Diplomarbeit

Inflammatory biomarkers in metastatic colorectal cancer: Prognostic and predictive role beyond the first line setting

eingereicht von

Jakob Riedl

zur Erlangung des akademischen Grades

Doktor(in) der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an der

Universitätsklinik für Innere Medizin

Klinische Abteilung für Onkologie

unter der Anleitung von

FA Dr. med.univ. Michael Stotz, Dr.med.univ. Florian Posch, MSc und Assoz. Prof. Priv.-Doz. Dr.med.univ. et scient.med. Armin Gerger, MBA

Graz, am 15.12.2017

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 15.12.2017 *Jakob Riedl eh*

Danksagung:

An dieser Stelle möchte ich mich bei all jenen bedanken, die mich in der Zeit meines Studiums und im Rahmen dieser Arbeit begleitet und unterstützt haben. Ganz besonderer Dank gilt meinen Betreuern Herrn Dr.med.univ. Michael Stotz, Herrn Dr.med.univ. Florian Posch, MSc und Herrn Assoz. Prof. Priv.-Doz. Dr.med.univ. et scient.med. Armin Gerger, MBA, die mir ermöglicht haben an der Abteilung für Onkologie forschen und arbeiten zu dürfen und mich zu jeder Zeit, nicht zuletzt beim Verfassen dieser Arbeit mit großer Motivation und Herzlichkeit unterstützt haben. Ich hätte mir wahrlich keine besseren Betreuer vorstellen können. Danken möchte ich auch Herrn ao.Univ.-Prof. Dr.med.univ. Herbert Stoeger und Herrn Assoz.-Prof. Priv.-Doz. Mag.rer.nat. Dr.med.univ. Martin Pichler, die mir die Möglichkeit gaben, zahlreiche onkologische Fortbildungen und Kongresse zu besuchen, und mir in vielen Situationen mit wertvollem Rat zur Seite standen. Ganz besonderer Dank gebührt meinen Eltern Arete und Bernhard, sowie meinem Bruder Philip, ohne deren unendliche Unterstützung und Beistand dies alles nicht möglich gewesen wäre. Zuletzt möchte ich noch meinen Freunden und langjährigen Mitbewohnern Markus und Lukas dafür danken, dass sie mich während meines Studium mit großer Hilfsbereitschaft, Nachsicht und beispielloser Freundschaft begleitet haben.

Zusammenfassung

Hintergrund

Inflammatorische Biomarker sind potentiell wertvolle Prädiktoren für das Therapieansprechen und den Krankheitsverlauf bei KrebspatientInnen. Der prognostische und prädiktive Wert dieser Biomarker wurde bei PatientInnen mit metastasiertem Kolorektalkarzinom jedoch bis dato nur für die Erstlinientherapie untersucht. In dieser Studie untersuchen wir den prognostischen und prädiktiven Wert der Neutrophilen-Lymphozyten Ratio (NLR), der Lymphozyten/Monozyten Ratio (LMR), der Thrombozyten-Lymphozyten Ratio (PLR), der Höhe des CRP (C reaktive Protein) Wertes und des „advanced-lung-cancer-inflammation-index“ (ALI) bei PatientInnen mit metastasiertem Kolorektalkarzinom in den ersten drei Therapielinien, sowie nach Beendigung der tumorspezifischen Therapie im Sinne des „best supportive care“ (BSC).

Material und Methoden

In diese retrospektive Studie wurden 258 PatientInnen mit metastasiertem Kolorektalkarzinom eingeschlossen. Die primären Endpunkte waren die objektive Ansprechrate und das 6-Monats-progressionsfreie-Überleben während der Erst-, Zweit- und Drittlinientherapie, sowie das 6-Monats-Gesamtüberleben nach Beendigung der tumorspezifischen Therapie.

Ergebnisse

In der multivariablen Analyse zeigte sich nach Adjustierung für die Kovariable Polychemotherapie, dass ein Anstieg der NLR um eine Standardabweichung mit einer 8,5% niedrigeren absoluten Ansprechrate in der Erstlinie (11-7, p<0,0001), einer 3% niedrigeren Ansprechrate in der Zweitlinie (4-2, p<0,0001) und einer 3% niedrigeren Ansprechrate in der Drittlinie (8-2, p=0.24) assoziiert ist. Zudem war eine Zunahme der NLR in allen Therapielinien signifikant mit steigenden Hazard Ratios (HR) für ein schlechteres progressionsfreies Überleben assoziiert. (HR 1.30, p= 0.021 Erstlinie); (HR 1.37, p<0.0001 Zweitlinie); (HR 1.44, p=0.042 Drittlinie) Auch die PLR war mit dem 6-Monats-progressionsfreien Überleben in allen Therapielinien signifikant assoziiert. (HR 1.43 (95%CI 1.09–1.88, p= 0.009 Erstlinie); HR 1.67 (95%CI 1.34–2.09,p< 0.0001 Zweitlinie) und HR 1.43 (95%CI 1.04–1.98, p=0.029 in Drittlinie)) Für das CRP zeigte

sich eine signifikante Assoziation des 6-Monats-progressionsfreien Überlebens in der Erst- und Zweitlinientherapie, sowie des 6-Monats-Geamtüberlebens nach Beendigung der tumorspezifischen Therapie. (HR 1.49 (95%CI 1.23–1.80, p<0.0001 Erstlinie); HR 1.25 (95%CI 1.06–1.47, p=0.007 Zweitlinie); HR 1.09 (95%CI 0.81–1.48, p=0.552 Drittlinie und HR 1.43 (1.15–1.79, p= 0.002 BSC))

Conclusio

Diese Studie zeigt, dass inflammatorische Biomarker sowohl in der Erst-, Zweit- und Drittlinientherapie, als auch nach Beendigung tumorspezifischer Therapie wertvolle Prädiktoren für das Therapieansprechen, respektive den Krankheitsverlauf bei PatientInnen mit metastasiertem Kolorektalkarzinom sind.

Abstract

Introduction

Inflammation based biomarkers are useful prognostic tools in cancer patients. However, the prognostic and predictive value of inflammatory biomarkers beyond the first line setting in metastatic colorectal cancer has not been investigated yet. In this study we aim to quantify the significance of the neutrophil/lymphocyte ratio (NLR), the lymphocyte/monocyte ratio (LMR), the platelet/lymphocyte ratio (PLR), the C-reactive protein (CRP) level and the advanced lung cancer inflammation index (ALI) as prognostic markers for therapy response and disease outcome over the first three treatment lines in metastatic colorectal cancer (mCRC) patients.

Materials and Methods

Two-hundred-fifty-eight patients with mCRC undergoing palliative chemo(immuno-)therapy were included in this retrospective study. The primary endpoints were 6-month PFS and ORR during 1st-line, 2nd-line, and 3rd-line treatment, and 6-month overall survival during BSC.

Results

In multivariable analysis adjusting for polychemotherapy, 1 standard deviation increase in NLR was associated with an 8.5% absolute lower objective response rate (ORR) in first line (11-7, $p<0.0001$), 3% lower ORR in second line (4-2, $p< 0.0001$), and 3% lower ORR in third line (8-2, $p=0.24$), respectively. Regarding progression free survival (PFS), an increase in the NLR was significantly associated with rising hazard ratios (HR) of progression over all treatment lines (HR 1.30, $p= 0.021$ first line); (HR 1.37, $p<0.0001$ second line); (HR 1.44, $p=0.042$ third line). The PLR was associated with 6-month PFS over all lines. (HR 1.43 (95%CI 1.09–1.88, $p= 0.009$ first line); HR 1.67 (95%CI 1.34–2.09, $p< 0.0001$ second line) and HR 1.43 (95%CI 1.04–1.98, $p=0.029$ in third line)) For CRP, the prognostic association was significant in the first two chemotherapy lines and in case of best supportive care (BSC). (HR 1.49 (95%CI 1.23–1.80, $p<0.0001$ first line); HR 1.25 (95%CI 1.06–1.47, $p=0.007$ second line); HR 1.09 (95%CI 0.81–1.48, $p=0.552$ third line and HR 1.43 (1.15–1.79, $p= 0.002$ in BSC))

Conclusion

Our data suggest that inflammatory biomarkers are useful predictors for disease outcome and treatment response over several treatment lines and best supportive care in mCRC patients.

Index

Abbrevations:

ALI ..... *advanced lung cancer inflammation index*
BMI ..... *body mass index*
BSC ..... *best supportive care*
CEA ..... *carcinoembryonic antigen*
CI ..... *confidence interval*
CR ..... *complete remission*
CRP ..... *C-reactive-protein*
DCR ..... *disease control rate*
EGFR ..... *epidermal growth factor receptor*
EMVI ..... extramural vascular invasion
ESMO ..... *European Society for Medical Oncology*
FAP ..... *familial adenomatous polyposis*
gFOBT ..... *Guaiac-based fecal occult blood test*
HNPCC ..... *hereditary non polyposis colorectal cancer*
HR ..... *hazard ratio*
KI ..... *Karnofsky index*
LMR ..... *lymphocyte-to-,onocyte ratio*
MAP ..... *MUTYH associated polyposis*
mCRC ..... *metastatic colorectal cancer*
METs ..... *metabolic equivalents*
MRF ..... mesorectal fascia
NE ..... *not evaluable*
NLR ..... n*eutrophil-to-lymphocyte Ratio*
ORR ..... *objective response rate*
OS ..... *overall survival*
PD ..... *progressive disease*
PFS ..... *progression free survival*
PLR ..... *platelet-to-lymphocyte*
PR ..... *partial remission*
SD ..... *stable disease*
TME ..... *total mesorectal excision*
UICC ..... *Union for Internation Cancer Control*
VEGF ..... *vascular endothelial growth factor*
WHO ..... *World Health Organization*

Figures:

Tables:

Introduction

Colorectal cancer (CRC) is the third most common cancer in males and second most common in females worldwide. In developed countries the mortality rates have constantly decreased over the last years, mainly due to extensive colorectal cancer screening and improved treatment options. (1) Yet, around 20 percent of patients with CRC have synchronous metastasis at initial diagnosis and more than half of all CRC patients die from their disease. (2)

Up to date only limited data exist to predict therapy response and survival outcome in CRC patients. Since inflammation was shown to play a crucial role in the pathogenesis and promotion of cancer progression, inflammatory biomarkers have gained more attraction as potential predictive and prognostic parameters in recent years. (3,4) A variety of routinely available blood based markers of inflammation such as hypalbuminaemia, C-reactive protein level (CRP), blood cell counts and its ratios like the neutrophil-to-lymphocyte ratio (NLR), the lymphocyte-to-monocyte ratio (LMR), or the platelet-to-lymphocyte ratio (PLR) have been investigated in different cancer entities as prognostic tools. (5–10) However, only few data exist regarding the prognosis of survival outcomes and prediction of therapy response in metastatic colorectal cancer beyond the first-line treatment setting.

The aim of this study is to examine the value of blood-based inflammatory biomarkers as prognostic and predictive markers for therapy response and disease outcome during the first three chemotherapy lines, and after start of best-supportive-care (BSC) only treatment concept in mCRC patients.

Epidemiology

Incidence

With around 1.4 Million newly diagnosed cases per year worldwide, colorectal cancer is the third most common cancer in males and the second most common in females. In males only lung cancer and prostate cancer have a higher incidence, whereas in females breast cancer is the most common cancer entity. The rates of colorectal cancer are highest in well developed areas such as Australia, New Zealand, North America and parts of Europe and

lowest in China, India and Africa with rates ranging from 40 per 100.000 to less than 5 per 100.000. (11)(12) This indicates that environmental exposure surroundings and lifestyle including diet and physical examination may play a crucial role in the development of colorectal cancer. The temporary trends vary within different regions. In Austria for example the incidence rates have continuously declined over the last years, whereas in Eastern European countries they are rising. (13) In general, the incidence of colorectal cancer is higher in men than in women and increases with age. More than 90% of all colorectal cancers occur in people aged 50 or older. (12)

Mortality

In the US colorectal cancer leads to around 50.000 deaths per year, which accounts for 8 % of all cancer deaths. (14) In the Western World mortality rates have been decreasing in most of the countries, presumably due to better screening options, better therapy and reduced prevalence of risk factors like smoking, unhealthy diet and obesity. (12) As an example, in Austria the mortality rate fell from 48.2 per 100.000 in 1983 to 26.3 per 100.000 in 2014. (15)

Aetiology, Pathogenesis and risk factors

Molecular pathogenesis

Colorectal cancer is one of the best understood solid tumors regarding patterns of origin and development, especially in terms of molecular pathogenesis. This is mainly due to good acceptability of tissue samples and the mostly slow progression from normal colonic epithelium to invasive cancer via an intermediate precursor, the adenomatous polyp.

This knowledge allows us to differentiate three different models of colorectal cancer pathogenesis:
sporadic, familial and inherited. (16)

- Sporadic colorectal cancer occurs in patients who do not suffer from any genetic syndrome with a predisposition to colorectal cancer and who have a negative family history. Accounting for 70 % of all colorectal cancer patients, this is the most common case. The main risk factors for sporadic colorectal cancer are listed below.

- In 25 % colorectal cancer patients have a positive family history, without having an inherited syndrome. The risk of developing colorectal cancer in patients with one first degree relative having a positive history is almost twice as high as in general population. (17)

- The third and less common group of colorectal cancer patients includes those, who have a congenital syndrome leading to an altered risk of colorectal cancer development e.g. the Lynch Syndrome or the familial adenomatous polyposis (FAP), which will be described more detailed in chapter "Hereditary CRC syndroms".

In most cases colorectal cancer develop on the basis of adenomas that become dysplastic. This model is called adenoma carcinoma sequence and was first described by Vogelstein et al. in 1990. It describes the development from normal colon mucosa via low grad adenomas and high grade adenomas to invasive colorectal cancer as a multilevel process. For each step one further mutation leading to a selective growth advantage of the mutated cells is acquired. (18) Commonly mutated genes found in colorectal cancer are the oncogenes RAS, MYC and SRC and the tumor suppressor genes APC, p53 and SMAD4. (18–21)

Today it is believed that around 80% of colorectal cancers develop on the basis of the adenoma carcinoma sequence, whereas the rest arises from serrated polyps in which other molecular mechanism such as BRAF mutation and widespread gene promoter hypermethylation leads to carcinogenesis. (22)

Non genetic risk factors

Age

Higher age is one of the major risk factors for colorectal cancer. The incidence rate doubles every ten years after the age of 40 and is 50 times higher in patients aged 60 to 70 than in those under 40. (12)

Gender

Colorectal cancer risk and mortality is higher in men than in women. Interestingly males more often suffer from distal colorectal cancer than females. This might be due to sex specific exposures to risk factors and the difference in sexual hormone status. (23)

Lifestyle

A study of Parkin et al. showed that lifestyle factors are responsible for more than half of colorectal cancer cases in Great Britain. (24) The most important lifestyle risk factors are:

Dietary habits and obesity

There is strong evidence that nutritional behaviour plays an important role in the development of colorectal cancer. An increased Body Mass Index (BMI) is highly associated with both colon and rectal cancer. A pooled analysis including data from 23 studies showed a 1.1 higher relative risk per 8kg/m2 increase in BMI. (25) An even stronger association was found for increased abdominal fat measured by waist circumference with a 50% enhanced risk for colorectal cancer in the group with the highest waist circumference. (26)

Various observational studies have investigated the effects of dietary habits on colorectal cancer risk. However, in most cases the results are inconsistent. Most strongly associated with an increased colorectal cancer risk seems to be the long term consumption of red or processed meats. (27) As potentially protective, greater intake of fiber containing foods, fruits and vegetables, folic acid, Vitamin B6, Calcium, Magnesium, Garlic, and omega 3 fatty acids are discussed. (28–35)

Smoking

As in many other malignancies, smoking is one of the major risk factors for colorectal cancer. Botteri et al. included 106 observational studies in their meta-analysis and showed that smoking is associated with an absolute risk increase of 10.8 cases per 100.000 person-years. They further proved that the risk is dose related and enhances with an increasing number of pack years. In addition to the increased risk of developing CRC smoking also leads to higher mortality rates in CRC patients. (36)

Alcohol

Several studies have shown a significant association between alcohol consumption and an increased risk of CRC. Compared with people, who never drink alcohol, moderate drinkers (two to three drinks per day) have a 20 % higher risk of developing CRC and heavy drinkers (4 or more drinks per day) have a 50% higher risk. Having one drink or less per day does not seem to increase the risk. (37) The patterns how alcohol consumption leads to an elevated CRC risk are not entirely understood. It is discussed that alcohol works as a direct colorectal carcinogen by inducing increased mucosal cell proliferation and activation of intestinal carcinogens. (38) Another explanation model may be the interference of alcohol with the folate acid metabolism and the decreased folate intake, which often comes along with high alcohol consumption. Lack of folate seems to result in hypomethylation of the DNA and as a consequence leads to an elevated risk of CRC. (39)

Physical Activity

Various studies have pointed out the protective effect of physical activity in different cancer entities. (40,41) Concerning colorectal cancer, Moore et al. showed that people with high levels of physical activity measured by metabolic equivalents (METs) per week have a 16% reduction in risk compared to those who hardly do any sports. (42) In another meta-analysis implemented by Wolin et al., physical activity was associated with an even higher decrease of 26% in colorectal cancer risk. (43) In addition to its preventive effect physical activity also seems to improve the cancer specific and overall survival in colorectal cancer patients. (44)

The underlying physiological mechanism how physical activity reduces cancer risk and mortality still remains unclear.

Medication

Several drugs such as Aspirin and other nonsteroidal anti-inflammatory drugs have been shown to have chemopreventive effects in colorectal cancer patients. However in most cases the data are conflicting. (45,46) As a result, up to date there are no general recommendations for chemoprevention in persons with an average risk for CRC.

