Targets in the microenvironment of rectal cancer

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Rectal and colon cancer: Not just a different anatomic site

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Abstract

Due to differences in anatomy, primary rectal and colon cancer require different staging procedures, different neo-adjuvant treatment and different surgical approaches. For example, neoadjuvant radiotherapy or chemoradiotherapy is administered solely for rectal cancer. Neodjuvant therapy and total mesorectal excision for rectal cancer might be responsible in part for the differing effect of adjuvant systemic treatment on overall survival, which is more evident in colon cancer than in rectal cancer. Apart from anatomic divergences, rectal and colon cancer also differ in their embryological origin and metastatic patterns. Moreover, they harbor a different composition of drug targets, such as v-raf murine sarcoma viral oncogene homolog B (BRAF), which is preferentially mutated in proximal colon cancers, and the epidermal growth factor receptor (EGFR), which is prevalently amplified or overexpressed in distal colorectal cancers. Despite their differences in metastatic pattern, composition of drug targets and earlier local treatment, metastatic rectal and colon cancer are, however, commonly regarded as one entity and are treated alike.

In this review, we focused on rectal cancer and its biological and clinical differences and similarities relative to colon cancer. These aspects are crucial because they influence the current staging and treatment of these cancers, and might influence the design of future trials with targeted drugs.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer globally, accounting for 10.0% of the estimated 14.1 million new cancer cases registered in 2012 [1]. Moreover, it is the third leading causes of cancer-related death in women and the fourth in men, with more than 693,600 deaths occurring worldwide in 2012. About one-third of CRCs are rectal cancers, which in 2008 corresponded to approximately 450,000 new cases worldwide.

Several biological and clinical hallmarks indicate that rectal cancer is different from colon cancer. The rectum and colon have a different embryological origin, anatomy and function [2-4]. Consequently, the treatments for primary rectal and colon cancer are different. Primary rectal cancer requires specific surgical treatment: total mesorectal excision (TME), preceded by neoadjuvant radiotherapy or chemoradiotherapy [5, 6]. This reduces the risk of local recurrence, but does not improve survival compared to surgery alone [7]. Adjuvant systemic chemotherapy following curative surgery improves survival of lymph node-positive (stage III) colon cancer patients [8, 9]. At present, fluoropyrimidine-based adjuvant chemotherapy is also recommended in stage II-III rectal cancer by the European Society for Medical Oncology (ESMO) and the National Comprehensive Cancer Network (NCCN) guidelines [10, 11]. In case of rectal cancer, however, the maximal overall survival benefit at 10 years is only 3.4%. Presently, the divergent treatment for localized rectal and colon cancer is not accompanied by therapy differences in the metastatic setting. Metastasized rectal and colon cancer are commonly regarded as one entity and treated alike [11-13].

Despite a substantial rise in survival over the last two decades, the 5-year disease-specific overall survival rate is approximately 59% for colon cancer and 61% for rectal cancer [14]. This indicates that there is still much room for improvement. In this review, we have summarized the reported differences and similarities in rectal and colon cancer biology as

well as the differences and similarities in their clinical behavior. This could provide further guidance for the design of novel clinical approaches.

Search strategy

PubMed and Google Scholar were searched for research articles, reviews and meta-analyses published in English up to May 2015. We used the following search terms: "rectal cancer", "colon cancer", "epidemiology", "histology", "gene", "(neo)adjuvant treatment", "metastasis", "targeted drugs", and "tumor microenvironment", in various combinations. We also consulted current ESMO [10, 13, 15], Dutch [16, 17] and NCCN [11, 12] Clinical Practice Guidelines for rectal and colon cancer, and the registry for clinical studies of the ClinicalTrials.gov site.

