] Folkesson J, Birgisson H, Pahlman L, et al. Swedisch rectal cancer trial: Long lasting benefits from radiotherapy on survival and local recurence rate. Journal of Clinical Oncology. 2005;**23**:5644-5650

[74] Kapiteijn E, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. The New England Journal of Medicine. 2001;**345**:6386-6346

[75] De Caluwé L, van Nieuwenhove Y, Ceelen WP et al. Preoperative chemoradiation versus radiation alone for stage II and III resectable rectal cancer. Cochrane Database of Systematic Reviews. 2013;**2**:CD006041

[76] Bosset JF, Collette L, Calais G, et al. Chemotherapy with preoperative radiotherapy in rectal cancer. The New England Journal of Medicine. 2006;**355**:1114-1123

[77] Boulis-Wassif S, Gerard A, Loygue J, et al. Final results of a randomized trial on the treatment of rectal cancer with preoperative radiotherapy alone or in combination with 5-fluorouracil, followed by radical surgery. Trial of the european organization on research and treatment of cancer Gastroninestinal tract cancer group. Cancer. 1984;**53**:1811-1818

[78] Bujko K, Nowacki MP, Nasierowska-Guttmejer A, et al. Long-term results of a randomized trial comparing preoperative short-course radiotherapy with preoperative conventionally fractionated chemoradiation for rectal cancer. The British Journal of Surgery. 2006;**93**:1215-1223

Chapter 6

Our Experience in Self-Management Support following Colorectal Cancer Treatment

Racho Ribarov

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72194

"Clinical services, systems, processes and environments must all convey to patients the message: 'You have a part to play. We are partners. We respect your role and will support you to be part of the team.'" [1]

Abstract

The aim of this chapter is to present information and data from our studies on the analysis and assessment of the necessity of self-management support and promoting the awareness of Bulgarian patients with colorectal cancer. This survey covered a total of 315 patients with stoma, making use from consultations at specialized offices in 8 Bulgarian towns. An anonymous questionnaire was conducted, covering a total of 31 questions. The chapter presents results from nonparametric analysis for the more important questions searching for statistically significant relationship with other comparable questions listed in the questionnaire. The necessity of self-management support is assessed on the basis of the received answers. The activity of the established consultation room's network is described, and information is provided concerning the realized self-management support through enhancing the patients' and health-care specialists' awareness on recent scientific achievements referring to dietary preventive and risk factors. Additional studies are needed in order to involve effectively each patient's potential in the struggle for successful disease outcome and to select the best and most effective approaches for self-management support in compliance with the individual demands of patients with colorectal cancer.

Keywords: colorectal cancer, self-management support, consultation rooms, patients' awareness, dietary factors

1. Introduction

The current diverse picture of colorectal cancer epidemiology outlines prevailing data showing systematic recent increase of the global colorectal cancer incidence rate with certain

emphasis reflecting already some reduction of the prevalence characteristic only for some well-developed countries [2, 3]. In this aspect the evidence revealing synchronous growth of age and concomitant chronic disease provokes great interest. For them the predictions of the Institute for the Future, Health and Health Care are that the population aged 65+ will increase from 35 million in 2000 to 53 million in 2020 [4]. The outlined data substantiate the necessity to enhance the variety of approaches to both the prevention and treatment of colorectal cancer and particularly on the involvement of self-management. The complexity of care, intrinsic for colorectal cancer, with its personal, clinical, and social aspects outlines the necessity of self-management support. To respond to this necessity, we focused on involvement of self-management in the struggle against colorectal cancer and provision of the necessary support to achieve an effective disease outcome.

The aim of this paper was to provide information and data from our surveys on the analysis and assessment of the necessity of self-management support and improving the awareness of Bulgarian patients with colorectal cancer.

The survey covered a total of 350 patients with stoma consulted at specialized offices in eight Bulgarian towns: Sofia, Plovdiv, Varna, Burgas, Ruse, Pleven, Haskovo, and Stara Zagora. An anonymous questionnaire study was conducted with a total of 315 respondents delivering correctly completed questionnaire, and 35 questionnaires were discarded because of incorrectly supplied information. Questionnaire studies are among the most informative approaches particularly to problems depending on numerous factors such as colorectal cancer.