Non hereditary predisposing diseases and other risk factors

Chronic inflammatory bowel diseases

The risk for CRC is both elevated in ulcerative colitis and Crohn's disease. In ulcerative colitis it raises with increasingly extensive disease at diagnosis and disease duration. Patients with a pancolitis have a 15 fold higher risk than the general population and the increase of risk begins about 10 years after diagnosis. Colitis limited to the left colon results in a 3 fold higher risk, which starts to increase 15 years after diagnosis. The cumulative risk after 30 years of disease is about 18%. (47)

In Crohn's disease it is not entirely understood if and how strong the risk for CRC is elevated. In some studies the risk of longstanding Crohn disease showed to be comparable with ulcerative colitis, whereas in others no increased risk was found. (48,49) One aspect that hardens the estimation of CRC risk in Crohn's disease patients is, that many of them undergo colectomy early during their disease to relieve symptoms.

In inflammatory bowel diseases CRC mostly does not develop from polyps, but from intraepithelial dysplasia, which makes the endoscopic and pathological diagnosis much more difficult. (50,51)

Diabetes Mellitus

Recent studies have shown an association of diabetes mellitus with an altered risk of CRC. This may be due to the increased levels of insulin coming along with diabetes. Insulin is a crucial growth factor for colonic mucosal cells and was shown to stimulate tumor cell mitosis in vitro. (52,53) Dehal et al. further sowed that CRC patients with diabetes mellitus type II have a higher mortality compared to those who do not have this comorbidity. (54)

Abdominal radiation

Childhood cancer survivors, who have received abdominal radiation therapy have a higher risk to develop colorectal cancer and other gastrointestinal malignancies in their later life. Hence, intensified screening is recommended for those patients. (55)

Hereditary CRC syndromes

Inherited syndromes leading to an increased CRC risk are rare. However together they account for around 5 to 10 % of all colorectal cancer cases. The most important ones are:

Lynch syndrome

Also called hereditary non polyposis colorectal cancer (HNPCC), the lynch syndrome is an autosomal dominant inherited disease caused by germline mutation of DNA mismatch repair genes, which lead to microsatellite instability. The most commonly mutated genes in Lynch syndrome patients are MLH1, MSH2, MSH6, and PMS2. (56)

Patients who suffer from Lynch syndrome have an almost 80% lifetime risk of developing CRC. (57) The tumors are typically located in the right colon and the median age of diagnosis is 44 years. (58) In addition to CRC, HNPCC is associated with an elevated risk of endometrial cancer, gastric cancer, ovarial cancer, small bowel cancer and urothelial carcinoma. The risk is highest for endometrial cancer, which occurs in 40 to 60 % of female Lynch syndrome patients. (57,59,60) Special forms of Lynch syndrome are the Muir Torre syndrome, which is characterized by a combination of hereditary CRC and sebaceous tumors or subcutane keratocanthomas, and the Turcot syndrome, which is associated with brain tumors, most commonly gliomas. (61,62)

To identify Lynch syndrome patients Amsterdam II or Bethesda diagnosis criteria are generally used. Both include several aspects of family history and the age of diagnosis. To establish the diagnosis, genetic analysis on microsatellite instability is required. (63)

Familial adenomatous polyposis (FAP)

Caused by a germline mutation of the adenomatous polyposis coli (APC) tumorsuppressor gene, this inherited syndrome accounts for 1 % of all CRC cases. The mutation can be either acquired by autosomal dominant inheritance or by de novo mutation. FAP is characterized by the occurrence of multiple, often more than 100, colorectal adeonomas, which usually start to emerge after the age of 16 and eventually progress to cancer. With

almost 100% of the patients developing CRC after 30 years without treatment this syndrome is described as an obligate precancerosis. The mean age of cancer diagnosis is 39 years.

Typical extracolonic manifestations of FAP are the occurrence of polyps in the upper gastrointestinal tract and the congenital hypertrophy of the retinal pigment (CHRPE). CHRPE can be detected in up to 85% of the FAP patients by ophthalmoscopy and may be an early indicator of this syndrome.

A special subtype of FAP is the attenuated FAP, which is a milder form that comes along with less than 100 polyps, a later age of adenoma appearance and a slightly lower cancer risk. When combined with osteomas and epidermoid cysts FAP is called Gardner syndrome, whereas Turcot syndrome describes the association of familial colorectal cancer and brain tumors.

Most commonly, FAP is detected by colonoscopy. For a definite diagnosis APC gene testing is required. (64,65)

MUTYH associated polyposis (MAP)

MAP is a very rare syndrome caused by a germline mutation in the base excision repair gene mutY homolog. It is typically associated with 20 to a few 100 colorectal adenomas that occur at the mean age of about 50 years, but can also present without a polyposis. (66)

Other rare polyposis syndromes associated with an elevated risk of CRC are:

- Cronkhite Canada syndrome
- Birth Hogg Dube syndrome
- Peutz Jeghers syndrome
- Juvenile polyposis syndrome.

(66)

Histopathology

Localization

In Europe large bowel cancers with an aboral boarder located 16 centimeters or less proximal to the anocutaneous line measured by rigid proctoscopy are defined as colon cancers and those, which lie distal to that point as rectal cancers. Rectal cancers can be separated into three different groups. Tumors located 10 to 15 centimetres from the anal margin are defined as high, tumors located 5 to 10 centimetres from the anal margin as middle and tumors that are located less than 5 cm from the anal margin as low recta cancers. Accounting for about one third of all colorectal cancers the rectum is the most common localization of CRC. (67)

Macroscopic appearance

Colorectal carcinomas can present as polyps, as ulcers or with diffuse infiltrating growth. In the right colon a polypoid appearance is more common, whereas in the distal colon colorectal cancer tends to present as annular and endophytic lesions. However, also a mixture of both types is frequently seen.

Histology

Histologically colorectal carcinomas are defined as malignant epithelial tumors of the colorectal mucosa, which infiltrate the submucosa or deeper layers of the colonic wall. Carcinomas are the by far most common large bowel cancers in humans. Other rare non epithelial colorectal tumors are listed in the World Health Organization (WHO) classification below. Accounting for more than 95% of all colorectal carcinomas adenocarcinomas are the most common histological type of differentiation. They are gland forming and often produce mucus, which can remain in the cells or is secreted to the extracellular matrix. If more than 50% of the tumor mass is composed of mucus the carcinomas are defined as mucinous adenocarcimomas. (68) They account for around 10% to 15% of all CRCs and seem to have a poorer prognosis compared with other CRCs. (69,70) Another histological subtype is the signet ring cell carcinoma which composes of tumor cells characterized by a large vacuole that displaces the nuclei to the cell periphery looking like a signet ring. To be classified as a signet ring cell carcinoma more than 50% of all cancer cells must contain this intracytoplasmatic mucin. Signet ring cell carcinomas

are pretty rare, but typically show very aggressive tumor behaviour with a poor overall survival. (71) Other rare variants of the colorectal carcinoma are the medullary carcinoma, which is characterized by a non-glandular appearance, the squamous carcinoma, the adenosquamous carcinoma, the small cell carcinoma and the undifferentiated carcinoma.

Grading

Colorectal carcinomas can be divided into three grades of histologic differentiation. The grading takes into account how well formed the glands in the tumor are. High differentiated or also called low grade G1 adenocarcinomas consist of well defined glandular structures, whereas in low differentiated/high grade carcinomas little to no glandular formations can be detected. To facilitate the grading the three grades can be distinguished by the percentage of tubular formations in the tumor. If more than 95% of the tumor is built of tubular structures it is classified as G1, 50% to 95% as G2 and less than 5% as G3 respectively. G3 tumors typically show high mitotic rates and a high amount of cellular atypia. (68)

Tumor dissemination

In about 25% colorectal cancer patients show with distant metastasis at initial diagnosis and another quarter of all patients develop metachronous metastasis during the course of their disease. (72) The tumor cells can spread by hematogenous and lymphatic dissemination or per continuitatem in the surrounding structures.

Lymphatic metastasis strongly correlates with the depth of bowel wall invasion. Most common lymphatic metastases are found in the pericolonic and perirectal lymphnodes. However, distal rectal cancers also frequently spread to inguinal lymphnodes. (73)

As the major part of the large bowel is drained by the portal system the first site of hematogenic dissemination is usually the liver. From there the blood flows via the inferior vena cava to the heart and then to the lungs, which are the second most common site of distant metastasis. The distal rectum is not drained by the portal vein system, but by the iliac veins, which directly empty into the inferior vena cava. (74) Consequently, distal rectal cancer more often spread to the lungs than to the liver. Less common sites of

hematogenous metastatic dissemination are the bones, the brain, the skin and the adrenal glands.

The third way of colorectal cancer dissemination is via infiltration of contiguous organs, such as the stomach, the pancreas, the uterus, the urinary bladder or through the peritoneal fluid within the peritoneal cavity. (2)

Staging

Today, colorectal cancers are usually staged by the TNM staging system of the Union for Internation Cancer Control (UICC). No longer used are the Duke and the Astler Coller classification. The TNM staging system can be assessed by radiological imaging (rTNM) mostly used in the preoperative treatment planning of rectal cancers or by pathological examination (pTNM), which has higher validity. Both forms take three different factors into account. The T stands for the infiltration depth of the tumor, the N for the lymph node status and the M provides information about the occurrence of distant metastasis. By these three criterias colorectal cancer can be classified into five different stages. Stage 0 cancers are defined as carcinomas in situ (no infiltration of the submucosa) without lymph node or distant metastasis. Stage I characterizes tumors, which infiltrate the lamina propria or the submucosa, but do not reach the subserosa and have not disseminated. Cancers with lymph node metastasis, but no distant metastasis are classified as Stage III regardless of their infiltration depth, whereas stage IV carcinomas have already spread to other organs. The detailed TNM classification is listed below (75):

Table 1: TNM classification

Primary tumor: T	
TIS	Carcinoma in situ
T1	Tumor invades submucosa
T2	Tumor invades muscularis propria
T3	Tumor invades through the muscularis propria into pericolorectal tissues
T4a	Tumor penetrates to the surface of the visceral peritoneum

T4b	Tumor directly invades or is adherent to other organs or structure
Regional lymph nodes: N	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph nodes
N1	Metastasis in 1-3 regional lymph nodes
N1a	Metastasis in 1 regional lymph node
N1b	Metastasis in 2-3 regional lymph nodes
N1c	Tumor deposit(s) in the subserosa, mesentery, or nonperitonealized pericolic or perirectal tissues without regional nodal metastasis
N2	Metastasis in 4 or more lymph nodes
N2a	Metastasis in 4-6 regional lymph nodes
N2b	Metastasis in 7 or more regional lymph nodes
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis
M1a	Metastasis confined to 1 organ or site (eg, liver, lung, ovary, nonregional node)
M1b	Metastases in more than 1 organ/site or the peritoneum

Table 2: Tumor stage

Stage	**T**	**N**	**M**
0	Tis	N0	M0
I	T1	N0	M0
	T2	N0	M0
IIA	T3	N0	M0
IIB	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1-T2	N1/N1c	M0

	T1	N2a	M0
IIIB	T3-T4a	N1/N1c	M0
	T2-T3	N2a	M0
	T1-T2	N2b	M0
IIIC	T4a	N2a	M0
	T3-T4a	N2b	M0
	T4b	N1-N2	M0
IVA	Any T	Any N	M1a
IVB	Any T	Any N	M1b

Clinical management

Clinical presentation

In about 10% colorectal cancer patients present without any symptoms at initial diagnosis and are most commonly discovered by routine screening colonoscopy. In most cases these are early stage cancers that present with a small tumor size and low infiltration depth. The majority of the patients however are detected by diagnostic endoscopy after the onset of suspect symptoms. (76)

A challenge in recognizing CRC is that the symptoms are typically uncharacteristic for a long time, leading to a delayed medical consultation and diagnosis. Early suspect signs can be weight loss, changings in bowel habits, like diarrhoea or obstipation, loss of power, unexplained iron deficiency anemia and abdominal pain. More specific symptoms are bloody stools, typically in the form of hematochezia and the occurrence of a palpable tumor. (77,78)

The clinical manifestation also depends on the localization of the cancer. As the intestinal lumen calibre gets smaller and the faeces become harder from proximal to distal, left sided tumors are more prone to present with a change in bowel habits and signs of obstruction than proximal cancers. They further show a higher rate of rectal bleeding, whereas right sided tumors controversially seem to present with lower haemoglobin levels. (79)

Another 5% to 10% of all CRC patients present with an acute emergency and get diagnosed during surgery. Typical indications are complete intestinal obstructions, peritonitis, abscesses and intestinal perforation. (80,81) CRC patients diagnosed in this way most commonly suffer from a locally advanced tumor and have a poor prognosis independent from stage. (82)

Patients with metastatic disease can additionally show symptoms like right upper quarter pain, ascites, pathological fractures, bone pain and chronic cough depending on the site of metastasis.

Diagnostic tools

Digital rectal examination

In a patient with suspected colorectal cancer digital rectal examination should be part of the physical examination, in order to obtain information about the function of the anal sphincter and to detect an obstructed distal rectum, which is highly suspicious for rectal cancer and should lead to further examinations.

Guaiac-based fecal occult blood test (gFOBT)

This test is used for the detection of occult blood in the stool and is based on a peroxidase reaction in the presence of haemoglobin. Three consecutive stool samples are placed on a guaiac paper and get sprinkled with hydrogen peroxide, which in the presence of blood leads to a blue colouring of the test strip. If the test is positive a colonoscopy should be performed afterwards to detect the cause of bleeding. The guaiac test offers the advantage of an inexpensive and simple, non-invasive screening option for colorectal cancers. With a detection rate of about 30 to 40 % the sensitivity of a single test is not high. However, if repeated on a regular basis the sensitivity raises up to 90% and leads to a 30% reduced CRC mortality. (83) The weakness of the test is the high rate of false positive results that can be caused by the intake of raw meat and peroxidase containing vegetables like broccoli and cauliflower, microbleedings in the upper gastrointestinal tract associated with ASS intake and other gastrointestinal bleedings such as haemorrhoids.

Rectoscopy

Rectoscopes are straight and rigid metal tubes, used for the visual inspection of the rectum. In rectal cancers they are used to measure the distance of the aboral border of the tumor to the anocutaneous line. This has a major impact on the type of surgical resection.

Sigmoideoscopy

With a length of around 60 centimetres sigmoideoscopy allows to investigate and take biopsies of the rectum and the distal part of the colon up to the splenic flexure. In contrast to rectoscopy this examination is performed by the use of a flexible endoscope. It is mainly used for screening and detects about two thirds of all colorectal cancers. (84) The advantage of sigmoideoscopy compared to colonoscopy is that the examination is routinely performed without sedation, which lowers the complication rate. (85)

Colonoscopy

With a detection rate of almost 95% colonoscopy is the gold standard in the diagnosis of colorectal cancer. (86) It allows to examine the whole colon up to the ileocaecal valve, to take biopsies and to excise polyps and other suspect lesions. As a consequence colonoscopy is the preferred tool not only in the diagnosis of suspected CRC but also in screening. At an average follow up of 16 years Zauber et al. showed a 53% reduction of CRC mortality in patients with colonoscopic polypectomy compared with the expected CRC mortality in the general population. (87) Disadvantages of colonoscopy are the rigorous bowel preparation, which has to be done before the examination and the higher complication rate compared to sigmoideoscopy. Most feared complications are endoscopically non stoppable bleedings, perforations of the bowel wall and sedation associated complication, which all together, however, are quite rare. (88)

Barium enema

Barium enema has a lower detection rate of CRC than colonoscopy. However, it is widely available, cheaper and can be performed when complete colonoscopy is not possible due to obstructions.

Other imaging methods

So far rarely used diagnostic tools for CRC are the CT colonography and the PillCam capsule endoscopy.

Tumor markers

A variety of serum tumor markers for CRC have been investigated over the last years. The best data exist for the carcinoembryonic antigen (CEA), which describes a family of glycoproteins that seem to play an important role in cancer cell adhesion and dissemination. (89) In clinic, CEA is used as a prognostic and follow up marker; however, with a diagnostic sensitivity of only 46% its diagnostic value is very limited. (90)

Metastases detection

Most commonly used examinations for the detection of possible metastases are thorax radiography, abdominal ultrasound and computed tomography of the chest, abdomen and pelvis. In operated cancer patients with suspected disease recurrence positron emission tomography scan is an established examination. (91)

Screening

Colorectal cancer is a mostly slow growing tumor entity, that, in most cases, raises out of adenomas over years. (21) This provides the chance to prevent colorectal cancer development by detecting and removing premalignant lesions or to diagnose CRC at an early stage. Therefore screening plays a major role in the “treatment” of CRC. Today we have several commonly used tests for CRC screening:

- Digital rectal examination
- Guaiac based fecal occult blood test
- Fecal immunochemical test
- Sigmoideoscopy
- Colonoscopy
- CT colonography

The screening guidelines differentiate between non high risk and high risk patients by taking predisposing diseases into account.

Non high risk patients

Patients with a negative family history for CRC and no predisposing diseases are categorized as normal or non-high risk patients. In Austria recommendation for this collective includes yearly digital rectal examinations plus guaiac based fecal occult blood test after the age of 40 and total colonoscopy every five to seven years after the age of 50, if no abnormalities are detected. If adenomas or other suspicious lesions are found in colonoscopy, the examination has to be repeated within a shorter time. (92)

High risk patients:

Positive family history

Patients with first degree relatives with CRC are recommended to perform their first colonoscopy ten years before the age of cancer diagnosis of their relative, but by no later than 50.

Ulcerous colitis

Patients suffering from colitis of the whole bowel for eight years or from a left sided colitis for 15 years should perform colonoscopy yearly. In patients with high grade dysplasia a preventive proctocolectomy is recommended.

Lynch syndrome

Lynch syndrome patients should start with yearly colonoscopy screening at the age of 25. In female Lynch syndrome patients, a yearly biopsy of the endometrium is further recommended after the age of 35.