Epidemiology and lifestyle risk factors for sporadic colorectal cancer

According to the 2011 National Statistics of Cancer Incidence in the United Kingdom, approximately 31% of CRCs occur in the proximal colon and 25% in the distal colon, as divided at the splenic flexure, whereas approximately 34% are rectal and rectosigmoid junction tumors [18, 19]. In recent decades, however, tumors of the proximal colon and rectum have shown different incidence trends [20]. In a number of Western countries, including the United States of America (USA) [21-23], Canada [24, 25], Australia [26], New Zeeland [27], Japan [28, 29] and European countries [30-33], there has been a rising incidence of proximal colon cancer during the last five decades, but a decreasing incidence of rectal cancer during the last three decades. Proximal colon cancer is more common in women, whereas rectal cancer occurs more frequently in men [34-36]. Moreover, several studies addressing environmental factors such as diet, smoking, and physical activity, found that these factors might have a different effect in colon cancer than in rectal cancer. Most comprehensive systematic reviews and meta-analyses of epidemiological studies concluded that—at least in Western countries—physical activity decreased the risk of colon

but not of rectal cancer [37-39]. This observation is in line with a prospective cohort analysis of the National Institutes of Health of 506,488 participants followed between 1995-2006 in the USA [40]. In that analysis, behavioral factors (physical activity, diet, smoking) and body mass index were stronger mediators of risk for colon cancer than for rectal cancer. Overall, a healthy lifestyle seems to have less impact in preventing rectal cancer compared to colon cancer.

Primary tumor histology, molecular characteristics and anatomic site

Three major histological subtypes of CRC can be identified: intestinal type adenocarcinoma, mucinous adenocarcinoma and signet-ring cell carcinoma. The occurrence of mucinous and signet-ring cell tumors is higher in the proximal colon (approximately 45%) than in the distal colon or rectum (approximately 20%) [41].

Two main syndromes resulting from germ line mutations play a role in the occurrence of CRCs. The first is familial adenomatous polyposis syndrome (FAP), which is associated with mutations in the adenomatous polyposis coli (*APC*) tumor suppressor gene. In patients with this syndrome, tumors develop in the distal colon in approximately 60% of the cases, and in the rectum in 25% of patients [42]. The second is Lynch syndrome (hereditary nonpolyposis colorectal cancer, HNPCC), which results from inactivating mutations in DNA mismatch repair (MMR) genes (commonly MutL homolog 1 (*MLH1*) and MutS homolog 2 (*MSH2*); 55% of these tumors are present in the proximal colon and 15% in the rectum [42, 43].

(*Epi*)*genetic instability in colorectal cancer*

Three main types of (epi)genetic instability have been established so far in CRC (Table 1) [44-46]. The first type is chromosomal instability (CIN), characterized by aneuploidy and

Table 1. Types of (epi)genetic instability in colon and rectal carcinomas, and their molecular, histological and anatomical characteristics [44-55].

Parameter	CIN	MSI	CIMP	
Histology	Adenocarcinoma	Mucinous adenocarcinoma	sessile serrated adenocarcinoma	Traditional serrated adenocarcinoma
KRAS mutation	+++	+	−	−
BRAF mutation	+	+++	+++	+
MLH1 methylation	−	+++	+++	−
MGMT methylation	−	−	−	+++
Anatomical site	No preference	Proximal colon	Proximal colon	Distal colorectum

CIN, chromosomal instability; MSI, microsatellite instability; CIMP, CpG island methylator phenotype; *KRAS*, Kirsten rat sarcoma gene; *BRAF*, v-raf murine sarcoma viral oncogene homolog B; *MLH1*, MutL homolog 1 gene; *MGMT*, O-6-methylguanine-DNA methyltransferase gene; +++, present; +, can be present; −, absent.

loss of heterozygosity. CIN predominantly occurs in sporadic tumors developing from adenomas along the large bowel, irrespective of their anatomical site [47]. CIN is also present in the inherited condition FAP [48]. Activating Kirsten rat sarcoma (*KRAS*) oncogene mutations represent an important feature of sporadic CIN tumors [47], and constitute the major cause for clinical resistance to standard epidermal growth factor receptor (EGFR)-inhibitory therapy [49]. The second type is microsatellite instability (MSI), which results from deficient DNA mismatch repair. This can be caused by inactivating germ line mutations, as present in Lynch syndrome, or by *MLH1* promotor hypermethylation, as present in sporadic carcinomas. Activating mutations in v-raf murine sarcoma viral oncogene homolog B (*BRAF,* mainly mutation V600E) are enriched in sporadic tumors with *MLH1* hypermethylation [50, 51], which can render them resistant to currently used EGFR-inhibitors [52]. Sporadic tumors harboring MSI are very rare in the rectum; they are localized especially in the proximal colon, and are often mucinous adenocarcinomas [53]. The third type of epigenetic instability is the CpG island methylator phenotype (CIMP), characterized by excess methylation of some CpG islands. This type occurs in sporadic sessile serrated adenocarcinomas of the proximal colon that show *MLH1* hypermethylation [44, 51, 54, 55], or in traditional serrated adenocarcinomas of the distal colon and rectum that show O-6-methylguanine-DNA methyltransferase (*MGMT*) methylation [43].