The questionnaire contained 31 questions, some of them with sub-questions. The questions were distributed in three main directions: sociodemographic characteristics, treatment quality and patients' satisfaction, and institutions' arrangements, public attitudes, patient's own activity, and supporting environment. The results were processed with descriptive statistical approach presented in one of our previous studies [5]. In this paper we present results from nonparametric analysis of some of the more important questions, searching for statistically significant relationship with other compatible questionnaire questions. Those achieved results were listed in relevant tables.

2. Analysis and assessment of the necessity of self-management support

The role and importance of self-management among the current diversity of measures and approaches for effective CRC treatment is more and more often emphasized. Many studies have shown that patients with colorectal cancer have to be educated to self-manage their condition and improve the quality of their physical, mental, and social life after cancer [5–7].

Self-management is identified as an approach with many benefits in the aspect of patient and economic outcomes and is set as a key element in the current health-care reforms. This approach is often defined as activities performed by the individuals and care providers for themselves, their children, their families, and other individuals in order to be in good

http://dx.doi.org/10.5772/intechopen.72194

shape and maintain good physical and mental health, to respond to social and psychological demands, to prevent disease or accidents, to take care of minor health issues and chronic health states and to maintain health and well-being. In view of patients with colorectal cancer, self-management is an important problem because of the introduction of complex therapeutic regimes often including numerous combinations of chemotherapeutic drugs, and our previous evidence has shown poor patients' awareness concerning the role and effect of administered therapeutic and preventive approaches [5, 8].

Having in mind that during the last years there is greater interest to the use of chemotherapeutics administered orally and the patients' potential to implement chemotherapy at home, there will be a necessity to further activating of self-management. Those perspectives have certain claims to self-management as they require greater responsibility of the patients and their families in the administration of chemotherapy and associated risks. The complexity of the regimes also supposes that the patients will experience potentially toxic side effects requiring quick and effective self-control in order to prevent the unfavorable effects on the treatment and life quality.

Some of our previous studies have shown the necessity to trigger the activities associated with self-management of Bulgarian patients with colorectal cancer, because inadequate awareness about the disease, the risk, and preventive factors as well as the results revealed low level of trust in the administered therapeutic and health care [5, 8]. The realization of those activities requires numerous additional studies.

Patients' mental state, their living environment, and their activity in the treatment process are the most important conditions for successful course and maximal increase of the postoperative period and for good life quality. In this respect we selected three of the questions that to the greatest extent could provide an adequate response concerning the need of patient's active involvement in the processes of treatment and prevention of the concomitant aggravations: "Are you concerned about functions and abilities?" "Is the word 'cancer' a taboo for you and your family?" "Do the people with whom you have shared your diagnosis support you?" The replies to those questions were first assessed by descriptive statistics in percent, showing the trends, without statistical significance [5], providing an assessment of the momentary opinion of the respondents. This assessment, of course, is very important, but it is equally important to reveal what has affected those opinions, in order to undertake respective corrective activities.

The tools of the nonparametric statistical analysis (Fisher's exact test) were used to investigate the effect of most questions, compatible with each of the above-listed questions. The results obtained for each of the three questions are presented in tables covering only the questions with statistically significant effect on the formation of the responses to those three questions.

Table 1 presents the relationships with the question "Are you concerned about your functions and abilities?"

Analyzing the data in the table, the effect of patient's satisfaction with medical care as a significant factor is clearly highlighted ($p<0.005$). The assessment of the professionalism of

Independent variable	p <
How would you evaluate as a whole your satisfaction with the medical services you experienced by now?	0.005
Doctors' professionalism/competence in diagnostics and treatment	0.02
Doctors' attitude to the patients	0.05
Observing the confidentiality, discreetness, and keeping the disease secret	0.003
Information about the disease course and treatment results	0.003
Provision of psychological consultations	0.02
What do you think about the current scheme of prescribing the necessary drugs?	0.0001
Do you meet difficulties in finding the necessary medical specialist?	0.0001
To what extent are you informed about the character of your disease, and do you think you have chances to overcome the disease?	0.001
Do you know the effect of the prescribed treatment and what could be expected from it?	0.0001
Do you think that the state policy is sufficiently beneficial for cancer patients?	0.0001
Do you think that cancer patients should work in alleviated working conditions?	0.02
How do you envisage the future?	0.001

Table 1. Are you concerned about your functions and abilities? (Dependent variable).