Familial adenomatous polyposis

In FAP syndrome patients, the first colonoscopy should be performed at the age of 10 and repeated yearly until preventive proctocolectomy is done. (93)

Treatment

Over the last decades major advances have been made in the treatment of CRC especially due to the development of new systemic chemotherapies and the discovery of targeted therapy. Dependent from clinical stage and tumor localization CRC therapy today consists of surgical resection, radiotherapy, chemotherapy and the application of high selective monoclonal antibodies:

Surgery

A wide surgical resection of the tumor is the basis of CRC therapy and essential for curative treatment. The aim is to completely remove the tumor and the mesentery with the lymphatic drainage baisin of the affected colonic segment. This en block resection shall lower the recurrence and metastasis rate of the tumor and can be either performed by laparoscopy or open colectomy. Open colectomy has the advantage of a shorter operation time, whereas minimal invasive surgery comes along with faster recovery, lower need for analgetics and better cosmetic results. Disease free and overall survival does not significantly differ between the two methods. (94)

The location of the tumor and its supplying blood vessels decide, which part of the colon has to be removed. Information regarding vascularization and recommended surgery for each tumor site are listed in the table below (92):

Table 3. Surgical operation

Localization	Vascularization	Surgical operation
Cecum	Ileocolic artery, Right colic artery	Right hemicolectomy
Ascending colon	Ileocolic artery, Right colic artery	Right hemicolectomy
Hepatic flexure	Right colic artery, Middle colic artery	Extended right hemicolectomy
Transverse colon (proximal third)	Right colic artery, Middle colic artery	Extended right hemicolectomy
Transverse colon (mid third)	Middle colic artery	Transverse colectomy
Transverse colon (distal third)	Middle colic artery, Left colic artery	Extended left colectomy

Splenic flexure	Middle colic artery, Left colic artery	Extended left colectomy
Descending colon	Left colic artery	Left colectomy
Sigmoid colon (proximal)	Left colic artery	Left colectomy
Sigmoid colon (distal)	Left colic artery	Sigmoid colectomy

In rectal cancer the selected surgery depends on the localization, stage and the size of the tumor. Early stage cancer without high risk features can be treated with local excision. In patients with advanced rectal cancer sphincter sparing resection followed by a colorectal anastomosis should be performed if a negative distal resection margin can be achieved. Otherwise, an abdominoperineal resection with a permanent colostoma is necessary. (95)

Radiotherapy

Radiation is a precious therapy modality in various cancer entities such as brain tumors, breast cancer, lymphomas and sarcomas. Also in the treatment of CRC it is indispensable. The anti cancerous effect of radiotherapy is based on direct damage of ionising rays on cancer cell DNA through energy absorption, as well as on indirect radiation damage, which is obtained by the genesis of free radicals that destroy the DNA. Hyperthermia and the application of pharmacological radiosensitizers such as Misonidazol can further enhance the therapeutic effect. Radiotherapy can be either applied by single radiation or in the form of fractionated radiotherapy, in which higher doses can be used. (96)

In the curative treatment of colon cancer, radiotherapy does not play an important role and is only rarely used in an adjuvant setting. However, in order to lower the local recurrence rate all rectal cancer patients with stage II and III cancer should get neoadjuvant or adjuvant radiation combined with infusional fluoropyrimidines or their orally applied prodrug capecitabine. The radiation is usually administered in fractionation of 1.8 Gray per day over five weeks. Most common side effects of chemoradiotherapy in rectal cancer are diarrhoea, dermatitis and hematotoxicity. (97)

Chemotherapy

Besides surgery, chemotherapy has been the backbone of cancer treatment for now more than 50 years. In colorectal cancer various cytotoxic substances come into practice. They are either applied as single treatment or in combinational regimens. In the palliative setting

of colorectal cancer biologicals can be further added to systemic chemotherapy to improve treatment response and prolong survival. Commonly used therapeutics in CRC patients are:

Fluorouracil

Firstly synthesized in the 1950s fluorouracil or its derivatives are still part of almost every chemotherapy regimen in CRC treatment. By inhibiting the thymidylate synthase fluorouracil, which is needed for the synthetisation of thymidine, fluorouracil blocks DNA replication and cancer cell division. (98) Today, fluorouracil is usually applied in combination with leucovorin, a reduced form of folic acid that further prolongs the inhibition of the thymidylate synthase and thus enhances the cytotoxic effect. (99) Leucovorin modulated fluorouracil therapy can be either administered as bolus regimen or in infusional schedules. The best response rates in the palliative setting have been shown for a bimonthly regimen with a two hour leucovorin infusion followed by a bolus and 22 hour infusion of 5-fluorouracil every two weeks. (100) Most common adverse effects in this therapy are:

- Nausea
- Diarrhoea
- Mucositis
- Conjunctivitis
- Dermatitis
- Alopecia
- Myelosuppression

Capecitabine

Capecitabine is an orally applied fluorpyrimidine that gets converted into 5-fluorouracil in three enzymatic reactions. The final reaction is catalysed by the thymidine phosphorylase of which high levels were found in tumor tissue. Capecitabines are administered as adjuvant as well as palliative chemotherapy and show similar success rates compared to fluorouracil in both settings. (101,102) Most common side effects of capecitabines are:

- Hand foot syndrome
- Diarrhoea
- Hyperbilirubinemia

- Nausea
- Neutropenia
- Stomatitis

Oxaliplatin

Oxaliplatin is a platin derivative that blocks DNA replication by working as an alkylating agent. In colorectal cancer it is applied only in combination with parenteral or oral fluoropyriminidines. Multiple studies have shown that adding oxaliplatin to fluoropyrmidine regimen leads to significantly better treatment response and disease outcome in both localized and metastatic CRC compared to application of fluoropyrimidines alone. (103,104) Common side effects of oxaliplation containing chemotherapy are:

- Paraesthesia
- Myelosuppression
- Nausea
- Diarrhoea
- Stomatitis
- Dermatitis
- Alopecia

Irinotecan

The cytotoxic effect of irinitocan is based on an inhibition of the topoisomerase I an enzyme that is essential for DNA replication and transcription. Like oxaliplatin irinotecan is usually administered in combination with a fluoropyrimidine regimen and is an alternative to oxaliplatin containing chemotherapy. In metastatic CRC patients that did not benefit from fluoropyrimidine based therapy in prior lines application of irinotecan alone showed a survival benefit compared to best supportive care and should therefore be considered. (105) Important side effects of irinotecan are:

- Diarrhoea
- Nausea

- Myelosuppression
- Alopecia
- Anorexia
- Fatigue
- Stomatitis
- Cholinergic syndrome

Therapy regimens

Commonly used combinational chemotherapy regimens in CRC are:

- FOLFOX
    - Folinic acid
    - Fluorouracil
    - Oxaliplatin
- FOLFIRI
    - Folinic acid
    - Fluorouracil
    - Irinotecan
- XELOX
    - Capecitabine
    - Oxaliplatin
- FOLFOXIRI
    - Folinic acid
    - Fluorouracil
    - Irinotecan
    - Oxaliplatin

Targeted therapy

This relatively new cancer treatment approach has led to major advances in the prognosis of various cancer entities over the last two centuries. Targeted therapy or also called biologic therapy aims at inhibiting cancer cell division by blocking or antagonising specific

molecules which are essential for carcinogenesis. The great advantage of targeted therapy is that it does not simply kill all rapidly dividing cells like systemic chemotherapy, but only targets cancerous cells and therefore has a much better toxicity profile. Targeted therapy agents can be roughly divided into two groups: small molecules and monoclonal antibodies. Small molecules are usually orally administered substances that typically target intracellular molecules. An example is the famous BCR-Abl tyrosine kinase inhibitor imatinib, which is one of the oldest biologics used in cancer therapy. Monoclonal antibodies on the other hand are larger structures that cannot enter cancer cells and thus are usually used to target cell surface molecules such as receptors. As their protein structure would denaturate in the gastrointestinal tract, monoclonal antibodies have to be applied intravenously. (106) One major problem coming along with targeted therapy are the high costs of its therapy agents. Bevacizumab for example is reported to extend life of metastatic colorectal cancer patients by 4.7 months at a cost of about $50.000. (107)

Biologics administered in CRC are:

Bevacizumab

Bevacizumab is a recombinant humanized monoclonal antibody that works as an angiogenesis inhibitor by targeting the vascular endothelial growth factor A. (VEGF) Angiogenesis plays a crucial role in cancer development and is one of the main aims in targeted therapy. In CRC bevacizumab is administered as palliative therapy in combination with a fluoropyrimidine based chemotherapy. Adding bevacizumab leads to significantly better treatment response and overall survival compared to systemic chemotherapy alone. (108) In the adjuvant treatment setting the additional application of bevacizumab does not show any benefit. (109)

Most feared adverse effects of bevacizumab are:

- Hypertension
- Proteinuria
- Bleedings
- Thrombotic events
- Delayed healing
- Gastrointestinal perforation

Cetuximab

Cetuximab is a chimeric monoclonal antibody that binds and inhibits the epidermal growth factor receptor (EGFR). Overexpression of EGFR leads to uncontrolled cell division and is found in various cancers. In metastatic CRC a survival benefit of cetuximab added to first line system chemotherapy was shown in the CRYSTAL trial. However, subgroup analysis revealed that cetuximab is effective only in KRAS wildtype tumors (110). Today it is known that only patients with wildtypes in all RAS genes (KRAS and NRAS) benefit from cetuximab therapy. (111) In the adjuvant setting of CRC cetuximab did not show any additional treatment effect and is therefore not indicated. (112)

Feared side effects of cetuximab containing therapy are:

- Infusion related reactions
- Acne like rash
- Fatigue
- Malaise
- Pain
- Dyspnoea
- Diarrhea

Panitumumab

Like cetuximab, panitumumab is an anti epidermal growth factor receptor agent used in the therapy of RAS wildtype metastatic CRC. The ASPECCT study showed that these two agents provide similar overall survival benefit and comparable toxicity profiles. (113)

Common side effects of pannitumumab are:

- Acne like rash
- Hypomagnesemia
- Diarrheoa
- Pruritus
- Fatigue
- Nausea
- Decreased appetite

Regorafenib

Regorafenib is a tyrosine kinase inhibitor used as salvage therapy in metastatic CRC patients, which have been previously treated with a systemic chemotherapy and a monoclonal antibody. The CORRECT trial showed a significant survival benefit for these patients when comparing regorafenib monotherapy versus best supportive care. (114)

Feared adverse effects of regorafenib are:

- Fatigue
- Hand foot skin reaction
- Dairrhea
- Anorexia
- Voice change
- Hypertonia
- Oral mucositis
- Rash

Trifluridine tipiracil

This agent combination of the thymidylate synthetase inhibitor trifluridine and the thymidine phosporylase inhibitor tipiracil seems to promote cancer cell apoptosis and inhibits angiogenesis. Like Regorafenib Trifluridine tipiracil is used as a salvage therapy in mCRC patients who are refractory to fluoropyrimidines, irinotecan, oxaliplatin, anti VEGF and anti EGFR therapy. In this patient collective the RECOURSE trial could show a significant overall survival benefit for trifulridine tipiracile against placebo. In detail the median overall survival in the therapy group was 1.8 monts longer than in the control group. (115)

Most common side effects in this trail were:

- Nausea
- Reduced appetite
- Fatigue
- Neutropenia
- Anemia

Stage dependent treatment

Stage I colon cancer

The adequate therapy for stage I colon cancer contains radical surgical resection of the tumor followed by regular posttreatment surveillance including clinical, laboratory and imagining examinations. Adjuvant chemo- or radiotherapy is not recommended for stage I colon cancer patients. (116)

Stage II colon cancer

In patients with stage II colon cancer, who have undergone curative excision of the tumor the application of adjuvant chemotherapy has to be discussed. The QUASAR trial showed a prolonged overall survival for stage II patients treated with fluorurcail plus leucovorin compared to observation. However, with a three to four percent absolute survival benefit the absolute improvements are small and in addition to this the chemotherapy associated morbidity has to be considered. (117) Therefore, there is no general recommendation for adjuvant chemotherapy in stage II colon cancer patients. To facilitate the decision making which patient would profit from chemotherapy several clinicopathologic variables associated with higher recurrence rates have been determined as risk factors:

- T4 stage
- Bowel obstruction or perforation at initial diagnosis
- High grade histology
- Lymphovascular invasion
- Perineural invasion
- Less than 13 sampled lymph nodes
- Inadequate tumor margins
- High preoperative CEA levels

In about 20% of all stage II colon cancer patients a microsattelite instability can be detected. Those patients seem to have a lower recurrence rate and might not benefit from adjuvant chemotherapy. (118)

Stage III colon cancer

In stage III colon cancer adjuvant chemotherapy should be recommended to all patients, who are fit enough for the treatment. Biomarkers like the risk factors used in stage II colon cancer do not have any impact on the decision making. Clinical factors, in particular biological age and comorbitities have an influence on which treatment protocol is applied. Fit patients profit most from a more intense regimen such as FOLFOX or XELOX. (104,119) For patients who do not tolerate oxaliplatin a fluoropyrimidine-alone protocol is an acceptable option. If possible chemotherapy should be started within eight weeks after surgery. Adding a monoclonal antibody to systemic chemotherapy does not show any benefit and is therefore not indicated. (109,112)

Stage I – III rectal cancer

According to the most recently published European Society for Medical Oncology (ESMO) clinical practice guidelines for non-metastatic rectal cancer patients can be divided into five different risk adapted treatment groups:

Very early disease

Very early disease is defined as clinically staged cT1 tumors that are lymph node negative (N0) and low grade (G1 or G2). For this patient collective the standard of care are local excision procedures such as transanal endoscopic microsurgery or direct tumor excision. There is no general recommendation for perioperative radiotherapy, chemoradiotherapy and chemotherapy. In patients who rare not operable or reject surgery and in patients with an unfavourable ssessment following tumor excision these options might be considered. (67)

Early disease

This group includes all tumors with no mesorectal fascia (MRF) involvement and no extramural vascular invasion (EMVI) that do not fit the criteria for local excision:

- cT1, N0, G3 regardless of tumor location
- cT3 with 5mm or less invasion depth beyond the mucularis propria, N0, G1-3 if middle or high
- cT3 with 5mm or less invasion depth beyond the mucularis propria, N1, G1-3 if high

Patients with early disease should be treated by radical total mesorectal excision (TME) implying the excision of the tumor and the complete mesorectal fat including all lymphnodes. Considering the surgeon's preference, the location of the tumor and the general health condition of the patient TME can be either performed as laparoscopic or open surgery. In patients with cT1 or cT2 tumors smaller than four centimetres neoadjuvant radiotherapy or chemoradiotherapy followed by local tumor excision is a possible treatment alternative. Otherwise neoadjuvant therapy is not generally recommended. (67)

Intermediate disease:

Inclusion criteria are:

- cT3 with 5mm or less invasion depth beyond the mucularis propria, N0 – N2, no EMVI, MRF clear if low
- cT3 with 5mm or less invasion depth beyond the mucularis propria, N1 – N2, no EMVI, MRF clear if middle or high

Standard treatment for intermediate rectal cancer consists of neoadjuvant treatment followed by TME. The downsizing therapy can be either applied as short course preoperative radiotherapy with 25 Gray total dose at five fractions during one week or as chemoradiotherapy with a recommended total radiation dose of 45 to 50 Gray combined with concurrent fluoropyrimidine chemotherapy. Both approaches are valid. If the surgeon is confident that a good quality TME can be achieved without neoadjuvant therapy TME alone can be considered. (67)

Locally advanced disease:

All cT3 tumors with 5 mm or more invasion depth beyond the muscularis propria or with positive EMVI are staged as locally advanced rectal cancers if the MRF is clear and the levators are not threatened. Like intermediate disease locally advanced tumors should be treated with neoadjuvant downstaging therapy followed by TME. (67)

Advanced disease:

This group composes of tumors with threatened resection margins implying cT4b stage, MRF infiltration and threatened levators. In this case preoperative chemoradiotherapy

followed by TME is the standard of care, as it alters the chance of an R0 resection compared to radiotherapy alone. (67)

Postoperative therapy:

Postoperative chemoradiotherapy should be considered in all patients who did not receive preoperative treatment and further showed unfavourable histopathological features such as stage pT4b disease or positive circumferential resection margin after tumor resection.

In stage III or high risk stage II patients who did receive neoadjuvant treatment postoperative chemotherapy is recommended and can be either applied as single fluoropyrimidine regimen or combined with oxaliplatin depending on tumor stage and patient performance status. (67)

Stage IV colorectal cancer

Around a quarter of all CRC patients present with synchronous metastasis at initial diagnosis and an additional 25% will develop metastases during the course of their disease. (11) For a long time it was thought that there is no cure for CRC patients with distant metastasis. However, in recent years it became clear that metastectomy in patients with liver or lung only metastases is a potentially curative treatment with five years survival rates up to 50% and at least a one in 6 chance of cure . (120) (121) Roughly patients with distant metastasis can be divided into three groups:

Firstly, patients with initially resectable metastases, which account for around 15 % of all metastatic CRC patients. In this situation guidelines recommend upfront resection followed by adjuvant chemotherapy or perioperative treatment with FOLFOX consisting of six two-week cycles before and after surgery. The EORTC study showed a benefit in progression free survival for the addition of perioperative chemotherapy, but no benefit in overall survival. (122)

Secondly, patients with potentially resectable metastases, which however do not fit surgical resection criteria at presentation. In these patients the most effective induction chemotherapy with the aim of maximal tumor shrinkage should be suggested. Possible regimens are FOLFIRI, FOLFOX or FOLFOXIRI plus a targeted therapy agent. In RAS wildtype patients Stintzing et al. could show a significant benefit in objective response

rate, early tumor shrinkage and median depth of response for cetuximab plus FOLFIRI compared to bevacizumab plus FOLFIRI, indicating that for those patients this may be the best therapy. (123)

The third group are metastatic CRC patients with not resectable metastatic disease and primary palliative treatment intention. For patients with a high tumor burden or symptomatic disease more intense first line chemotherapy such as FOLFOX or FOLFIRI plus bevacizumab or plus an EGFR antibody in RAS wildtype cancer is the recommended treatment strategy to induce tumor shrinkage and symptom relief. If the tumor responds therapy can be de-escalated after a few months and continued as maintenance therapy in order to reduce toxicity. In asymptomatic patients with slow tumor progression a less toxic regimen seems to be the best option as first line therapy. In case of progression further line treatment has to be decided individually, whilst taking prior therapy, RAS status and the treatment's goal into account. The following principles should be considered (92):

- After a oxaliplatin based first line therapy an irinitocan containing regimen should be administered and vice versa
- After an irinotecan based bevacizumab free first line therapy FOLFOX plus bevacizumab should be used
- Continuing bevacizumab therapy after first line progression leads to a significant survival benefit
- Patients with RAS wildtype who have not been given an EGFR antibody as first line therapy should receive a combination of chemotherapy plus an EGFR antibody
- The application of cetuximab in patients who did not benefit from a panitumumab containing therapy is not indicated and vice versa
- In patients who are refractory to all established chemotherapies and monoclonal antibodies the application of the tyrosine kinase inhibitor Regorafenib leads to a prolonged overall survival

Prognosis

Colorectal cancer patients have a 90%, 65% and 15% five year relative survival rate in localized, regional and advanced tumor stage. Localized tumor stage is defined as cancer without metastatic spread, regional tumors present with lymph node metastases and advanced tumor stage inludes all patients with distant metastases. Overall five year survival rate in CRC patients is 63%. (124)

Prognostic and predictive biomarkers in metastatic CRC

RAS mutation status

RAS is a family of oncogenes, that play a crucial role in the regulation of cell growth and differentiation. In humans three different RAS genes are identified: HRAS, KRAS and NRAS. Inappropriate activation of these genes leads to uncontrolled cell growth and therefore promotes cancer development. Mutations in RAS genes are found in around 25% of all human cancers. (125) In colorectal cancer mutations occur in approximately 40%. (126) As the RAS proteins are strongly involved in the endothelial growth factor receptor pathway RAS mutation status is predictive for EGFR antibody therapy response. Several studies could demonstrate, that patients with KRAS exon 2 mutation do not benefit from cetuximab or panitumuab therapy. (127,128) More recently it was shown that not only exon 2 mutations in KRAS but all RAS mutations lead to unresponsiveness of EGFR therapy. (129) Hence, today extensive RAS gene testing is recommended for all patients with a planned anti EGFR therapy.