A comprehensive characterization of human colon and rectal carcinomas was carried out by the Cancer Genome Atlas Network to identify possible genetic differences between them [56]. Genome-wide analysis of 224 colorectal tumor/normal tissue pairs showed that 84% of the colon and rectal tumors had a low mutation rate < $8.24/10^6$ bases (defined as non-hypermutated). The remaining 16% of the tumors had a high mutation rate (>$12/10^6$ bases, defined as hypermutated). The mutation frequency of the well-known CRC-related genes *APC*, tumor protein *TP53*, *KRAS* oncogene and *BRAF* was respectively 81%, 59%, 43%, and

3% in the non-hypermutated tumors, and 51%, 17%, 30%, and 47% in the hypermutated tumors. The mutational profile of non-hypermutated colon and rectum tumors was similar, whereas three quarters of the hypermutated and most of the hypermethylated tumors originated in the proximal colon. As a possible explanation for these results, the authors proposed their differing origins: the proximal colon originates in the embryonic midgut, while the distal colorectum originates in the embryonic hindgut [56]. These data suggest that the non-hypermutated tumors in the Cancer Genome Atlas Network study basically correspond to the CIN phenotype, while the hypermutated tumors correspond with MSI phenotypes [57].

Overall, several histological, genetic and methylation findings support the idea that rectal and distal colon carcinomas share characteristics and are different from tumors of the proximal colon [58-61]. The concept of abrupt dichotomy at the splenic flexure [62] has recently been challenged by a study in 1,443 stage I-IV CRC patients [63]. In that study, the incidence of MSI-high, *BRAF* mutations and CIMP-high in tumors gradually decreased from the proximal colon to the rectum (Table 2). An assessment of molecular features along anatomical sites in colon carcinomas of patients enrolled in the Pan European Trial Adjuvant Colon Cancer-3 (PETACC3) chemotherapy trial found that proximal tumors were more often MSI, hypermutated, *BRAF* mutant, of serrated signature, densely infiltrated by tumor infiltrating lymphocytes. Distal tumors were CIN, human epidermal receptor (HER) 1 and 2 amplified, with an active EGFR signaling, and largely non-BRAF-like [64]. This analysis supported the gradual decrease of MSI-high distribution from the ascending colon to the rectum (n = 194), and reported a dichotomous character of distribution for *BRAF* mutations in proximal (higher frequency) vs distal carcinomas (lower frequency) as divided at the splenic flexure (n = 110). In a pooled analysis of 560 stage I-IV CRCs from three independent population-based studies, the molecular difference between microsatellite stable primary tumors according to site were studied [65]. Differences were apparent in the overexpression

of homeobox (*HOX*) genes, which was decreased in a gradient from the proximal colon toward the distal colon and rectum [65]. Consolidating an answer on discrete vs gradual molecular differences in CRCs according to their location in the bowel is needed because it can influence stratification of patients in studies with targeted drugs.

Table 2. Microsatellite instability-high (MSI-high), *BRAF* mutation and CpG island methylator phenotype-high (CIMP-high) frequency (%) in colon and rectal carcinomas according to anatomical subsites [63].

Parameter	MSI-high (%)	*BRAF*mutant (%)	CIMP-high (%)
Cecum	22	12	22
Ascending colon	37	36	40
Hepatic flexure	29	32	35
Transverse colon	20	23	30
Splenic flexure	19	19	14
Descending colon	6.7	11	8.1
Sigmoid	2.8	3.8	3.6
Rectosigmoid junction	3.3	4.3	2.3
Rectum	1.6	1.6	2.2

MSI-high, microsatellite instability-high (instability in ≥30% of markers); *BRAF*, v-raf murine sarcoma viral oncogene homolog B; CIMP-high, CpG island methylator phenotype-high (≥6/8 methylated promoters). Adapted by permission from BMJ Publishing Group Limited.