the medical doctors engaged in the treatment process is also a factor for overcoming the patients' concern ($p < 0.02$) as their trust in the positive health outcome is to a great extent based on the doctor's knowledge and skills. The good attitude together with understanding of the patient's state contributes to overcome the concern ($p < 0.05$) and increases the extent of trust in the treatment process and associated health care. Observing confidentiality and discreetness and keeping the disease secrets by the medical specialists are also important factors ($p < 0.003$) to cope with patients' concern. In fact this result could be regarded as patient's confidence in the positive outcome of the disease in the future when the present disease will possibly not be commented. A very important requirement for self-management is the patients' awareness of the disease course and treatment results ($p < 0.003$). The clarification of the disease course and the role of implemented treatment approaches causes marked decrease of patients' concern. The poorly informed patients will have, respectively, the greatest extent of concern. The difficulties in finding the necessary medical specialist affect significantly ($p < 0.0001$) the patients' concern about their functions and abilities. In this aspect it is necessary to clarify the possible ways to realize specific medical consultations complying with the cultural competence of the individual patient that is an accent on self-management support.

The closer and better psychological consultations are an important factor for overcoming the patients' anxiety and raising their trust in the further disease development and outcome ($p < 0.001$). The knowledge on the effect of the administered treatment and the expected results helps significantly ($p < 0,001$) to relieve patients' anxiety.

One of the main components of the psychological status of the colon cancer victim is confidentiality, focusing on one side on the consciousness about the vicious character of the disease and, on the other side, giving hope, though small, for a positive outcome.

Table 2 presents the relationships between patients' answers referring to their requirement for confidentiality ("Is the word 'cancer' taboo for you and your family?"). This table also clearly outlines the significant relationships between the answer to the question and the patients' assessment of doctor's competence and professionalism ($p < 0.02$), substantiating their trust and possibility to overcome the "disease taboo." From emotional point of view, the personal attitude of the doctor to the patient as to an ill person but also as to a personality is of particular importance ($p < 0.05$). The patient's demand to accomplish the doctors' professionalism with sympathy and personal approach to the victim is clearly manifested. This table, like the previous one, shows the necessity of psychological consultations which is a focus for self-management support for the studied patients.

In many cases of grave diseases, the patients do not want the people around them to be informed about their status, a standpoint particularly characteristic for cancer patients. This discreetness means that they do not want to be considered doomed, as their hope for getting well is stronger than the feeling of hopelessness.

The defeatist thinking is characteristic for most ill people, but it is most clearly expressed in cancer patients. The causes are as follows: the disease is incurable in an advanced stage, and even in an initial stage, there is no guarantee that the remission will not be followed by disease recurrence. That is why the doomed thinking and the constant stay at hospitals, resection of some parts of the body, lead to disturbed normal life rhythm and make patients dependent on drugs and time without a clear view whether they could plan—even for a short period of time—their life activities. All that leads to a second-rate life when the patients look mainly for information that makes things more optimistic for them. That is why, besides their own awareness, they need the information and discussion with the monitoring physician that should be provided by the self-management support.

Independent variable	p <
How did you choose the respective hospital establishment?	0.001
How would you evaluate as a whole your satisfaction with the medical services you experienced by now?	0.005
Provision of psychological consultations	0.02
What do you think about the current scheme of prescribing the necessary drugs?	0.0001
To what extent are you informed about the character of your disease and do you think you have chances to overcome the disease?	0.001
What do you think about your obligation to visit the hospital once per month for treatment?	0.002

Table 2. Is the word "cancer" a taboo for you and your family? (Dependent variable).

Independent variable	p <
Doctors' professionalism/competence in diagnostics and treatment	0.02
How would you evaluate as a whole your satisfaction with the medical services you experienced by now?	0.005
If you do not succeed to visit the doctor for treatment every month, what are the reasons for that?	0.02
Please, share with us whether you have problems during the visit to the outpatients' and what they are	0.0001
Provision of psychological consultations	0.02
Doctors' attitude to the patients	0.05

Table 3. Are you supported by the people with whom you have shared about your diagnosis? (dependent variable).

The data from the analysis of the answers to the question whether patients get support are presented in **Table 3**.

Social support is very important in the case of chronic diseases—it is a tool to collect information associated with the disease and sufferings, to reduce uncertainty—with chronic diseases there is always uncertainty about who will administer the treatment and how and what the patient's future will be, to establish a sense of certainty. Social support is effective when the chronically ill individual assesses it as adequate and is satisfied with it.