BRAF mutation status

Like the RAS genes BRAF is a human proto oncogene that encodes for proteins involved in the regulation of cell growth. If mutated, BRAF proteins induce cell transformation and cancer development. BRAF mutations occur in around 8% of all human cancers with the highest frequency found in melanomas. (130) In CRC BRAF mutations that are mutually exclusive with KRAS mutations can be detected in 5 to 15%. With a 5 year survival rate of only 16% BRAF mutations are associated with a very poor survival in microsatellite stable CRC patients. These results suggest that BRAF mutated patients may benefit from a aggressive first line triplet chemotherapy such as FOLFOXIRI. In patients with microsatellite unstable tumors, BRAF mutations have no adverse impact on survival. (131) Besides, its prognostic value BRAF mutation status seems to be a valid predictor for anti EGFR therapy response. Pietrantonio et al. could show that RAS wildtype patients with BRAF mutation do not benefit from cetuximab or panitumumab therapy. (132) Therefore BRAF status should be tested in all CRC patients.

Tumor location

Recent studies revealed that primary tumor location may have a strong prognostic and predictive relevance in localized and metastatic CRC. A retrospective analysis of the FIRE 3 and CRYSTAL trials could show that in the RAS wildtype population patients with right sided tumors have a significantly worse prognosis than those with left sided and might not benefit from addition of cetuximab to systemic chemotherapy. (133)

Methods

Study design, patient cohort, and clinical outcomes

The current study is a single-center, retrospective observational cohort study including patients with histologically-proven metastatic colorectal adenocarcinoma who were treated with chemo(immune-)therapy at the Clinical Division of Oncology, Medical University of Graz, Austria, between March 2010 and January 2016. These patients were drawn from our in-house colorectal cancer cohort, which includes exactly 1000 patients with UICC stage II-IV adenocarcinomas of the colon or rectum who were seen at our Department since January 2010. Of these 1000 patients, 3 were lost-to-follow-up, and 612 were adjuvant patients who did not develop metastasis during a median follow-up of 2.9 years (95%CI: 2.8-3.11, **Supplementary figure 1**). Of the remaining 388 patients with metastatic disease, 130 patients did not get any type of palliative chemotherapy (reasons: reduced performance status (n=61), declined therapy (n=13), other reasons (n=6), not known: n=50)), leaving a final analysis population of 258 patients with mCRC undergoing first-line chemo(immuno-)therapy . Baseline and follow-up data were extracted from our hospital trust's electronic health record database (including all public hospitals in the province of Styria, Austria). For the main biomarker analysis, we considered 5 inflammatory biomarkers, namely the neutrophil-lymphocyte-ratio (NLR), the lymphocyte-monocyte-ratio (LMR), the platelet-lymphocyte-ratio (PLR), C-reactive protein (CRP), and the advanced lung cancer inflammation index (ALI), respectively. The ALI is defined as (body mass index * serum albumin) / NLR. We only considered laboratory data that were collected within a timeframe of a maximum at 14 days prior start of the respective chemotherapy line. In time-to-event analysis, we investigated response rates (RR) according to RECIST 1.1 criteria and rates of progression-free (PFS) and overall survival (OS) during the first three lines of treatment, and after start of a purely "best supportive care (BSC)" treatment concept. The primary endpoint was 6-month PFS during 1st-line, 2nd-line, and 3rd-line treatment, and 6-month OS during BSC.

Ethics statement

The study had been approved by the local ethics committee (Ethikkommission der Medizinischen Universität Graz, IRB00002556) before any patient-related activities were

performed (No.25-458 ex 12/13). Written informed consent was not obtained from individual patients, because the local ethics committee specifically granted a "waiver of consent" for this retrospective database study. All investigations took place in accordance with the priniciples embodied in the declaration of Helsinki.

Statistical analysis

All statistical analyses were performed using Stata (Windows version 14.0, Stata Corp., Houston, TX, USA). Continuous variables were summarized as medians [25^{th}-75^{th} percentile], whereas categorical variables were reported as absolute counts (%). The association between response rates and the biomarkers under study were analyzed with uni- and multivariable generalized linear models from the Bernoulli family with an identity link. Median follow-up was estimated according to the method of Schemper & Smith. Probabilities of progression-free and overall survival were computed with Kaplan-Meier estimators, and compared between two or more groups with log-rank tests. Uni- and multivariable modeling of PFS and OS was performed with Cox proportional hazards models. The proportionality of hazards assumption was assessed by fitting an interaction between linear follow-up time and the variables of interest. To compare the magnitude of association with PFS and OS between the different biomarkers, we Z-standardized these variables in order to render them on a common scale (mean=0, standard deviation=1).

Results

Analysis at baseline

Two-hundred-fifty-eight patients were included in this analysis (Table 1). The median age of the cohort at start of first line therapy was 66 years, and 36% were female. More than 80 % of patients had no evidence of medical comorbidity at initial diagnosis, and the median Karnofsky index (KI) was 90%. The most frequent tumor site was the rectum (n=90 (35%)), and 65 (26%) patients had right-sided tumors, which were defined as tumors located proximal to the splenic flexure. Two thirds of the patients had synchronous metastases, whereas the other third developed metastases after surgery in curative intent. Polychemotherapy regimens which were defined as either multiagent chemotherapy or single/multiagentchemotherapy plus molecular targeted therapy were administered as 1st-

line therapy in 70% of patients, as 2nd-line therapy in 62%, and as 3rd-line therapy in 56% of patients, respectively. The median NLR was 3.9 before start of first line chemotherapy. More detailed information concerning baseline demographic, tumor, treatment and laboratory variables are summarized in Table 1. We observed changes in the levels of the inflammatory parameters between the different treatment lines (Supplementary Table 1).The median NLR, for example, showed an 18% relative reduction from first to second line, remained at the same level after second line, but finally raised by more than 30% compared to baseline value, when entering BSC. Similar changes could be observed for the other biomarkers.

Table 4. Baseline characteristics of the study population.

Overall Distribution and by therapy line. The column "n (% miss.)" indicates the number of patients with observed values of the respective variable (% missing). Continuous variables are summarized as medians [25th percentile (Q1) – 75th percentile (Q3)], whereas categorical variables are reported as absolute frequencies and percentages. *ALI = (body mass index * serum albumin) / NLR. Abbreviations: BMI – Body mass index, NLR – Neutrophil to lymphocyte ratio, LMR – Lymphocyte to monocyte ratio, PLR – Platelet to lymphocyte ratio, CRP – C reactive protein, ALI – Advanced lung cancer index

		1st line (n=258)			2nd line (n=153)			3rd line (n=72)			BSC (n=183)	
Variable		N (%miss.)	Summary measure		N (%miss.)	Summary measure		N (%miss.)	Summary measure		n (% miss.)	Summary measure
Demographic variables												
Female gender		258 (0%)	92 (36%)		153(0%)	53(35%)		72(0%)	27(38%)		183(0%)	63(34%)
Age (years)		258(0%)	66 [58-73]		153(0%)	65 [59-72]		72(0%)	64 [60-71]		183(0%)	66 [59-73]
BMI (kg/m²)		221(14%)	24 [22-27]		134(12%)	25 [22-27]		64(11%)	24 [21-27]		0 (100%)	/
Karnofsky Index		161 (38%)	90 [80-100]		95(38%)	90 [80-90]		41(43%)	90 [80-90]		0(100%)	/
No comorbidity		256(1%)	210(82%)		151(1%)	126(83%)		70(3%)	61(87%)		182(1%)	148(81%)
Smoker or ex smoker		132(49%)	56(42%)		77(50%)	34(44%)		37(49%)	15(41%)		83(55%)	44(53%)
Tumor variables												

Synchronous metastases		258(0%)	172(67%)		153(0%)	104(68%)		72(0%)	48(67%)		183(0%)	121(66%)
Location of primary tumor		256(1%)	/		151(1%)	/		71(1%)	/		183(0%)	/
---Right ascending		/	43(17%)		/	22(14%)			11(15%)			33(18%)
---Right flexure		/	17(7%)		/	14(9%)			5(7%)			11(6%)
---Transverse colon		/	10(4%)		/	6(4%)			3(4%)			9(5%)
---Left flexure		/	13(5%)			7(5%)			3(4%)			13(7%)
---Left descending		/	6(2%)			5(3%)			2(3%)			5(3%)
---Sigma		/	71(28%)			37(25%)			18(25%)			45(25%)
---Rectum		/	90(35%)			56(37%)			28(39%)			62(34%)
---Multilocular		/	6(2%)			4(3%)			1(1%)			5(3%)
Kras wildtype		232(10%)	123(53%)		140(8%)	80(57%)		66(8%)	40(61%)		163(11%)	85(52%)
Nras wildtype		64(75%)	54(84%)		31(80%)	25(81%)		11(85%)	9(82%)		38(79%)	30(79%)
Treatment variables												
Number of chemotherapy cycles		241(7%)	8 [4-10]		141(8%)	8 [6-10]		68(6%)	8 [6-11]		/	/
Polychemotherapy		257(1%)	181(70%)		153(0%)	95(62%)		72(0%)	40(56%)		/	/
Laboratory variables												
Hemoglobin		232(10%)	12.4 [11.2-13.4]		119(22%)	12.7 [11.7-13.9]		59(18%)	13.1 [11.2-14.0]		164(11%)	11.4 [10.3-12.8]
Leucocyte count		194(25%)	8.8 [6.9-11.7]		120(22%)	7.1 [5.6-9.4]		59(18%)	7.6 [5.9-8.9]		165(10%)	8.5 [6.0-11.9]
Absolute neutrophil count		143(45%)	6.1 [4.4-8.7]		114(25%)	4.6 [3.4-6.3]		57(21%)	4.9 [3.5-6.0]		152(17%)	5.8 [3.9-9.2]

Absolute lymphocyte count		129(50%)	1.4 [1.1-1.9]		114(25%)	1.4 [1.0-1.7]		57(21%)	1.4 [1.0-2.0]		151(17%)	1.1 [0.8-1.7]
Absolute monocyte count		140(46%)	0.7 [0.5-0.9]		114(25%)	0.7 [0.6-0.9]		57(21%)	0.8 [0.6-1.0]		150(18%)	0.9 [0.6-1.2]
Absolute platelet count		193(25%)	312 [249-398]		120(22%)	223 [184-304]		60(17%)	252 [193-333]		164(10%)	264 [207-374]
NLR		120(53%)	3.9 [2.6-5.5]		114(25%)	3.2 [2.2-5.4]		57(21%)	3.2 [1.9-5.9]		151(17%)	5.2 [3.1-8.5]
LMR		110(57%)	1.9 [1.5-2.8]		113(26%)	1.8 [1.2-2.8]		57(21%)	1.9 [1.3-3.0]		149(19%)	1.2 [0.8-2.0]
PLR		110(57%)	212 [147-401]		114(25%)	164 [123-245]		57(21%)	171 [115-270]		150(18%)	239 [155-359]
Albumin		80(69%)	4.1 [3.6-4.4]		130(15%)	4.0 [3.7-4.2]		62(14%)	3.9 [3.5-4.1]		129(30%)	3.5 [3.0-3.8]
CRP		241(7%)	11.7 [4-34]		149(3%)	11 [4-34]		69(4%)	13.7 [5.0-48]		174(5%)	43 [12-96]
ALI*		170(34%)	26.9 [15.1-42.0]		88(43%)	30.9 [19.8-51.5]		46(36%)	29.5 [12.4-56.9]		N/A	N/A
Uric acid		112(57%)	5.2 [4.2-6.5]		63(59%)	5.3 [4.2-6.3]		24(67%)	5.7 [4.5-6.2]		70(62%)	5.2 [3.9-6.7]
CEA		154(40%)	17 [4-100]		135(12%)	52 [13-211]		62(14%)	67 [12-277]		130(29%)	78 [17-498]
CA19 9		143(45%)	45 [10-529]		135(12%)	83 [16-1237]		62(14%)	147 [25-1111]		127(31%)	445 [28-4406]

Table 5. Changes of inflammatory biomarkers over the first three treatment lines and BSC

		1st line (n=258)		2nd line (n=153)			3rd line (n=72)			BSC (n=183)		
		Median [25th-75th percentile]		Median [25th-75th percentile]	% relative change compared to first line	P for comparison with first line	Median [25th-75th percentile]	% relative change compared to first line	P for comparison with first line	Median [25th-75th percentile]	% relative change compared to first line	P for comparison with first line
NLR		3.9 [2.6-5.5]		3.2 [2.2-5.4]	-18 %	0.691	3.2 [1.9-5.9]	-19%	0.935	5.2 [3.1-8.5]	+32%	0.010
LMR		1.9 [1.5-2.8]		1.8 [1.2-2.8]	-6%	0.145	1.9 [1.3-3.0]	-2%	0.028	1.2 [0.8-2.0]	-36%	<0.0001
PLR		212 [147-401]		164 [123-245]	-23%	0.025	171 [115-270]	-19%	0.559	239 [155-359]	+13%	0.215
CRP		11.7 [4-34]		11 [4-34]	-9%	0.095	13.7 [5.0-48]	+17%	0.013	43 [12-96]	+268%	<0.0001
ALI*		26.9 [15.1-42.0]		30.9 [19.8-51.5]	+15%	0.345	29.5 [12.4-56.9]	+10%	0.934	N/A	N/A	N/A

Analysis of response patterns and their association with inflammatory biomarkers

During first-line treatment of 258 patients with chemo(immuno-)therapy, we observed 5 complete remissions (CR, 2%), 70 partial remissions (PR, 27%), 67 stable disease (SD, 26%), and 77 primary disease progressions (PD, 30%), respectively (Supplementary Figure 1). Response was not evaluable in 39 patients (NE, 15%). Response data for further lines of treatment are reported also in Supplementary Figure 1. Among the patients assessable for response, we estimated objective response rates (ORR) of 34% (95%CI: 30-41), 19% (13-26), and 17% (7-27), during 1st-line, 2nd-line, and 3rd-line treatment. Corresponding disease control rates (DCR, i.e. a composite of CR+PR+SD as best response) were 65% (59-71), 50% (42-59), and 37% (24-50), respectively.