Clinical outcome in relation to molecular subtype and site of colorectal cancer

CRC can develop through (in)activation of several pathway, involving combinations of genetic and epigenetic changes. Biologically distinct subtypes of CRC could translate into stage-independent survival differences in patients. A recent analysis of 2,720 stage III colon cancer samples of patients participating in the randomized phase 3 NCCTG (Alliance) N0147

adjuvant chemotherapy trial found that stage III colon cancer patients with a MMR proficient primary tumor harboring mutant *KRAS* (*n* = 945) or *BRAF* (*n* = 189) had a shorter 5-year disease-free survival than patients whose tumors lacked these mutations (HR 1.48 and 1.43 respectively) [66]. Patients with MMR proficient tumors without *BRAF* or *KRAS* mutations (*n* = 1331) and those with MMR deficient tumors (*n* = 255) had a similar 5-year disease-free survival. A trend toward a better 5-year disease-free survival rate was observed in stage III patients with distal (*n* = 880; 73.7%) vs proximal MMR proficient tumors lacking *KRAS* or *BRAF* mutations (*n* = 437; 65.0%), and in those with BRAF mutation (*n* = 45; 66.0% for distal and *n* = 140; 50.9% for proximal), as divided at the splenic flexure.

Patients with stage II proximal colon carcinoma (cecum to hepatic flexure; *n* = 353) enrolled in the PETACC3 adjuvant chemotherapy trial relapsed less frequently than patients with distal tumors (splenic flexure to sigmoid colon; HR 0.6; *n* = 488) [64]. In contrast, patients with stage III disease displayed no site-dependent risk for relapse. Survival after relapse was worse in proximal stage III colon tumors than in distal tumors in a multivariate analysis including MSI, *KRAS* and *BRAF* mutation status (HR 1.95, 95% CI 1.6-2.4; *n* = 285), possibly in relation to a protective behavior of MSI in stage I-II, before any metastasis is present.

A large-scale, community-based analysis carried out by the Colorectal Cancer Subtyping Consortium (CRCSC) on 4,000 stage II-III CRC samples identified four molecular subtypes of CRC (colorectal cancer molecular subtypes, CMS 1-4) [74]. These subtypes were distinct in their (epi)genetic characteristics, disturbed signaling pathways, and clinical presentation (Table 3). Subtype CMS1 is a MSI, immune-activated tumor, hypermutated and enriched for *BRAF* mutations with propensity for the proximal colon. The CMS2 subtype is a microsatellite stable tumor with high CIN, strong WNT/MYC pathway activation, *EGFR* amplification or overexpression and mutant *TP53*, and is located especially in the distal

colorectum. The CMS3 subtype is a tumor with low CIN, moderate WNT/MYC pathway activation, mutant *KRAS* and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha gene (*PIK3CA*), and insulin-like growth factor binding protein 2 (IGFBP2) overexpression. The CMS4 subtype is a CIN/MSI heterogeneous tumor of mesenchymal type, with transforming growth factor beta (TGF-β) activation, and neurogenic locus notch homolog protein 3 (NOTCH3)/vascular endothelial growth factor receptor 2 (VEGFR2) overexpression. CMS3 and CMS4 showed no anatomical site preference. Patients with a CMS2 tumor had better survival, and those with a CMS4 tumor had worse survival, whereas survival of patients with a CMS1 or CMS3 tumor was intermediate [67].

Table 3. Molecular subtypes of colon and rectal carcinomas: genetic instability, signaling pathway and clinical features [67].

Molecular subtype (%)	Parameters
CMS1 (14%)	MSI, immune pathway activation/expression, right-side tumors, older age at diagnosis, females, hypermutation, *BRAF* mut, intermediate survival
CMS2 (41%)	High CIN, MSS, strong WNT/MYC pathway activation, left-side tumors,*TP53* mut, *EGFR* amplification/overexpression, better survival
CMS3 (8%)	Low CIN, moderate WNT/MYC pathway activation, *KRAS* mut, *PIK3CA* mut, IGFBP2 overexpression, intermediate survival
CMS4 (20%)	CIN/MSI heterogeneous, mesenchymal/TGF-beta activation, younger age at diagnosis, NOTCH3/VEGFR2 overexpression, worse survival