The psychological ban to use the word "cancer" is usually imposed by the victim or the people he/she lives with. The most frequent case is that his close relatives do not want to suggest him/her that he/she is ill and that the probability for a lethal exit is quite high. At the same time, the patient himself does not want to feel inferior, and in this aspect, he/she rejects talking about his disease, accepting his/her state as natural.

The patient prefers to talk about this topic only with people with the same diagnosis. They want to hear how other people who have been in the same situation have overcome the situation successfully. Only a person who has had cancer can understand the experience carried by the diagnosis, illness, and treatment. The strongest support is usually provided by their closest relatives, but in this case, there are many embarrassing facts depending on the victim's state that could impede his sincerity to his relatives.

3. Self-management support through establishing consultation rooms

Considering the difficulties experienced by each patient with colorectal cancer, we primarily started organizing consultations on the use of anus praeter pouches after surgical intervention. During those consultations it was established that the patients needed not only practical training but also support in various aspects: information about the disease itself, about the treatment course and disease development, the effect of administered drugs and therapeutic approaches, the importance of self-management, and role of psychological control.

Our consultation activities started with the first office in Sofia, followed in the subsequent years by similar consultation rooms in the towns: Plovdiv, Varna, Burgas, Pleven, Ruse, Stara Zagora, and Haskovo. The consultations are held by a doctor, a pharmacist, or a nurse—specially trained to work with patients with stoma. The Sofia office has employed the largest staff of consulting specialists followed by Varna and Plovdiv offices employing two different specialists. The offices at the other towns have only one consultant. The total number of patients who have visited the consultation rooms exceeds 5000, unevenly distributed in the years with a marked trend to increase during the recent years. Thus, at this stage the number of patients who have visited the consultation offices exceeds 1500 per year. The expectations envisage significant increase in compliance with the current data from epidemiological studies on colorectal cancer incidence rate. The preoperative consultations cover informing the patient about the necessity of specific tests aiming at precise diagnosis, eventually surgical intervention, revealing the particular options to delay the disease development process or the disease outcome. The patient is introduced to the possibilities of the postoperative therapeutic approaches focusing on handling the stoma bag. The matter is visualized by specially prepared brochures, photos, films, stoma model, and products for servicing the stoma, though without detailed training. The postoperative consultations mainly refer to handling the stoma through the use of various products, cosmetics, and accessories facilitating the patient's work. The patients participate in training courses, and in the majority of cases, their relatives are also trained because after the surgery, most of the patients cannot perceive correctly the recommendations due to the stress they are experiencing. This state is about new products and accessories. The patients are also advised about the procedures to reimburse the products. The role of the consultant who must help the patient in the postoperative recovery period is particularly important. That type of patients, especially the younger ones, is to be resocialized as quickly as possible, to go back to work, to have the same engagements as before the intervention. They must be sure that there is no difference between them and the other people and to have normal lifestyle. This is particularly valid for their sexual life due to the embarrassing presence of the stoma. The so-called "emotional self-care" incorporates approaches associated with clarification and enrichment of the information about the therapeutic interventions in order to comprehend their effect on the patient's physical and mental well-being and to help him to rationalize the effect of the comprehensive treatment process. The consultations affected the normalization of the patients' lifestyle and strengthened their sense of identity. In all above aspects, the patients get the necessary advice and current scientific information. Thus their awareness, respectively, the effectiveness of the self-management, is enhanced, achieving successful risk management. In addition to the basic consultation activity of the offices established in the towns, medical nurses were employed by contracts for home visits. Those visits are postoperative, and each patient is entitled to two free visits, paid by us. The office staff also organizes lectures engaging leading specialists in nutrition because of the particular patients' interest associated with possibilities and changes in their dietary regime, suggested by the disease. The series of activities provided by the specialists at the consultation offices substantiates the effectiveness of risk management at colorectal carcinoma. Although the importance of self-management is widely acknowledged and the patients are actively encouraged to take greater responsibility for their self-care, the scientific publications show that there is little empirical evidence and self-care is not in the patients' center of attention [9].

We would like to underline, listing the results of this survey, that the understanding of the meaning, content, and importance of self-management and self-management support, concerning particularly Bulgarian patients treated for colorectal cancer, is still insufficient. We would accentuate on the recommendations for broader scope of self-management upgrading it with the psychological and emotional aspects of health care and health management, facilitating it with appropriate effective self-management support.