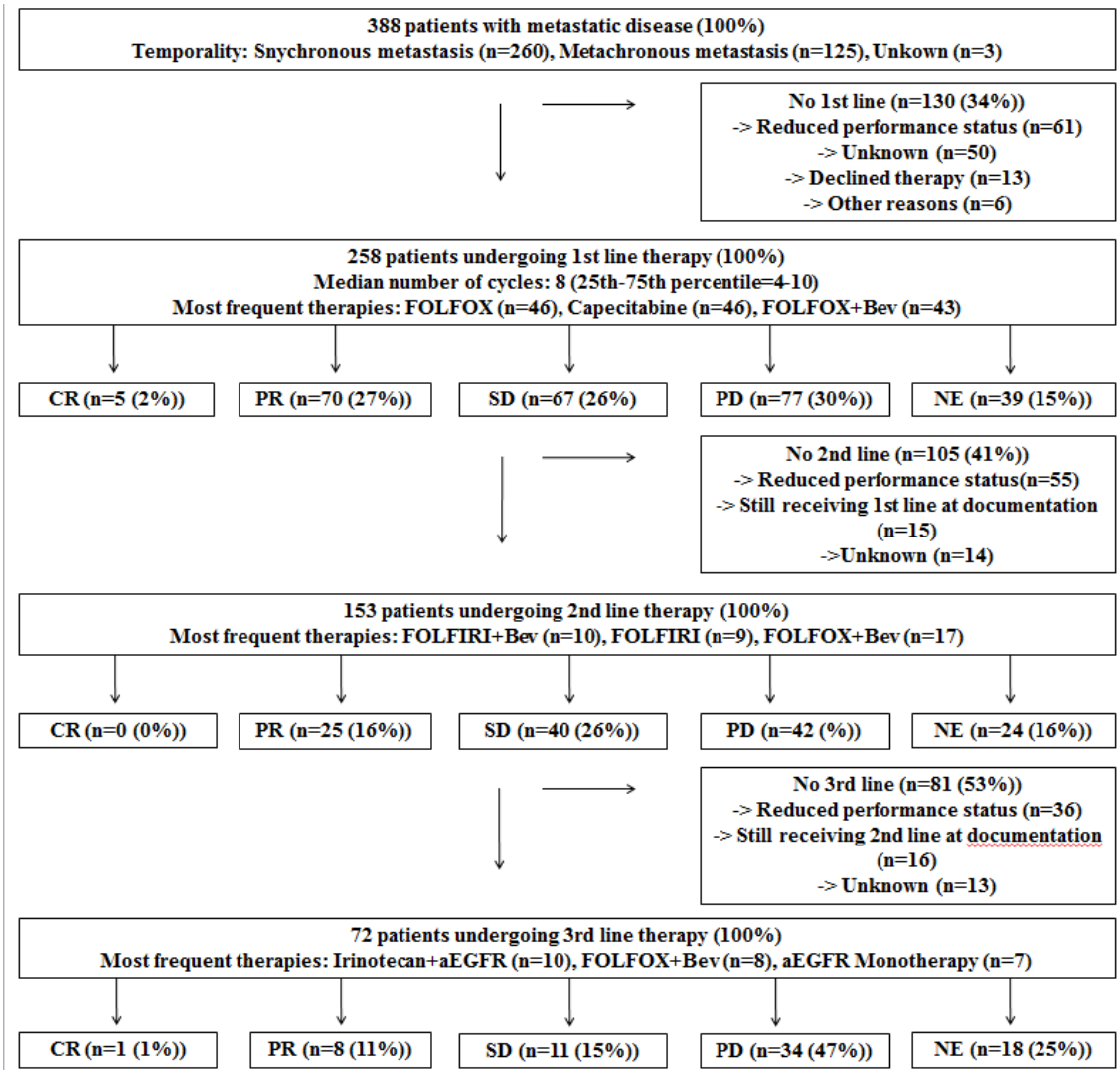

Figure 1. Flowchart of the study population

In univariable analysis of absolute response rates, we observed associations between inflammatory biomarkers and ORR (Table 2). In detail and after z standardization, 1 standard deviation (SD) increase in NLR was associated with a 7% absolute lower ORR in first line (95%CI: 6-9, p<0.0001), 4% lower ORR in second line (3-5, p< 0.0001), and 2% lower ORR in third line (-1-11, p=0.68), respectively. Corresponding results for the LMR, PLR, CRP and ALI are reported in the Table 2. Another strong univariable predictor of response was polychemotherapy (23% higher response rates in first line (p<0.0001), 13% higher ORR in second line (p=0.05), 20% in third line (p=0.02)). Right side location of the tumor and age were not significantly associated with ORR in all patients, but highly associated with a 26% lower 1st-line ORR in the subgroup of patients with KRAS-wildtype tumors (Table 2). In multivariable analysis adjusting for polychemotherapy, associations between inflammatory biomarkers and ORR prevailed (Table 2). This suggests that inflammatory biomarkers are important and independent predictive markers of response to antineoplastic chemotherapy, not only in first but also in later lines of treatment.

Table 6. Uni and multivariable predictors of clinical response rates in first, second and third line

Absolute change of ORR (objective response rate) per 1 standard deviation increase of the respective biomarker. Abbreviations: ORR – Objective response rate, CI – Confidence interval, P – P Value, SD – Standard deviation, NLR – Neutrophil to lymphocyte ratio, LMR – Lymphocyte to monocyte ratio, PLR – Platelet to lymphocyte ratio, CRP – C reactive protein, ALI – Advanced lung cancer index

Variable		Δ_{abs} in 1st-line ORR (95%CI, p)		Δ_{abs} in 2nd-line ORR (95%CI, p)		Δ_{abs} in 3rd-line ORR (95%CI, p)
Inflammatory biomarkers – Univariable analysis						
NLR (per 1SD increase)		-7.4% (-9.1-(-5.7), p<0.0001)		-3.6% (-4.5-(-2.7), p<0.0001)		-2.0% (-11.9-7.8, p=0.68)
LMR (per 1SD increase)		5.1% (-4.2-14.5, p=0.28)		3.9% (-4.7-12.6, p=0.38)		-5.1% (-10.6-0.5, p=0.07)
PLR (per 1SD increase)		-2.5% (-11.7-6.7, p=0.60)		-4.8% (-6.8-(-2.9), p<0.0001)		-3.3% (-5.3-(-1.2), p=0.002)
CRP (per 1SD increase)		-2.5% (-9.1-4.1, p=0.45)		-7.8% (-9.5-(-6.0), p<0,0001)		4.2% (-7.0-15.5, p=0,46)

ALI (per 1SD increase)		8.0% (0.4-15.5, p=0.04)		10.0% (-2.0-22.0, p=0.10)		-7.9% (-13.0-(-2.8), p=0.002)
Other predictors – Univariable analysis						
Age (per 10 years increase)		-5.7% (-11.7-0.0, p=0.06)		-2.7% (-9.6-4.2, p=0.44)		-12.8% (-20.0-(-5.5), p=0.001)
Right side		-11.1% (-25.1-3.0, p=0.12)		2.9% (-13.0-18.8, p=0.72)		-15.4% (-33.2-2.4, p=0.09)
Right side in KRAS wildtype		-25.5% (-45.4-(-5.6), p=0.01)		7.0% (-19.2-33.2, p=0.60)		-22.7% (-40.2-(-5.2), p=0.01)
Polychemotherapy		22.6% (10.2-35.0, p<0.0001)		12.7% (0.0-25.6, p=0.05)		20.5% (3.1-37.8, p=0.02)
Inflammatory biomarkers – Multivariable analysis adjusted for polychemotherapy						
NLR (per 1SD increase)		-8.5% (-10.5-(-6.6), p<0.0001)		-3.0% (-4.4-(-1.6), p<0.0001)		-3.1% (-8.3-2.0, p=0.24)

LMR (per 1SD increase)		4.6% (-4.6-13.8, p=0.33)		1.9% (-6.7-10.5, p=0.67)		1.1% (-8.2-10.5, p=0.81)
PLR (per 1SD increase)		-4.0 (-12.4-4.4, p=0.35)		-3.0% (-7.7-(1.6), p=0.20)		-3.7% (-8.5-1.0, p=0.13)
CRP (per 1SD increase)		-3.4% (-5.3-(-1.5), p<0.0001)		-8.4% (-8.4-(-8.3), p<0.0001)		2.1% (-5.2-9.4, p=0.58)
ALI (per 1SD increase)		8.4% (1.0-15.8, p=0.03)		7.9% (-4.0-19.8, p=0.20)		-5.1% (-12.8-2.7, p=0.20)

Univariable Analysis of 6-month PFS and OS across treatment lines

Median PFS was 6.7 months in 1 st line, 4.2 months in 2nd line and 3.2 months in 3rd line therapy, respectively. Six month PFS was 58% (52-64), 31% (23-38) and 22% (12-33) in first, second and third line, respectively. Median OS was 2.7 months in BSC, and 6-month OS in BSC was 32%. (Supplementary Figure 2)

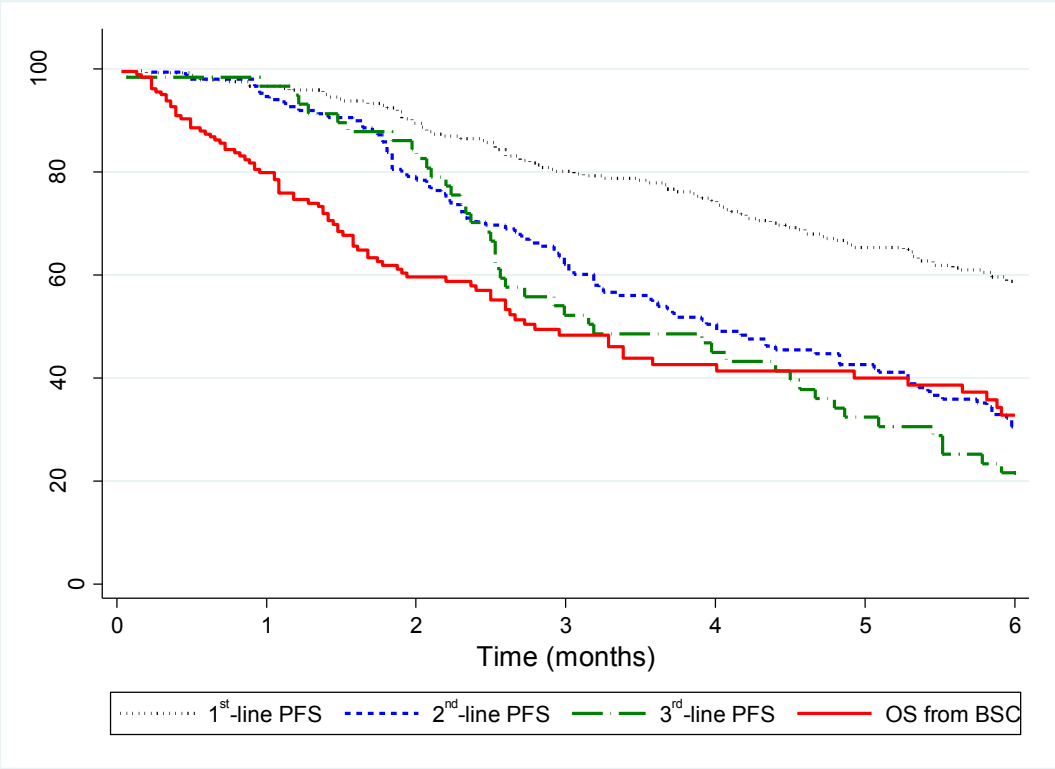

Figure 2. Cumulative probability of progression free survival in 1st, 2nd and 3rd of palliative chemotherapy and overall survival in best supportive care.

Associations between inflammatory biomarkers and PFS in the first three treatment lines and OS are reported in Table 3. In univariable Cox regression analysis, the NLR was associated with a numerically impaired 6-month PFS during first three treatment lines. However, this was only statistically significant in the 2nd-line setting with the numbers we had. No association between the NLR and 6-month OS could be observed in BSC (Table 3).

Table 7. Uni and multivariable predictors of clinical outcomes in first, second, third line and best supportive care.

Hazard ratio of 6-month PFS (progression free survival) per 1 standard deviation increase of the respective biomarker. Abbreviations: PFS – Progression free survival, HR – Hazard ratio, CI – Confidence interval, P – P Value, SD – Standard deviation, NLR – Neutrophil to lymphocyte ratio, LMR – Lymphocyte to monocyte ratio, PLR – Platelet to lymphocyte ratio, CRP – C reactive protein, ALI – Advanced lung cancer index

Variable		6-month PFS in 1st line (HR (95%CI, p))		6-months PFS in 2nd line (HR (95%CI, p))		6-months PFS in 3rd line (HR (95%CI, p))		6-months OS in BSC (HR (95%CI, p))
Inflammatory biomarkers – Univariable analysis								
NLR (per 1SD increase)		1.21 (0.98 – 1.15, p= 0.083)		1.39 (1.18 – 1.65, p< 0.0001)		1.42 (1.00 – 2.02, p= 0.051)		1.08 (0.91 – 1.28, p=0.376)
LMR (per 1SD increase)		0.74 (0.52 – 1.06, p= 0.099)		0.76 (0.58 – 1.01, p= 0.060)		0.71 (0.48 – 1.05, p= 0.084)		0.64 (0.46- 0.89, p=0.008)
PLR (per 1SD increase)		1.33 (1.02 – 1.74, p= 0.036)		1.68 (1.35 – 2.10, p< 0.0001)		1.41 (0.48 – 1.05, p= 0.033)		1.16 (0.97-1.38, p= 0.095)
CRP (per 1SD increase)		1.40 (1.16 – 1.69, p< 0.0001)		1.25 (1.06 – 1.47, p= 0.009)		1.09 (0.81 – 1.47, p= 0.559)		1.46 (1.18 – 1.81, p=0.001)
ALI (per 1SD increase)		0.7 (0.52 – 0.95, p< 0.024)		0.77 (0.55 – 1.08, p= 0.128)		0.74 (0.5 – 1.08, p= 0.117)		N/A
Other predictors –								

Univariable analysis								
Age (per 10 years increase)		1.04 (0.85 – 1.26,p= 0.714)		1.03 (0.85 – 1.26,p= 0.754)		1.09 (0.77 – 1.55, p= 0.613)		0.77 (0.63 – 0.94, p = 0.010)
Right side		1.31 (0.85 – 2.01, p= 0.218)		0.84 (0.53 – 1.32, p= 0.440)		1.32 (0.70 – 2.48, p= 0.390)		1.46 (0.94 -2.28, p=0.092)
Right side in KRAS-wildtype		0.92 (0.49 – 1.74,p= 0.807)		0.53 (0.26 – 1.08,p= 0.081)		0.67 (0.27 – 1.65, p= 0.386)		1.62 (0.90 – 2.92, p= 0.111)
Polychemotherapy		0.48 (0.32 – 0.72,p< 0.0001)		0.70 (0.47 – 1.04, p= 0.075)		0.82 (0.45 – 1.48, p= 0.501)		N/A
Metachronous metastases		1.03 (0.67 – 1.57,p= 0.895)		0.99 (0.65 – 1.49,p= 0.947)		1.15 (0.62 – 2.15,p= 0.654)		0.65 (0.41-1.03, p=0.067)
Inflammatory biomarkers – Multivariable analysis adjusted for polychemotherapy		Adjusted for polychemotherapy		Adjusted for polychemotherapy		Adjusted for polychemotherapy		Adjusted for age and metachronous metastases
NLR (per 1SD increase)		1.30 (1.04 – 1.62,p= 0.021)		1.37 (1.16 – 1.62, p<0.0001)		1.44 (1.01 – 2.05, p=0.042)		1.11 (0.93 – 1.33, p= 0.248)
LMR (per 1SD increase)		0.71 (0.49 – 1.03,p= 0.072)		0.78 (0.59 – 1.03,p= 0.080)		0.71 (0.48 – 1.04,p= 0.076)		0.62 (0.44 – 0.87, p= 0.006)
PLR (per 1SD increase)		1.43 (1.09 – 1.88,p= 0.009)		1.67 (1.34 – 2.09,p< 0.0001)		1.43 (1.04 – 1.98,p= 0.029)		1.18 (0.98 – 1.43, p=0.084)

CRP (per 1SD increase)		1.49 (1.23 – 1.80, p<0.0001)		1.25 (1.06 – 1.47,p= 0.007)		1.09 (0.81 – 1.48, p=0.552)		1.43 (1.15 – 1-79, p= 0.002)
ALI (per 1SD increase)		0.70 (0.51 – 0.95,p= 0.022)		0.78 (0.55 – 1.09,p= 0.139)		0.86 (0.56 – 1.33,p= 0.501)		N/A

Importantly, this was only found when using the NLR as continuous variable. When using the NLR as a dichotomized variable with empirically chosen cut-offs at the 25th or 75th percentile an upper quarter NLR was not significantly associated with an impaired PFS over the first three therapy lines, but with an impaired OS in BSC (Figure 1A-D).

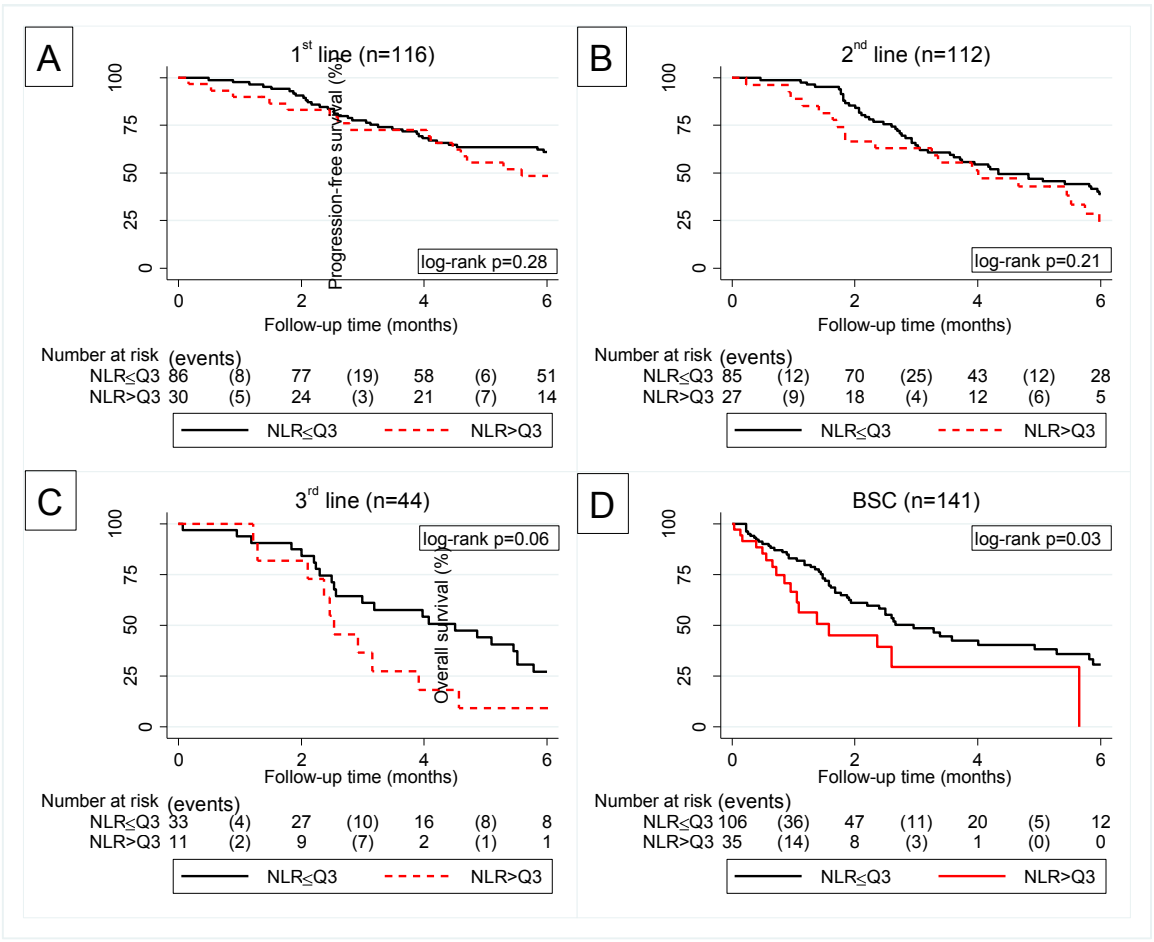

Figure 3.Kaplan Meier curve according to NLR > Q3 vs. NLR ≤ Q3 for progression free survival in 1st (A), 2nd (B) and 3rd (C) line of palliative chemotherapy and overall survival in best supportive care (D).