(%), percentage of samples; CMS, 1-4 colorectal cancer molecular subtypes 1-4; MSI, microsatellite instability; *BRAF* mut, mutant v-raf murine sarcoma viral oncogene homolog B; CIN, chromosomal instability; MSS, microsatellite stability; *TP53* mut, mutant tumor protein p53; *EGFR*, epidermal growth factor receptor; TGF-β, transforming growth factor beta; *KRAS*, Kirsten rat sarcoma oncogene; *PIK3CA* mut, mutant phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha gene; IGFBP2, insulin-like growth factor binding protein 2; NOTCH3, neurogenic locus notch homolog protein 3; VEGFR2, vascular endothelial growth factor receptor 2. Reprinted with permission.

Apart from influencing patient's survival, biologically distinct subtypes of colorectal cancer might be also important for the design of future clinical trials. For instance, MSI tumors produce neo-proteins, often called neo-antigens in literature, due to multiple frame shift mutations and nucleotide repeat replication deficiency. These neo-proteins are subsequently processed into neo-peptides that are presented on major histocompatibility complexes 1 (MHC-1) and result in more tumor infiltrating lymphocytes [68]. Patients with MSI CRCs might be optimal candidates for immune therapy trials. In the phase 2 trial with the programmed cell death protein-1 (PD-1) inhibitor pembrolizumab, objective response rate was 40% (n = 10) in MMR-deficient metastatic CRC patients, whereas no objective response was observed in the MMR-proficient cases (n = 18) [69].

Metastatic patterns

Venous drainage of the large bowel is achieved via the portal system. Therefore, the first site of hematogenous dissemination for CRCs is usually the liver, followed by the lungs, bone, and many other sites, including the brain [70]. However, tumors arising in the distal rectum can metastasize initially to the lungs because the inferior rectal vein drains into the vena cava inferior rather than into the portal venous system. An analysis of 567 patients with colon cancer and 1,013 with rectal cancer showed that 11.5% of rectal cancer patients had pulmonary metastasis, compared to only 3.5% of colon cancer patients [71]. In an autopsy study including 1,238 patients with metastatic colon and 441 patients with metastatic rectal cancer, no differences were found in the frequency of liver metastases according to the primary tumor site (colon, 69.6% vs rectal, 67.4%) [41]. However, for adenocarcinoma and mucinous carcinoma histological subtypes, intra-abdominal metastases were more frequent in case of colon cancer (peritoneal 28.8% vs 16.0%, omental 9.1% vs 2.9%, and ovarian 3.2% vs 1.1%), whereas extra-abdominal metastases occurred more often in rectal cancer patients

(lungs 42.0% vs 30.7%, and brain 5.0% vs 2.6%). Another study reported an increased risk for lung-only metastasis among rectal adenocarcinoma patients (odds ratio (OR) 3.32; *n* = 35) relative to colon adenocarcinoma patients (*n* = 108) [72]. This study found a *KRAS* mutational status discordance rate of 32.4% between the paired 37 primary tumors and lung metastases, compared to the 12.3% discordance rate between the paired 106 primary tumors and metastatic sites other than lungs. Another study described a concordance rate of 95% for *KRAS* status of primary colorectal tumors and matched liver metastasis [73]. These studies suggest a difference in *KRAS* mutational status between the primary tumor and hepatic vs extrahepatic metastases, and *KRAS* mutational status discordances between the primary tumor and lung metastases can be more common in rectal cancer patients.

Staging procedures, neoadjuvant treatment and surgery of the primary tumor

Following detection by endoscopy and confirmation by histopathology, rectal and colon tumors present important staging and treatment differences.

The rectum is located in the narrow pelvis and is surrounded by numerous vital structures such as large vessels, nerves, bladder, internal genital organs or sacrum. Therefore, the local treatment for rectal cancer is more aggressive than that for colon cancer. Neoadjuvant short-course radiation or chemoradiation followed by total mesorectal excision (TME) is the current standard of care for locally advanced rectal cancer, with a 5-year local recurrence rate of <10% [5,6,74].