4. Self-management support through promoting the awareness of colorectal cancer patients

The quick development of science provides many interesting data and facts that have to be clear not only to the therapist, manager, and health-care specialists but also to the patients and individuals at risk. In this aspect we attempted to introduce to our patients certain topics that we shall present briefly in this paper. The topics that were discussed with the patients and health-care specialists were intake of vitamins, antioxidants, and fibers as they are implemented broadly even without being prescribed by a doctor in the diet of cancer patients. The most frequent questions during the consultations were focused on those topics. Patients' awareness in this respect is recommendable not only for the self-management of the individual patient but also for the effective health management and health care.

4.1. Thiamine (vitamin B_1): colorectal cancer

The reason to focus on this problem is the patients' question "Why must we not intake vitamins of the group B?" as well as the growing amount of data showing a relationship between thiamine deficiency and the low extent of cancer incidence. Very often the recent recommendations for the nutrition of cancer patients include the recommendation to avoid the intake of vitamins of the group B and particularly vitamin B1 [10–12]. It is necessary to make it clear to patients that one and the same compound could be essential for the normal functioning of the organism and a risk factor at the same time. Are those facts due to the chemical nature of the vitamin itself or to the processes it is involved in? In this aspect many researchers are striving to find the exact answer, but there are still disputable items.

Thiamine is an essential, water-soluble vitamin, necessary for supporting the carbohydrate metabolism. It is essential for the activity of four key enzymes: pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase in the pathway of the tricarbonic acids, transketolase in the pentose-phosphate pathway, and branched chain alpha-keto acids—dehydrogenase complex, engaged in the amino acid catabolism.

The importance of thiamine for cancer cell proliferation has been proven with the use of the thiaminase enzyme. It has been confirmed that adding thiamine to a cell culture containing thiamine has a significant suppressing effect on the growth of cancer cells. Thiaminase causes reduction of ATP in the cancer cells underlining the key role of thiamine in maintaining the bioenergetic status of cancer cells. The role of thiamine was most clearly studied

through using its analogue—oxythiamine. It can suppress tumor growth both in vivo and in vitro. Transketolase inhibition by oxythiamine causes reduction of DNA and RNA synthesis through reduction of riboso-5-phosphate. This pentose is involved in the synthesis of all nucleotides. It has also been proven that oxythiamine is involved in apoptosis initiation in an experimental study on rats [13–15].

Together with those announcements come very interesting data from epidemiological studies showing a mono-directional relationship between low thiamine intake and very low level of cancer disease incidence rate [3, 12, 16]. It is accepted that the low dietary intake of vitamin B1 can be due both to its low content in the dietary foods and the high content of the thiaminase enzyme, decomposing thiamine. In the Asian and African countries, many food products characteristic for the local population diet contain thiaminase in higher amounts (fish, vegetables, nuts, seeds, and insects) that is the reason for the low dietary import of the vitamin. In this respect the clearest data are obtained by epidemiological studies, conducted in Gambia and Nigeria where the seasonal thiamine deficiency is a well-known health problem [10]. According to the reports of the National Cancer Register in America, providing data at global level as well, the lowest extent of prevalence of colorectal cancer, prostate cancer, and breast cancer is just in those countries (Gambia and Nigeria). Compared to them the prevalence of those cancer diseases in the Western countries is 50–100 times greater [3, 14, 15]. The exact mechanism of thiamine involvement in carcinogenesis processes is still disputable, but the epidemiological data are sufficient to make us cautious when administering vitamins of group B to population groups at risk for colorectal cancer.

It should be outlined that the scientific publications have reported data that did not reveal such relationship between thiamine deficiency and carcinogenesis [17, 18]. The relationship between changes in the thiamine status and the enhanced proliferation of the cancer tissue directs the scientific research efforts to a more detailed investigation of the role of thiamine and its involvement in the biochemical mechanisms of carcinogenesis [14, 16, 19]. The analysis of recent scientific publications proves that, in spite of the relatively small number of studies on the dependence between thiamine diet supplementation and the occurrence of cancer diseases, the majority of them confirm that thiamine deficiency in the organism could be accepted as a preventive factor against the development of various cancer diseases. The metabolic investigations reveal the dependence of cancer cells on the availability of thiamine-dependent enzymes for the processes of anabolism and proliferation and for their existence as a whole.