An elevated LMR showed a weak favourable prognostic association with PFS in all three treatment lines. (Figure 2A-C), and was strongly associated with favourable OS prognosis in BSC (Figure 2D).

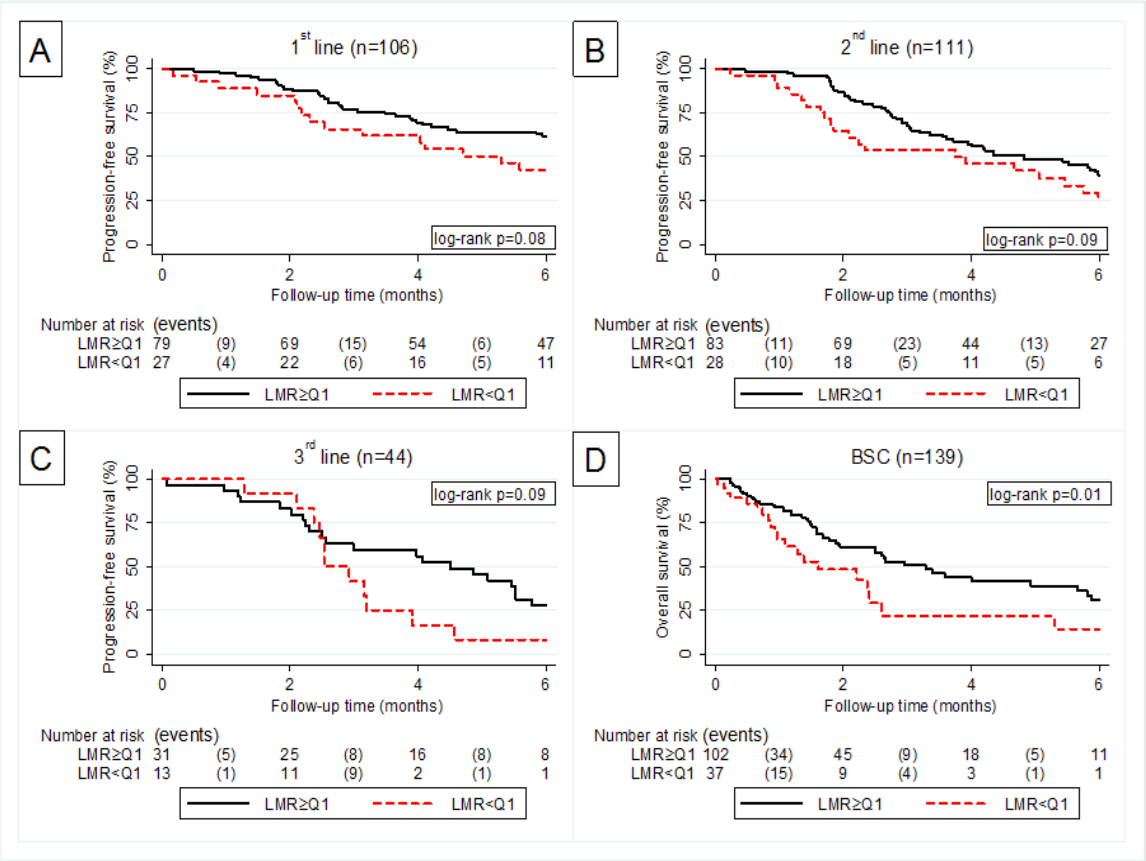

Figure 4. Kaplan Meier curve according to LMR > Q3 vs. LMR ≤ Q3 for progression free survival in 1st (A), 2nd (B) and 3rd (C line of palliative chemotherapy and overall survival in best supportive care (D).

An elevated PLR was a strong predictor for PFS during the first two treatment lines (Figure 3A, 3B). However this prognostic value weakened during third line and as a predictor for OS in BSC (Figure 3C, 3D).

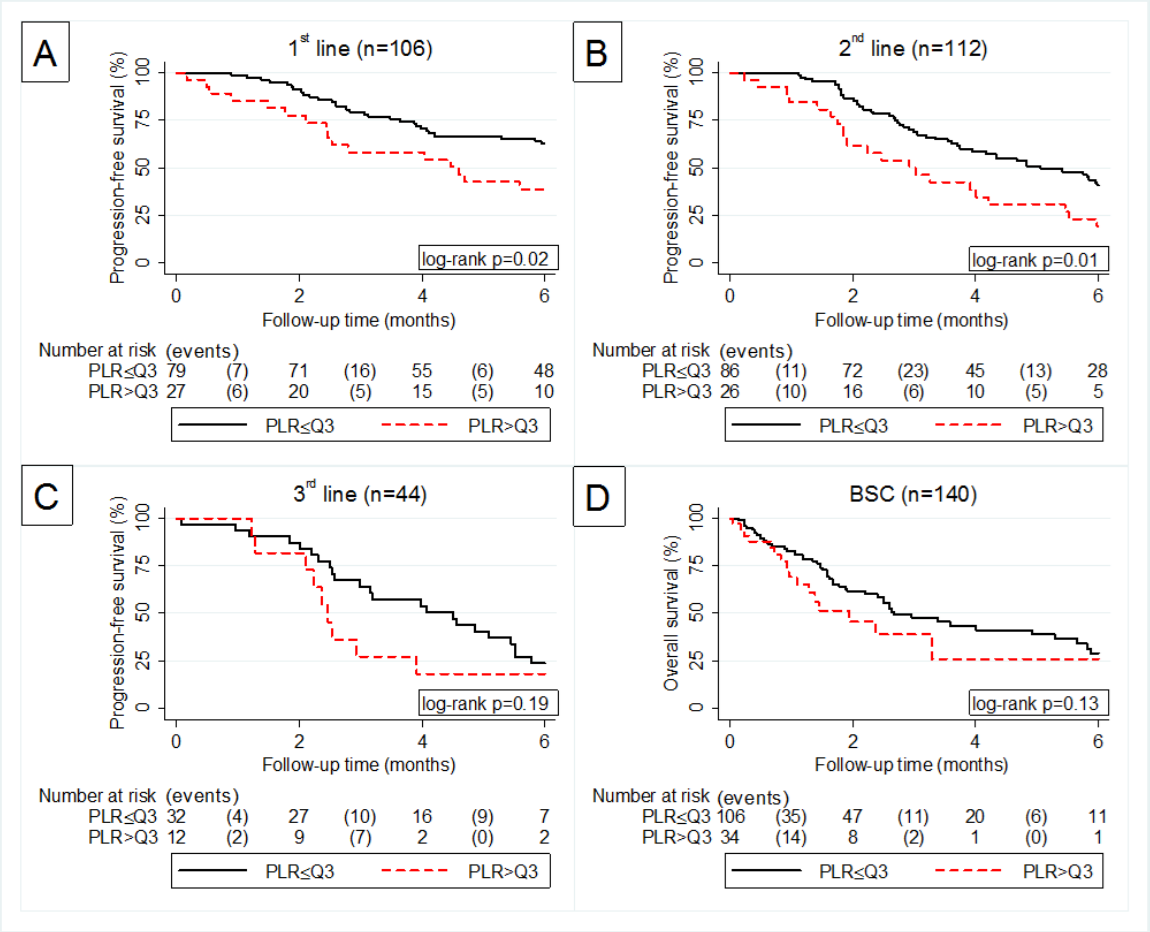

Figure 5. Kaplan Meier curve according to PLR > Q3 vs. PLR ≤ Q3 for progression free survival in 1st (A), 2nd (B) and 3rd (C) line of palliative chemotherapy and overall survival in best supportive care (D).

High CRP was strongly significantly associated with shorter PFS in first and second line and emerged as a predictor for poor OS in BSC (Figure 4A, 4B, 4D). In third line no association between the CRP value and PFS could be shown (Figure 4C).

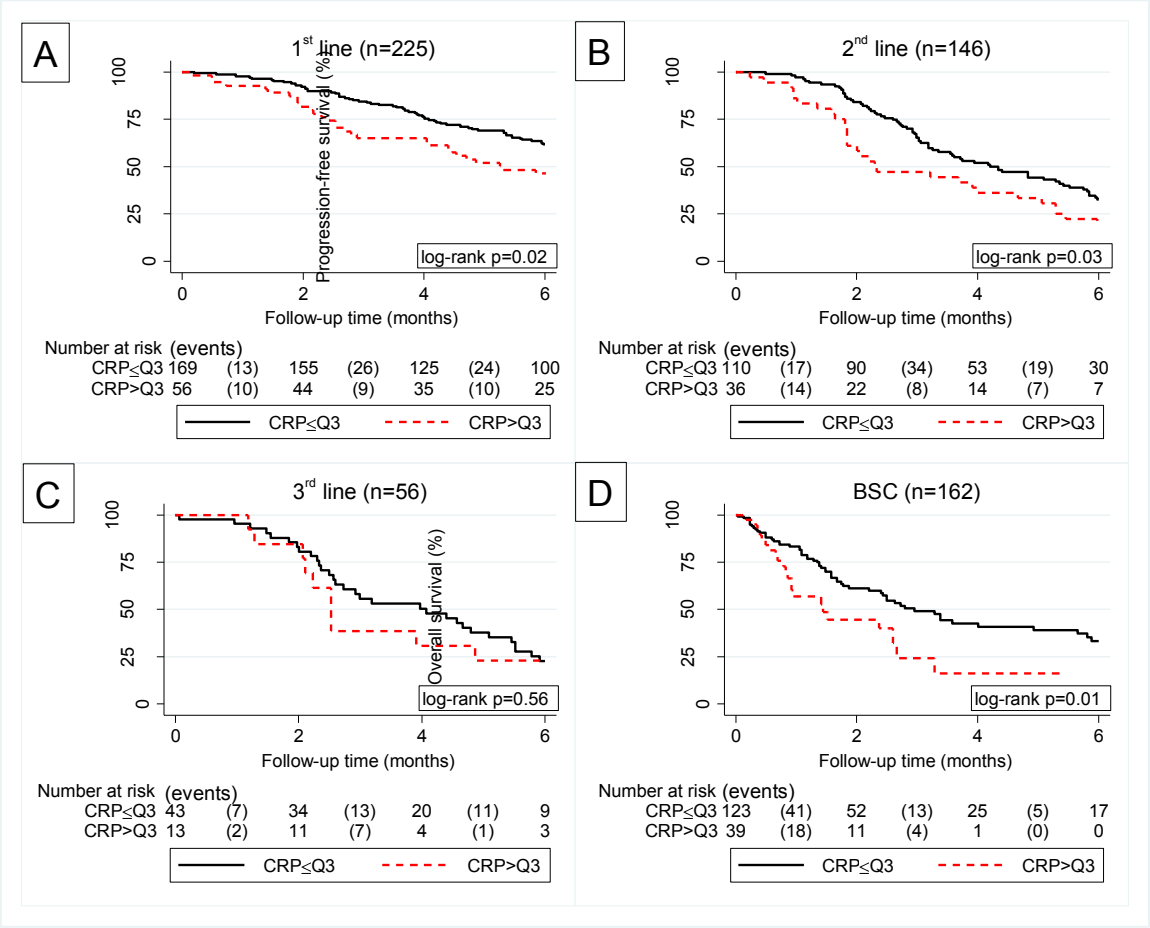

Figure 6. Kaplan Meier curve according to CRP > Q3 vs. CRP ≤ Q3 for progression free survival in 1st (A), 2nd (B) and 3rd (C) line of palliative chemotherapy and overall survival in best supportive care (D).

As the BMI was not recorded for patients entering BSC, the ALI was assessable only for the first three treatment lines. In first line an elevated ALI was significantly associated with prolonged PFS, whereas in second and third line an elevated ALI was only non-significantly in favor of a better PFS experience (Figure 4A-C).

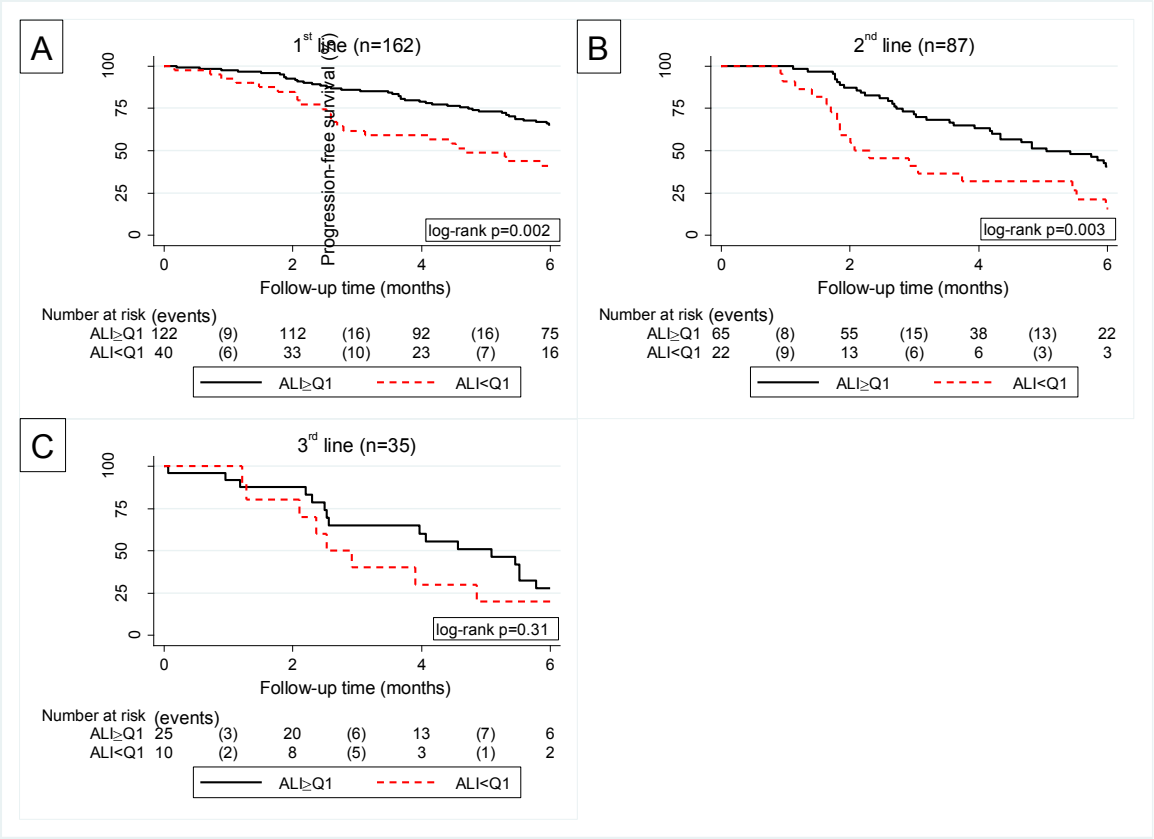

Figure 7. Kaplan Meier curve according to ALI > Q3 vs. ALI ≤ Q3 for progression free survival in 1st (A), 2nd (B) and 3rd (C) line of palliative chemotherapy

Multivariable analysis of 6-month PFS and OS across treatment lines

Besides the inflammatory biomarkers, only chemotherapy (mono- vs. polychemotherapy), but not age, tumor location or metachronous metastasis predicted for outcome (Table 3). Therefore, associations between inflammatory biomarkers and risk of progression or death across the first three treatment lines and BSC were multivariably adjusted for polychemotherapy. In this analysis, associations between inflammatory biomarkers and

outcome became consistently stronger (Table 3). For instance, an elevated NLR was now strongly significantly associated with poor 6-month PFS in all three treatment lines.

Discussion

Multiple studies have shown that inflammatory biomarkers are useful prognostic tools in the first line setting of mCRC. (134) However, the prognostic potential of these biomarkers in further lines of treatment and in the BSC setting of mCRC remains poorly defined. In this retrospective observational cohort study, we demonstrated that markers of systemic inflammation, namely the NLR, LMR, PLR, CRP and ALI retain their prognostic potential across multiple treatment lines in the mCRC setting, and thus appear to be useful outcome predictors beyond the first line. Furthermore, the same biomarkers emerged as predictive biomarkers for treatment response. These results support the use of inflammatory biomarkers as readily available predictors of outcome and therapy response in mCRC patients across treatment lines and during treatment with BSC.