4.2. Antioxidants: colorectal cancer

The second aspect of the application of scientific achievements refers to the role of antioxidants. Antioxidants are a subject of comprehensive research of cancer diseases as the oxidative stress is the first step involved in the mutagenesis and carcinogenesis processes, confirmed by numerous studies [20–22]. A detailed analysis and evaluation of the recent scientific evidence concerning antioxidant effect in the case of colorectal cancer are presented in our previous works [23].

According to the "antioxidant hypothesis," the reduction agents protect the organism against oxidative damage, and their higher level is a warranty to reduce the risk for development of many diseases. Oxidative stress plays an important role in the pathogenesis of cancer diseases as it is a disbalance between the effect of active oxygen radicals and that of the antioxidant's defense system. Because of the substantial increase of colorectal cancer incidence rate and the associated elevated mortality rate in the last decades, a number of studies have been dedicated to the role of antioxidants in the diet of patients with colorectal cancer [20, 24, 25]. The spectrum of those compounds contains a broad variety of vitamins, amino acids, minerals, and bioactive compounds—flavonoids, carotenoids, glucosinolates, etc.

The general antitumor therapies such as surgical intervention and chemo- and radiation therapy have been and still are subjected to improvements, but it is still necessary to develop innovative approaches for the effective cancer therapy as well as for provision of healthy life style. One of the promising more recent approaches is associated with the administration of antioxidants; thus, during the last years, their chemopreventive potential was analyzed in-depth and implemented successfully in a number of cases [26, 27]. New, particular information is needed, characterizing the rich variety of antioxidant-active compounds as well as information about the approaches and specificity of their administration. It is a mass practice nowadays that patients, upon their desire, without doctor's advice or prescription use various antioxidants that challenges the medical science to clarify those issues. Because of the existing numerous, different standpoints concerning antioxidant implementation in primary and secondary prevention of cancer diseases, it is necessary that the patients receive particular information from their doctor and dietician complying with their health status.

Numerous research studies have confirmed that the high consumption of fruits and vegetable has a certain preventive role against the development of cancer diseases that is associated with their rich content of various antioxidants [28, 29]. Having in mind that the main route of intake of exogenous antioxidants is with food which undergoes different metabolic processes in the digestive system, it is logical to assume their direct effect on the particular organs building that system. The diet for cancer diseases depends to a large extent on the involved organ determining its specificity. The most frequently applied diets are those rich in vitamins and minerals, and recently, their spectrum was enhanced with some bioactive compounds contained in the foods and food supplements [2, 25].

After the culmination of data and information about the positive effect of antioxidant implementation against the development of various cancer diseases, other evidence, not confirming similar effects, were communicated [24, 26]. Differences were outlined in the positive results from experimental studies and those from and clinical studies revealing negative effects.

Antioxidant intake is not recommended during chemo- and radiation therapy courses in order to prevent reduction of their power. In the case of diagnosed colorectal cancer after surgery and chemo- and radiation therapy, very high doses of individual antioxidants or combinations of synergically acting bioactive compounds with antioxidant activity must be administered depending on the patient's status. In risk groups, with family history it is recommendable to include high antioxidant doses in the primary prevention programs. The

successful health management requires the administration of high doses of antioxidants also in the secondary prevention of colorectal cancer in the form of a cocktail of several antioxidants with upgrading activity.

4.3. Fibers: colorectal cancer

The third aspect of scientific evidence covers the clarification of the role of dietary fibers as the knowledge in this respect undergoes serious changes proven by scientific research [30, 31].

The necessity to know the evolution of knowledge on risk factors on one side and their contradistinction with relevant preventive factors on the other are important elements of health management in the case of colorectal cancer. Logically, serious attention is given to food which, following its metabolic pathways, has direct effect on the gastrointestinal system as well as systemic effect through the nutrients and bioactive compounds contained in it. Many scientific investigations associated with the analysis and assessment of risk and preventive factors are focused on fibers. In one of our publications, we have presented very detailed information and analysis of existing scientific views on the "fibers and colorectal cancer" issue [32]. We have presented the assessment of the scientific information in two major aspects: mechanisms of fibers activity and studies on patients with colorectal cancer.

The general classic explanations of the biological activity of fibers are establishment of a larger area for development of intestinal microflora, activation of the peristaltic, and creation of a sensation of satiety. In relation to oncogenesis and colorectal cancer in particular, those explanations have their specificity determined by the anatomy and physiology of the colon. Of particular importance are the fibers' composition, their solubility, and ability to ferment, to modify the acid-alkaline balance, and to participate indirectly in the transformation of bile acids. An important factor is also the direct physical effect on the inner lining of the colon, a fact that must not be neglected especially after surgical intervention.

Almost all studies reveal the lack of consensus on the issue and need of further studies in order to provide a particular explanation of the mechanisms involved by fibers to realize their preventive effect against colorectal cancer. The recommendations for consumption of dietary fibers after surgery should be particularly careful because of their direct effect on the colon. The lack of unified test models, the significant methodological errors in the assessment of the diet of the investigated patients, as well as the differences between the experimental and clinical trials seriously challenge the science to plan further comprehensive studies covering all dimensions of the problem dietary fibers—carcinogenesis.

5. Conclusion

This survey on the necessity of activating the self-management of patients with colorectal cancer shows a definite need to promote patients' awareness on the etiopathogenesis of the disease, individual disease course, the role and importance of the administered drug treatment, and implementation of various therapeutic approaches and health care.

The repeating of the relationships between the discussed questions is identified as a primary task in the orientation of the patients to doctors and health-care specialists with proven professionalism with cultural competence allowing particular personal attitude to each patient.

The presented data reveal the need to conduct studies at individual level as each patient is characterized by the specificity of the disease course, awareness, psychological status, and cultural competence. Those diverse characteristics require also different self-management support in order to encourage the patients with colorectal cancer to improve and maintain a healthy lifestyle.

Author details

Racho Ribarov

Address all correspondence to: r.ribarov@rsr-bg.com

Faculty of Public Health, Medical University – Sofia, Sofia, Bulgaria

References

[1] Grazin N. Long-term conditions: Help patients to help themselves. The Health Service Journal. 2007;**117**:28-29

[2] Tarraga P, Solera J, Rodriguez-Montes J. Primary and secondary prevention of colorectal cancer. Clinical Medicine Insights: Gastroenterology. 2014;**7**:33-46

[3] Ribarov R. Interesting facts related to colorectal cancer epidemiology. Archives of the Balkan Medical Union. 2015;**50**(1):45-49

[4] Bodenheimer T, Loring K, Holman H. Patient self-management of chronic disease in primary care. Journal of the American Medical Association. 2002;**288**(19):2469-2475. DOI: 10.1001/jama.288.19.2469

[5] Ribarov R, Tz V, Ivanov A, Ivanova N. Necessity of self-management support following colorectal cancer treatment. Archives of the Balkan Medical Union. 2017;**52**(3):285-291

[6] Foster C, Fenlon D. Recovery and self-management support following primary cancer treatment. British Journal of Cancer. 2011;**105**:S21-S28

[7] Kidd LA. Consequences, control and appraisal: Cues and barriers to engaging in self-management among people affected by colorectal cancer-A secondary analysis of qualitative data. Health Expectations. 2014;**17**(4):565-578

[8] Ribarov R. Study on the awareness of patients with colorectal cancer. The 33 Balkan medical week, 8-11 October, 2014, Bucharest, Romania. Archives of the Balkan Medical Union. 2014;**49**(1):A119-A120

[9] Kidd L, Kearney N, O'Carroll R, Hubbard G. Experiences of self-care in patients with colorectal cancer: A longitudinal study. Journal of Advanced Nursing. 2008;**64**(5): 469-477

[10] Boros L. Population thiamine status and varying cancer rates between Western, Asian and African counters. Anticancer Research. 2000;**20**:2248

[11] Cascante M, Centelles J, Velch R. Role of thiamin (vitamin B1) and trans ketolase in tumor cell proliferation. Nutrition and Cancer. 2000;**36**(2):150-154

[12] Lee B, Yanamandra K, Bocchini J. Thiamin deficiency: A possible major cause of some tumors? Oncology Reports. 2005;**14**:1589-1592

[13] DeBorardinis R, Lum J, Hatzivassillon G, Thompson C. The biology of cancer & metabolic reprogramming fuels cell growth and proliferation. Cell Metabolism. 2008;**7**:11-20

[14] Yang CM, Liu YZ, Liao JW, ML H. The in vitro and in vivo anti-metastatic efficacy of oxythiamine and the possible mechanisms of action. Clinical & Experimental Metastasis. 2010;**27**:341-349