The interaction between inflammation and cancer has noticeably become the focus of cancer research in recent years. (3,4) Strong evidence for the crucial role of inflammation in cancer development is found in colon carcinogenesis. Patients, suffering from chronic bowel disease such as ulcerative colitis have a several times higher risk of developing CRC. (47) It is widely believed that reactive oxygen species built by leucocytes in chronically inflamed tissue induce DNA damage resulting in oncogenesis. In addition, cancer cells themselves release various proinflammatory cytokines to attract leucocytes, which infiltrate the tumor and orchestrate the tumor microenvironment. Those inflammatory cells, in particular tumor associated macrophages, produce a number of different angiogenic and growth stimulating cytokines and chemokines, which induce cancer cell proliferation and foster tumor spread. (4) In 2001, McMillan et al. could show that a high load of systemic inflammation response determined as an elevated CRP level comes along with a poor outcome in patients with advanced cancer. (135) As a consequence over recent years multiple studies have investigated the prognostic validity of various readily available inflammatory biomarkers in different cancer entities. (136–139) In metastatic colorectal cancer elevated levels of Interleukin 6, CRP and the NLR emerged as predictors of impaired disease outcome, whereas high levels of LMR seem to be

associated with prolonged survival. (140–142) However, all of these studies only focused on the first line setting of palliative chemotherapy. In the present study including a large cohort of mCRC patients we observed the prognostic potential of several inflammatory biomarkers over the first three therapy lines and for BSC in mCRC. First we could show that the inflammatory load measured by circulating biomarkers changes during the course of disease. The median NLR for example, which seems to be a good indicator of systemic inflammation response, was slightly higher in patients entering first line, than in those before second and third line. However, it was highly elevated in patients entering BSC. The apparent reduction of the NLR from first to second and third line has to be interpreted critically as only patients who were fit enough received further chemotherapy lines. On the contrary, most patients sooner or later entered BSC, which makes it legit to compare the values of the respective biomarkers before first line and BSC. Here, we observed a strong rise of systemic inflammation burden as indicated by these biomarkers. This supports the hypothesis that inflammation is a major contributor of progression and impaired survival outcome in CRC patients. (143)

Most studies use scores or cut offs determined by receiver operating characteristic (ROC) curve analysis to analyze the association between biomarker and cancer outcome. However, it is not entirely clear which threshold values are most appropriate. For instance Chua et al. who were first to investigate the prognostic and predictive value of the NLR in a large cohort of mCRC patients treated with different types of chemotherapy regimens as first line palliative treatment used a cut off NLR >5 to divide their cohort. Patients with NLR >5 had lower response rates, an increased risk of progression and a worse survival. (142) Another retrospective study by Formica et al., who observed the prognostic and predictive impact of the NLR in mCRC patients treated with FOLFIRI plus Bevacizumab as first line chemotherapy, determined 3.5 as optimal NLR cut off. (144) This diversity hardens a physician's decision which threshold to use in clinical practice. We tried to address this issue by using two different statistical methods. First we calculated the prognostic impact of various inflammatory biomarkers by using them as continuous variables. Here we observed that high levels of NLR, PLR and CRP are not only associated with poor PFS during the first therapy line which is highly consistent with previously reported data but also in later lines of chemotherapy. High LMR and ALI seem to be favourable prognostic markers, but did not reach statistical significance. In the BSC setting an elevated CRP and low LMR emerged to be the most accurate predictors of poor OS.

These data may be helpful for individual risk assessment in mCRC patients and could be used for more accurate patient stratification in clinical trials. Furthermore, our use of continuous and Z-standardized biomarkers may enable other researchers to use our results for biomarker meta-analyses.

When using biomarkers as dichotomized variables (with empirically chosen cut-offs at the 25th or 75th percentile), mainly the same levels of significance could be observed for the respective biomarkers except for the NLR, where an NLR above the 3rd quartile was only non-significantly associated with an impaired disease outcome compared to a NLR in the lower three quartiles during the first three therapy lines. This may be due to the loss of information and power coming along with categorization of continuous variables. (145)

The most important finding of our study was that inflammatory biomarkers do not only appear to be prognostic but also predictive tools. This concept is supported by our treatment response analysis. Here we found that particularly the NLR is a good indicator for therapy response over the first three chemotherapy lines. In detail, after adjusting for polychemotherapy 1 SD increase in NLR was associated with an 8.5% absolute lower ORR in first line, 3% lower ORR in second line and 3.1% lower ORR in third line. These results were highly significant in the first and second chemotherapy line, whereas in third line only a non-significant trend for an elevated NLR and poor therapy response could be observed. However, this lack of statistical significance might be explained by the small sample size of patients entering third line chemotherapy, and should therefore not be interpreted as absence of evidence for an association. Our results are in line with previously published works on the predictive validity of the NLR in the first line setting of palliative chemotherapy. (142) However, to the best of our knowledge we were the first to investigate the predictive role of inflammatory biomarkers in further lines. These data could therefore be of clinical relevance, as they might help oncologists to identify patients who would profit from further treatment, whilst sparing patients with a low predicted benefit from side effects coming along with cytotoxic therapy treatment.

Besides, the NLR right side tumor location in KRAS wildtype patients appeared to be a strong predictor of limited chemotherapy response. In detail, KRAS wildtype patients with right sided tumor location had a 25% lower ORR than those with left sided tumors in the first line of palliative chemotherapy. These results are highly consistent with a recently published retrospective analysis of the CRYSTAL and FIRE 3 trial, where right side

location in RAS wildtype tumors was associated with poor treatment response and disease outcome in mCRC patients. (146)

Yet, there are some limitations that need to be discussed. Firstly, due to its retrospective study design a selection bias in our study cohort cannot be fully excluded. Secondly, we did not assess potential confounding factors such as local or systemic infections, which might have affected the laboratory data collected on the inflammatory biomarkers. However, as the patients were eligible for chemotherapy in routine clinical practice, it is highly unlikely that they were suffering from severe infection at the time of biomarker measurement, which was performed within a timeframe of a maximum of 14 days prior to start of the respective chemotherapy line. Thirdly, we lack an external validation cohort to verify our findings on an independent data sat. Therefore, further studies have to be performed to validate our findings. Moreover, the chemotherapy regimens administered to the patients were heterogenous. However, we aimed to test the prognostic and predictive potential of inflammatory biomarkers not only for a selected cohort receiving polychemotherapy, but for all mCRC patients treated at a Middle-European academic center. According to our opinion this might be more reflective of daily routine clinical practice.

Within the limitations of a retrospective cohort study, we conclude that our data provide strong evidence that inflammatory biomarkers are useful predictors of disease outcome and treatment response over several treatment lines and best supportive care in mCRC patients and merit further validation.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin [Internet]. 2017;67(1):7–30. Available from: http://dx.doi.org/10.3322/caac.21387

2. Riihimäki M, Hemminki A, Sundquist J, Hemminki K. Patterns of metastasis in colon and rectal cancer. Sci Rep [Internet]. The Author(s); 2016 Jul 15;6:29765. Available from: http://dx.doi.org/10.1038/srep29765

3. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. England; 2008 Jul;454(7203):436–44.

4. Coussens LM, Werb Z. Inflammation and cancer. Nature. England; 2002 Dec;420(6917):860–7.

5. Wang H, Wang L, Chi P-D, Wang W, Chen X-Q, Geng Q-R, et al. High level of interleukin-10 in serum predicts poor prognosis in multiple myeloma. Br J Cancer. England; 2016 Feb;114(4):463–8.

6. Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dunlop DJ. Evaluation of cumulative prognostic scores based on the systemic inflammatory response in patients with inoperable non-small-cell lung cancer. Br J Cancer. England; 2003 Sep;89(6):1028–30.

7. Shrotriya S, Walsh D, Bennani-Baiti N, Thomas S, Lorton C. C-Reactive Protein Is an Important Biomarker for Prognosis Tumor Recurrence and Treatment Response in Adult Solid Tumors: A Systematic Review. PLoS One. United States; 2015;10(12):e0143080.

8. Proctor MJ, McMillan DC, Morrison DS, Fletcher CD, Horgan PG, Clarke SJ. A derived neutrophil to lymphocyte ratio predicts survival in patients with cancer. Br J Cancer. England; 2012 Aug;107(4):695–9.

9. Stotz M, Pichler M, Absenger G, Szkandera J, Arminger F, Schaberl-Moser R, et al. The preoperative lymphocyte to monocyte ratio predicts clinical outcome in patients with stage III colon cancer. Br J Cancer. England; 2014 Jan;110(2):435–40.

10. Absenger G, Szkandera J, Pichler M, Stotz M, Arminger F, Weissmueller M, et al. A derived neutrophil to lymphocyte ratio predicts clinical outcome in stage II and III colon cancer patients. Br J Cancer. England; 2013 Jul;109(2):395–400.

11. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. United States; 2015 Mar;65(2):87–108.

12. Haggar FA, Boushey RP, Ph D. Colorectal Cancer Epidemiology : Incidence , Mortality , Survival , and Risk Factors. 2009;6(212):191–7.

13. Statistik Austria. Dickdarm und Enddarm (C18-C21) - Krebsinzidenz (Neuerkrankungen pro Jahr), Österreich ab 1983. 2016. p. 2016.

14. Howlader ,N; Noone ,AM; Krapcho ,M; Miller ,D; Bishop ,K; Kosary ,CL; Yu ,M; Ruhl, J; Tatalovich, Z; Mariotto, A; Lewis, DR; Chen HFECK (eds). SEER Cancer Statistics Review, 1975-2014, National Cancer Institute. Bethesda, MD, [Internet].

Available from: https://seer.cancer.gov/csr/1975_2014/

15. Statistik Austria. Dickdarm und Enddarm (C18-C21) - Krebsmortalität (Sterbefälle pro Jahr), Österreich ab 1983. 2016;2016.

16. Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and Familial Colon Cancer. Gastroenterology [Internet]. 2010 Jun;138(6):2044–58. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3057468/

17. Tuohy TMF, Rowe KG, Mineau GP, Pimentel R, Burt RW, Samadder NJ. Risk of colorectal cancer and adenomas in the families of patients with adenomas: a population-based study in Utah. Cancer. United States; 2014 Jan;120(1):35–42.

18. Fearon EF, Vogelstein B. for Colorectal Tumorigenesis. 1990;61:759–67.

19. Takayama T, Ohi M, Hayashi T, Miyanishi K, Nobuoka A, Nakajima T, et al. Analysis of K-ras, APC, and beta-catenin in aberrant crypt foci in sporadic adenoma, cancer, and familial adenomatous polyposis. Gastroenterology. United States; 2001 Sep;121(3):599–611.

20. Cartwright C. Intestinal cell growth control: role of Src tyrosine kinases. Vol. 114, Gastroenterology. United States; 1998. p. 1335–8.

21. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. United States; 1988 Sep;319(9):525–32.

22. Young J, Jenkins M, Parry S, Young B, Nancarrow D, English D, et al. Serrated pathway colorectal cancer in the population: genetic consideration. Gut. England; 2007 Oct;56(10):1453–9.

23. Murphy G, Devesa SS, Cross AJ, Inskip PD, Katherine A, Cook MB. NIH Public Access. 2011;128(7):1668–75.

24. Parkin DM, Boyd L, Walker LC. The fraction of cancer attributable to lifestyle and environmental factors in the UK in 2010 Summary and conclusions. Br J Cancer [Internet]. Nature Publishing Group; 2011;105(S2):S77–81. Available from: http://dx.doi.org/10.1038/bjc.2011.489

25. Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, et al. Meta-analyses of colorectal cancer risk factors. Cancer Causes Control. Netherlands; 2013 Jun;24(6):1207–22.

26. Dai Z, Xu Y-C, Niu L. Obesity and colorectal cancer risk: a meta-analysis of cohort studies. World J Gastroenterol. United States; 2007 Aug;13(31):4199–206.

27. Chao A, Thun MJ, Connell CJ, McCullough ML, Jacobs EJ, Flanders WD, et al. Meat consumption and risk of colorectal cancer. JAMA. United States; 2005 Jan;293(2):172–82.

28. Peters U, Sinha R, Chatterjee N, Subar AF, Ziegler RG, Kulldorff M, et al. Dietary fibre and colorectal adenoma in a colorectal cancer early detection programme. Lancet (London, England). England; 2003 May;361(9368):1491–5.

29. Terry P, Giovannucci E, Michels KB, Bergkvist L, Hansen H, Holmberg L, et al. Fruit, vegetables, dietary fiber, and risk of colorectal cancer. J Natl Cancer Inst. United States; 2001 Apr;93(7):525–33.

30. Lee JE, Willett WC, Fuchs CS, Smith-Warner SA, Wu K, Ma J, et al. Folate intake and risk of colorectal cancer and adenoma: modification by time. Am J Clin Nutr. United States; 2011 Apr;93(4):817–25.

31. Larsson SC, Orsini N, Wolk A. Vitamin B6 and risk of colorectal cancer: a meta-analysis of prospective studies. JAMA. United States; 2010 Mar;303(11):1077–83.

32. Weingarten MA, Zalmanovici A, Yaphe J. Dietary calcium supplementation for preventing colorectal cancer and adenomatous polyps. Cochrane database Syst Rev. England; 2004;(1):CD003548.

33. Larsson SC, Bergkvist L, Wolk A. Magnesium intake in relation to risk of colorectal cancer in women. JAMA. United States; 2005 Jan;293(1):86–9.

34. Ngo SNT, Williams DB, Cobiac L, Head RJ. Does garlic reduce risk of colorectal cancer? A systematic review. J Nutr. United States; 2007 Oct;137(10):2264–9.

35. Wu S, Feng B, Li K, Zhu X, Liang S, Liu X, et al. Fish consumption and colorectal cancer risk in humans: a systematic review and meta-analysis. Am J Med. United States; 2012 Jun;125(6):551–9.e5.

36. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and colorectal cancer: a meta-analysis. JAMA. United States; 2008 Dec;300(23):2765–78.

37. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, et al. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. Ann Oncol Off J Eur Soc Med Oncol. England; 2011 Sep;22(9):1958–72.

38. Kune GA, Vitetta L. Alcohol consumption and the etiology of colorectal cancer: A review of the scientific evidence from 1957 to 1991. Nutr Cancer [Internet]. Routledge; 1992 Jan 1;18(2):97–111. Available from: http://dx.doi.org/10.1080/01635589209514210

39. Harnack L, Jacobs DRJ, Nicodemus K, Lazovich D, Anderson K, Folsom AR. Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. Nutr Cancer. United States; 2002;43(2):152–8.

40. Kyu HH, Bachman VF, Alexander LT, Mumford JE, Afshin A, Estep K, et al. Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose-response meta-analysis for the Global Burden of Disease Study 2013. BMJ. England; 2016 Aug;354:i3857.

41. Michaud DS, Giovannucci E, Willett WC, Colditz GA, Stampfer MJ, Fuchs CS. Physical activity, obesity, height, and the risk of pancreatic cancer. JAMA. United States; 2001 Aug;286(8):921–9.

42. Moore SC, Lee I, Weiderpass E, Campbell PT, Sampson JN, Kitahara CM, et al.

Association of Leisure-Time Physical Activity With Risk of 26 Types of Cancer in 1.44 Million Adults. 2016;20850:1–10.

43. Wolin KY, Yan Y, Colditz GA, Lee I-M. Physical activity and colon cancer prevention: a meta-analysis. Br J Cancer. England; 2009 Feb;100(4):611–6.

44. Ballard-Barbash R, Friedenreich CM, Courneya KS, Siddiqi SM, McTiernan A, Alfano CM. Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review. J Natl Cancer Inst. United States; 2012 Jun;104(11):815–40.

45. Dulai PS, Singh S, Marquez E, Khera R, Prokop LJ, Limburg PJ, et al. Chemoprevention of colorectal cancer in individuals with previous colorectal neoplasia: systematic review and network meta-analysis. BMJ [Internet]. 2016 Dec 5;355. Available from: http://www.bmj.com/content/355/bmj.i6188.abstract

46. Cooper K, Squires H, Carroll C, Papaioannou D, Booth A, Logan RF, et al. Chemoprevention of colorectal cancer: systematic review and economic evaluation. Health Technol Assess. England; 2010 Jun;14(32):1–206.

47. Ekbom A, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer. A population-based study. N Engl J Med. United States; 1990 Nov;323(18):1228–33.

48. Gillen CD, Walmsley RS, Prior P, Andrews HA, Allan RN. Ulcerative colitis and Crohn's disease: a comparison of the colorectal cancer risk in extensive colitis. Gut. England; 1994 Nov;35(11):1590–2.

49. Binder V, Hendriksen C, Kreiner S. Prognosis in Crohn's disease--based on results from a regional patient group from the county of Copenhagen. Gut. England; 1985 Feb;26(2):146–50.

50. Rembacken BJ, Fujii T, Cairns A, Dixon MF, Yoshida S, Chalmers DM, et al. Flat and depressed colonic neoplasms: a prospective study of 1000 colonoscopies in the UK. Lancet (London, England). England; 2000 Apr;355(9211):1211–4.

51. Odze RD. Pathology of dysplasia and cancer in inflammatory bowel disease. Gastroenterol Clin North Am. United States; 2006 Sep;35(3):533–52.

52. Giovannucci E. Insulin and colon cancer. Cancer Causes Control. Netherlands; 1995 Mar;6(2):164–79.

53. Yuhara H, Steinmaus C, Cohen SE, Corley DA, Tei Y, Buffler PA. Is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? Am J Gastroenterol. United States; 2011 Nov;106(11):1911–21; quiz 1922.

54. Dehal AN, Newton CC, Jacobs EJ, Patel A V, Gapstur SM, Campbell PT. Impact of diabetes mellitus and insulin use on survival after colorectal cancer diagnosis: the Cancer Prevention Study-II Nutrition Cohort. J Clin Oncol. United States; 2012 Jan;30(1):53–9.

55. Henderson TO, Oeffinger KC, Whitton J, Leisenring W, Neglia J, Meadows A, et al. Secondary gastrointestinal cancer in childhood cancer survivors: a cohort study. Ann Intern Med. United States; 2012 Jun;156(11):757–66, NaN-260.

56. Boland CR, Goel A. Microsatellite Instability in Colorectal Cancer. Vol. 138, Gastroenterology. 2010. p. 2073–2087.e3.

57. Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la Chapelle A, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J cancer. United States; 1999 Apr;81(2):214–8.

58. Hampel H, Stephens JA, Pukkala E, Sankila R, Aaltonen LA, Mecklin J-P, et al. Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology. United States; 2005 Aug;129(2):415–21.

59. Vasen HF, Stormorken A, Menko FH, Nagengast FM, Kleibeuker JH, Griffioen G, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol. United States; 2001 Oct;19(20):4074–80.

60. Barrow E, Robinson L, Alduaij W, Shenton A, Clancy T, Lalloo F, et al. Cumulative lifetime incidence of extracolonic cancers in Lynch syndrome: a report of 121 families with proven mutations. Clin Genet. Denmark; 2009 Feb;75(2):141–9.

61. Ponti G, Ponz de Leon M. Muir-Torre syndrome. Lancet Oncol. England; 2005 Dec;6(12):980–7.

62. Hamilton SR, Liu B, Parsons RE, Papadopoulos N, Jen J, Powell SM, et al. The molecular basis of Turcot's syndrome. N Engl J Med. United States; 1995 Mar;332(13):839–47.