[15] Daily A, Liu S, Bhatnagar S, Karabakhtsian RG, Moscow JA. Low-thiamine diet increases mammary tumor latency in FVB/N-Tg(MMTVneu) mice. International Journal for Vitamin and Nutrition Research. 2012;**82**:298-302

[16] Zastre J, Sweet L, Hanberry B, Ye S. Linking vitamin B1 with cancer cell metabolism. Cancer Metabolism. 2013;**1**:1-16

[17] Kabat G, Miller A, Rohan T. Dietary intake of selected B vitamins in relation to risk of major cancers in women. British Journal of Cancer. 2008;**99**:816-821

[18] Liu S, Monks NR, Hanes JW, Begley TP, Yu H, Moscow JA. Sensitivity of breast cancer cell lines to recombinant thiaminase I. Cancer Chemotherapy and Pharmacology. 2010;**66**:171-179

[19] Willett WC. Diet and cancer: An evolving picture. Journal of the American Medical Association. 2005;**293**:233-237

[20] Papaioannou D, Cooper K, Carroll C. Antioxidants in the chemoprevention of colorectal cancer and colorectal adenomas in the general population: A systematic review and meta-analysis. Colorectal Disease. 2011;**13**(10):1085-1099

[21] WCRF: World Cancer Research Fund and American Institute for Cancer Research. Colorectal Cancer Report. Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer. 2010. http://www.wcrf.org

[22] Zhang R, Kang K, Kim K. Oxidative stress causes epigenetic alteration of CDX1 expression in colorectal cancer cells. Gene. 2013;**524**(2):214-219

[23] Ribarov R. Antioxidants and colorectal cancer. Journal of Contemporary Medical Problems. 2015;**3**:5-11

[24] Pais R, Dumitrascu D. Do antioxidants prevent colorectal cancer ? A meta-analysis. Romanian Journal of Internal Medicine. 2013;**51**(3-4):152-163

[25] Saud S, Young M, Jones-Hall Y. Chemopreventive activity of plant flavonoid isorhamnetin in colorectal cancer is mediated by oncogenic Src and beta-catenin. Cancer Research. 2013;**73**(17):5473-5484

[26] Bjelakovic G, Nagorny A, Nikolowa D. Meta-analysis: Antioxidant supplements for primary and secondary prevention of colorectal adenoma. Alimentary Pharmacology & Therapeutics. 2006;**15**(24):281-291

[27] Wang Z, Ohnaka K, Morita M. Dietary polyphenols and colorectal cancer risk: the Fukuoka colorectal cancer study. World Journal of Gastroenterology. 2013;**19**(17):2683-2690

[28] Hou N, Huo D, Dignam J. Prevention of colorectal cancer and dietary management. Chinese Clinical Oncology. 2013;**2**:13-26

[29] Royston K, Tollefsbol T. The epigenetic impact of cruciferous vegetables on cancer prevention. Current Pharmacology Reports. 2015;**1**(1):46-51

[30] Peters U, Sinha R, Chatterjee N. Dietary fibre and colorectal adenoma in a colorectal cancer early detection programme. Lancet. 2003;**361**:1491-1495

[31] Schatzkin A, Mouw T, Park Y. Dietary fiber and whole–grain consumption in relation to colorectal cancer in NIH-AARP diet and health study. The American Journal of Clinical Nutrition. 2007;**85**:1353-1360

[32] Ribarov R, Tz V, Ivanov A. Dietary fibers and colorectal cancer. Archives of the Balkan Medical Union. 2015;**50**(3):410-413

Edited by Jindong Chen

With international experts sharing their experience and knowledge on these different aspects in the management of colorectal cancer, this book has this opportunity to offer all physicians treating colorectal cancer, as well as researchers, updated information concerning the biology, diagnosis, screening, and treatment of colorectal carcinoma. This book provides a detailed evaluation of diagnostic modalities, in-depth analysis of screening for colorectal cancer, recent advances in treatment, and principles and trends in the management of colorectal cancer. This updated knowledge will be an interesting and informative read for any clinician involved in the management of patients with colorectal cancer. In addition, readers such as related physicians, researchers, and colorectal cancer patients are potential beneficiaries of this book.

Published in London, UK

IntechOpen

ISBN 978-1-83881-320-8