63. American Gastroenterological Association medical position statement: hereditary colorectal cancer and genetic testing. Gastroenterology. United States; 2001 Jul;121(1):195–7.

64. Galiatsatos P, Foulkes WD. Familial adenomatous polyposis. Am J Gastroenterol. United States; 2006 Feb;101(2):385–98.

65. Hagen CE, Setia N, Lauwers GY. Familial adenomatous polyposis: a review of gastrointestinal manifestations. Diagnostic Histopathol [Internet]. Elsevier; 2016 Nov 25;21(4):152–60. Available from: http://dx.doi.org/10.1016/j.mpdhp.2015.04.005

66. Jasperson KW, Burt RW. APC-Associated Polyposis Conditions. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al., editors. Seattle (WA); 1993.

67. Glynne-Jones R, Wyrwicz L, Tiret E, Brown G, Rodel C, Cervantes A, et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol Off J Eur Soc Med Oncol. England; 2017 Jul;28(suppl_4):iv22-iv40.

68. Böcker W et al. Pathologie. 5th ed. München; 2012. 610-617 p.

69. Green JB, Timmcke AE, Mitchell WT, Hicks TC, Gathright JBJ, Ray JE. Mucinous carcinoma--just another colon cancer? Dis Colon Rectum. United States; 1993 Jan;36(1):49–54.

70. Secco GB, Fardelli R, Campora E, Lapertosa G, Gentile R, Zoli S, et al. Primary mucinous adenocarcinomas and signet-ring cell carcinomas of colon and rectum. Oncology. Switzerland; 1994;51(1):30–4.

71. Psathakis D, Schiedeck TH, Krug F, Oevermann E, Kujath P, Bruch HP. Ordinary colorectal adenocarcinoma vs. primary colorectal signet-ring cell carcinoma: study matched for age, gender, grade, and stage. Dis Colon Rectum. United States; 1999 Dec;42(12):1618–25.

72. Van Cutsem E, Nordlinger B, Cervantes A. Advanced colorectal cancer: ESMO Clinical Practice Guidelines for treatment. Ann Oncol Off J Eur Soc Med Oncol. England; 2010 May;21 Suppl 5:v93-7.

73. Steup WH, Moriya Y, van de Velde CJH. Patterns of lymphatic spread in rectal cancer. A topographical analysis on lymph node metastases. Eur J Cancer. England; 2002 May;38(7):911–8.

74. Schünke M, Schulte E, Schumacher U. Prometheus Innere Organe - LernAtlas der Anatomie. 4th ed. Stuttgart; New York: Thieme; 2015. 276-278 p.

75. Rose LJ. Colon Cancer Staging: TNM Classification for Colon Cancer [Internet]. [cited 2017 Oct 12]. Available from: http://emedicine.medscape.com/article/2006674-overview

76. Moreno CC, Mittal PK, Sullivan PS, Rutherford R, Staley CA, Cardona K, et al. Colorectal Cancer Initial Diagnosis: Screening Colonoscopy, Diagnostic Colonoscopy, or Emergent Surgery, and Tumor Stage and Size at Initial Presentation. Clin Colorectal Cancer. United States; 2016 Mar;15(1):67–73.

77. Hamilton W, Round A, Sharp D, Peters TJ. Clinical features of colorectal cancer before diagnosis: a population-based case-control study. Br J Cancer. England; 2005 Aug;93(4):399–405.

78. Majumdar SR, Fletcher RH, Evans AT. How does colorectal cancer present? Symptoms, duration, and clues to location. Am J Gastroenterol. United States; 1999 Oct;94(10):3039–45.

79. Saidi HS, Karuri D, Nyaim EO. Correlation of clinical data, anatomical site and disease stage in colorectal cancer. East Afr Med J. Kenya; 2008 Jun;85(6):259–62.

80. Smothers L, Hynan L, Fleming J, Turnage R, Simmang C, Anthony T. Emergency surgery for colon carcinoma. Dis Colon Rectum. United States; 2003 Jan;46(1):24–30.

81. Anderson JH, Hole D, McArdle CS. Elective versus emergency surgery for patients with colorectal cancer. Br J Surg [Internet]. John Wiley & Sons, Ltd.; 1992;79(7):706–9. Available from: http://dx.doi.org/10.1002/bjs.1800790739

82. Griffin MR, Bergstralh EJ, Coffey RJ, Beart RWJ, Melton LJ 3rd. Predictors of survival after curative resection of carcinoma of the colon and rectum. Cancer. United States; 1987 Nov;60(9):2318–24.

83. Church TR, Ederer F, Mandel JS. Fecal occult blood screening in the Minnesota study: sensitivity of the screening test. J Natl Cancer Inst. United States; 1997

Oct;89(19):1440–8.

84. Lieberman DA, Weiss DG. One-time screening for colorectal cancer with combined fecal occult-blood testing and examination of the distal colon. N Engl J Med. United States; 2001 Aug;345(8):555–60.

85. Gatto NM, Frucht H, Sundararajan V, Jacobson JS, Grann VR, Neugut AI. Risk of perforation after colonoscopy and sigmoidoscopy: a population-based study. J Natl Cancer Inst. United States; 2003 Feb;95(3):230–6.

86. Rex DK, Rahmani EY, Haseman JH, Lemmel GT, Kaster S, Buckley JS. Relative sensitivity of colonoscopy and barium enema for detection of colorectal cancer in clinical practice. Gastroenterology. United States; 1997 Jan;112(1):17–23.

87. Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, van Ballegooijen M, Hankey BF, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. N Engl J Med. United States; 2012 Feb;366(8):687–96.

88. Chukmaitov A, Bradley CJ, Dahman B, Siangphoe U, Warren JL, Klabunde CN. Association of polypectomy techniques, endoscopist volume, and facility type with colonoscopy complications. Gastrointest Endosc. United States; 2013 Mar;77(3):436–46.

89. Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clin Chem. United States; 2001 Apr;47(4):624–30.

90. Liu Z, Zhang Y, Niu Y, Li K, Liu X, Chen H, et al. A systematic review and meta-analysis of diagnostic and prognostic serum biomarkers of colorectal cancer. PLoS One. United States; 2014;9(8):e103910.

91. Whiteford MH, Whiteford HM, Yee LF, Ogunbiyi OA, Dehdashti F, Siegel BA, et al. Usefulness of FDG-PET scan in the assessment of suspected metastatic or recurrent adenocarcinoma of the colon and rectum. Dis Colon Rectum. United States; 2000 Jun;43(6):759–70.

92. Hofheinz R-D, Arnold D, Borner M, Folprecht G, Graeven U, Hebart, Holger Hegewisch-Becker S, et al. Deutsche Gesellschaft für Hämatologie und Onkologie - Onkopedia Kolonkarzinom Leitlinie. August 2017 [Internet]. Available from: https://www.onkopedia.com/de/onkopedia/guidelines/kolonkarzinom

93. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, Deutsche Krebshilfe A. S3-Leitlinie Kolorektales Karzinom, Langversion 1.0, AWMF Registrierungsnummer: 021-007OL [Internet]. 2013. p. 45–78. Available from: http://leitlinienprogrammonkologie.de/Leitlinien.7.0.html

94. Bonjer HJ, Hop WCJ, Nelson H, Sargent DJ, Lacy AM, Castells A, et al. Laparoscopically assisted vs open colectomy for colon cancer: a meta-analysis. Arch Surg. United States; 2007 Mar;142(3):298–303.

95. Herold G. Innere Medizin. Vol. 96, Innere Medizin. 2015. 488-493 p.

96. Reiser M, Kuhn F-P, Debus J. Duale Reihe Radiologie. 4th ed. Stuttgart; New York: Thieme; 2017. 107-127 p.

97. Sauer R, Becker H, Hohenberger W, Rodel C, Wittekind C, Fietkau R, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med. United States; 2004 Oct;351(17):1731–40.

98. Sobrero AF, Aschele C, Bertino JR. Fluorouracil in colorectal cancer--a tale of two drugs: implications for biochemical modulation. J Clin Oncol. United States; 1997 Jan;15(1):368–81.

99. Thirion P, Michiels S, Pignon JP, Buyse M, Braud AC, Carlson RW, et al. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: an updated meta-analysis. J Clin Oncol. United States; 2004 Sep;22(18):3766–75.

100. de Gramont A, Bosset JF, Milan C, Rougier P, Bouche O, Etienne PL, et al. Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer: a French intergroup study. J Clin Oncol. United States; 1997 Feb;15(2):808–15.

101. Hoff PM, Ansari R, Batist G, Cox J, Kocha W, Kuperminc M, et al. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. J Clin Oncol. United States; 2001 Apr;19(8):2282–92.

102. Twelves C, Wong A, Nowacki MP, Abt M, Burris H 3rd, Carrato A, et al. Capecitabine as adjuvant treatment for stage III colon cancer. N Engl J Med. United States; 2005 Jun;352(26):2696–704.

103. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol. United States; 2000 Aug;18(16):2938–47.

104. Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med. United States; 2004 Jun;350(23):2343–51.

105. Cunningham D, Pyrhonen S, James RD, Punt CJ, Hickish TF, Heikkila R, et al. Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. Lancet (London, England). England; 1998 Oct;352(9138):1413–8.

106. Gerber DE. Targeted therapies: a new generation of cancer treatments. Am Fam Physician. United States; 2008 Feb;77(3):311–9.

107. Mayer RJ. Two steps forward in the treatment of colorectal cancer. Vol. 350, The New England journal of medicine. United States; 2004. p. 2406–8.

108. Hurwitz HI, Tebbutt NC, Kabbinavar F, Giantonio BJ, Guan Z-Z, Mitchell L, et al. Efficacy and safety of bevacizumab in metastatic colorectal cancer: pooled analysis from seven randomized controlled trials. Oncologist. United States; 2013;18(9):1004–12.

109. Allegra CJ, Yothers G, O'Connell MJ, Sharif S, Petrelli NJ, Lopa SH, et al. Bevacizumab in stage II-III colon cancer: 5-year update of the National Surgical

Adjuvant Breast and Bowel Project C-08 trial. J Clin Oncol. United States; 2013 Jan;31(3):359–64.

110. Van Cutsem E, Kohne C-H, Hitre E, Zaluski J, Chang Chien C-R, Makhson A, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med. United States; 2009 Apr;360(14):1408–17.

111. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. England; 2010 Aug;11(8):753–62.

112. Alberts SR, Sargent DJ, Nair S, Mahoney MR, Mooney M, Thibodeau SN, et al. Effect of oxaliplatin, fluorouracil, and leucovorin with or without cetuximab on survival among patients with resected stage III colon cancer: a randomized trial. JAMA. United States; 2012 Apr;307(13):1383–93.

113. Price TJ, Peeters M, Kim TW, Li J, Cascinu S, Ruff P, et al. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study. Lancet Oncol. England; 2014 May;15(6):569–79.

114. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet (London, England). England; 2013 Jan;381(9863):303–12.

115. Mayer RJ, Van Cutsem E, Falcone A, Yoshino T, Garcia-Carbonero R, Mizunuma N, et al. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. N Engl J Med. United States; 2015 May;372(20):1909–19.

116. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, Deutsche Krebshilfe A. S3-Leitlinie Kolorektales Karzinom, Langversion 1.0, AWMF Registrierungsnummer: 021-007OL [Internet]. 2013. p. 131–46, 181–98. Available from: http://leitlinienprogrammonkologie.de/Leitlinien.7.0.html

117. Gray R, Barnwell J, McConkey C, Hills RK, Williams NS, Kerr DJ. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet (London, England). England; 2007 Dec;370(9604):2020–9.

118. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med. United States; 2003 Jul;349(3):247–57.

119. Haller DG, Tabernero J, Maroun J, de Braud F, Price T, Van Cutsem E, et al. Capecitabine plus oxaliplatin compared with fluorouracil and folinic acid as adjuvant therapy for stage III colon cancer. J Clin Oncol. United States; 2011 Apr;29(11):1465–71.

120. Abdalla EK, Adam R, Bilchik AJ, Jaeck D, Vauthey J-N, Mahvi D. Improving resectability of hepatic colorectal metastases: expert consensus statement. Ann Surg

Oncol. United States; 2006 Oct;13(10):1271–80.

121. Tomlinson JS, Jarnagin WR, DeMatteo RP, Fong Y, Kornprat P, Gonen M, et al. Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol. United States; 2007 Oct;25(29):4575–80.

122. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, et al. Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): long-term results of a randomised, controlled, phase 3 trial. Lancet Oncol. England; 2013 Nov;14(12):1208–15.

123. Stintzing S, Modest DP, Rossius L, Lerch MM, von Weikersthal LF, Decker T, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab for metastatic colorectal cancer (FIRE-3): a post-hoc analysis of tumour dynamics in the final RAS wild-type subgroup of this randomised open-label phase 3 trial. Lancet Oncol. England; 2016 Oct;17(10):1426–34.

124. Majek O, Gondos A, Jansen L, Emrich K, Holleczek B, Katalinic A, et al. Survival from colorectal cancer in Germany in the early 21st century. Br J Cancer [Internet]. Cancer Research UK; 2012 May 22;106(11):1875–80. Available from: http://dx.doi.org/10.1038/bjc.2012.189

125. Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer. England; 2003 Jan;3(1):11–22.

126. Markowitz SD, Bertagnolli MM. Molecular Basis of Colorectal Cancer. N Engl J Med [Internet]. Massachusetts Medical Society; 2009 Dec 17;361(25):2449–60. Available from: http://dx.doi.org/10.1056/NEJMra0804588

127. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. United States; 2008 Apr;26(10):1626–34.

128. Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. United States; 2008 Oct;359(17):1757–65.

129. Douillard J-Y, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med. United States; 2013 Sep;369(11):1023–34.

130. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature [Internet]. 2002 Jun 27;417(6892):949–54. Available from: http://dx.doi.org/10.1038/nature00766

131. Samowitz WS, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res. United States; 2005 Jul;65(14):6063–9.

132. Pietrantonio F, Petrelli F, Coinu A, Di Bartolomeo M, Borgonovo K, Maggi C, et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. Eur J Cancer. England; 2015 Mar;51(5):587–94.

133. Tejpar S, Stintzing S, Ciardiello F, al et. Prognostic and predictive relevance of primary tumor location in patients with ras wild-type metastatic colorectal cancer: Retrospective analyses of the crystal and fire-3 trials. JAMA Oncol [Internet]. 2017 Feb 1;3(2):194–201. Available from: http://dx.doi.org/10.1001/jamaoncol.2016.3797

134. Ishizuka M, Nagata H, Takagi K, Kubota K. Influence of inflammation-based prognostic score on mortality of patients undergoing chemotherapy for far advanced or recurrent unresectable colorectal cancer. Ann Surg. United States; 2009 Aug;250(2):268–72.

135. McMillan DC, Elahi MM, Sattar N, Angerson WJ, Johnstone J, McArdle CS. Measurement of the systemic inflammatory response predicts cancer-specific and non-cancer survival in patients with cancer. Nutr Cancer. United States; 2001;41(1–2):64–9.

136. Richtig G, Pichler M. Prediction of Response in Melanoma Therapy by Systemic Inflammatory Response - One Size Fits Not All. EBioMedicine. Netherlands; 2017 Apr;18:13–4.

137. Dalpiaz O, Luef T, Seles M, Stotz M, Stojakovic T, Pummer K, et al. Critical evaluation of the potential prognostic value of the pretreatment-derived neutrophil-lymphocyte ratio under consideration of C-reactive protein levels in clear cell renal cell carcinoma. Br J Cancer. England; 2017 Jan;116(1):85–90.

138. Stotz M, Szkandera J, Stojakovic T, Seidel J, Samonigg H, Kornprat P, et al. The lymphocyte to monocyte ratio in peripheral blood represents a novel prognostic marker in patients with pancreatic cancer. Clin Chem Lab Med. Germany; 2015 Feb;53(3):499–506.

139. Stotz M, Liegl-Atzwanger B, Posch F, Mrsic E, Thalhammer M, Stojakovic T, et al. Blood-Based Biomarkers Are Associated with Disease Recurrence and Survival in Gastrointestinal Stroma Tumor Patients after Surgical Resection. PLoS One. United States; 2016;11(7):e0159448.

140. Thomsen M, Kersten C, Sorbye H, Skovlund E, Glimelius B, Pfeiffer P, et al. Interleukin-6 and C-reactive protein as prognostic biomarkers in metastatic colorectal cancer. Oncotarget. United States; 2016 Nov;7(46):75013–22.

141. Shibutani M, Maeda K, Nagahara H, Ohtani H, Sakurai K, Yamazoe S, et al. Prognostic significance of the lymphocyte-to-monocyte ratio in patients with metastatic colorectal cancer. World J Gastroenterol. United States; 2015 Sep;21(34):9966–73.

142. Chua W, Charles KA, Baracos VE, Clarke SJ. Neutrophil/lymphocyte ratio predicts chemotherapy outcomes in patients with advanced colorectal cancer. Br J Cancer. England; 2011 Apr;104(8):1288–95.

143. Ghuman S, Van Hemelrijck M, Garmo H, Holmberg L, Malmstrom H, Lambe M, et al. Serum inflammatory markers and colorectal cancer risk and survival. Br J Cancer. England; 2017 May;116(10):1358–65.

144. Formica V, Luccchetti J, Cunningham D, Smyth EC, Ferroni P, Nardecchia A, et al.

Systemic inflammation, as measured by the neutrophil/lymphocyte ratio, may have differential prognostic impact before and during treatment with fluorouracil, irinotecan and bevacizumab in metastatic colorectal cancer patients. Med Oncol. United States; 2014 Sep;31(9):166.

145. Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. Stat Med [Internet]. John Wiley & Sons, Ltd.; 2006;25(1):127–41. Available from: http://dx.doi.org/10.1002/sim.2331

146. Tejpar S, Stintzing S, Ciardiello F, Tabernero J, Van Cutsem E, Beier F, et al. Prognostic and Predictive Relevance of Primary Tumor Location in Patients With RAS Wild-Type Metastatic Colorectal Cancer: Retrospective Analyses of the CRYSTAL and FIRE-3 Trials. JAMA Oncol. United States; 2016 Oct;