Optimal neoadjuvant and surgical treatment assignment for rectal cancer patients according to the risk for local recurrence requires a reliable preoperative assessment of the tumor status (T), the nodal status (N), and the surgical circumferential resection margin (CRM). Distinct from colon tumors, preoperative endorectal ultrasound (EUS) and magnetic resonance imaging (MRI) play important roles in the diagnostic management of rectal tumors.

EUS supports differentiation between superficial cancers invading into the submucosa (T1) that are treated by transanal endoscopic microsurgery (TEM), and those that breach the muscularis propria (T2) [75]. A meta-analysis of 90 studies published between 1985-2002 demonstrated a high pooled sensitivity (94%) of MRI for assessing the depth of tumor penetration through the rectal wall and for providing an accurate image of the surrounding soft pelvic structures [76]. Therefore, MRI is part of the current standard preoperative work-up for non-superficial rectal tumors, i.e. large tumors limited to the bowel wall (T2), tumors penetrating through the muscular wall (T3), those that penetrate the visceral peritoneum (T4a), or those invading adjacent organs (T4b) [10,11].

Several studies and meta-analyses have been conducted to define the accuracy of preoperative MRI in predicting the CRM and the nodal status [77-82]. A multivariate analysis correlated MRI and histopathological CRM assessment in 374 rectal cancer patients enrolled in the MERCURY trial [81]. This showed that primary tumors located at >1 mm from the mesorectal fascia on the MRI scan have a low risk for CRM tumor involvement as judged by the pathologist (hazard ratio (HR) 3.72, 95% confidence interval (CI) 1.43-9.71). Hence, MRI is currently the preoperative examination of choice for establishing the relationship between the edge of the rectal tumor and the mesorectal fascia, which is the anatomical cornerstone for the feasibility of curative TME.

Compared with colon cancer, the preoperative nodal status in rectal cancer has a higher impact on the choice of neoadjuvant treatment. A meta-analysis of 84 studies published between 1985-2004 on histologically proven rectal cancer patients showed a size-based N-staging accuracy for pelvic MRI of 57%-85% [80]. This moderate sensitivity could be explained by MRI overlooking mainly small (<5 mm) metastatic lymph nodes, and in rectal cancer, the majority of metastatic nodes are smaller than 5 mm [83]. A study in 42 rectal cancer patients who underwent TME suggested that defining MRI node positivity by

irregular border or heterogeneous signal rather than size might improve MRI sensitivity and specificity [84]. In current clinical practice, therefore, lymph nodes are defined as metastatic based on the above mentioned morphological criteria: if they are round-shaped with a diameter ≥5 mm, if they have a heterogeneous signal and/or show irregular border on MRI [85].

A palliative colectomy is often justified in case of patients with metastatic colon cancer. However, in rectal cancer patients, due to a high risk of postoperative morbidity, TME is usually only justified in a curative setting. Therefore, an accurate staging that takes into account the higher rate of lung metastases in rectal cancer is crucial before a curative TME surgery decision can be made. Hence, for screening of metastatic disease in the liver and lungs, several guidelines recommend state-of-the-art CT of the abdomen and chest (11-13,86).

Adjuvant chemotherapy

In lymph node-positive (stage III) colon cancer, systemic adjuvant chemotherapy can improve survival [8, 9]. When started within 8 weeks after surgery, 5-FU, leucovorin, oxaliplatin (FOLFOX) or capecitabine, oxaliplatin (CAPOX) administered in 3-weekly cycles over 24 weeks yielded overall survival benefits of 5-25% [87, 88]. In a recent retrospective multicenter study of 433 stage II-III MSI colon carcinoma patients, an improved relapse-free survival was found with FOLFOX therapy (HR 0.46, 95% CI 0.23-0.79; *n* = 119) compared to surgery alone (*n* = 263), whereas no survival benefit effect was observed with 5-FU therapy (HR 1.02, 95% CI 0.60-1.73; *n* = 51) [89]. Therefore, the Dutch national guideline for colon cancer recommends that patients with MSI stage II-III colon carcinoma who have a contraindication for oxaliplatin should not receive adjuvant 5-FU monotherapy [17]. Importantly, MSI status should not be determined or used to define treatment in stage III

colon cancer patients for whom a standard of care oxaliplatin-based regimen is being planned [12,15].

It is still controversial whether rectal cancer patients should receive adjuvant chemotherapy after neoadjuvant radiotherapy or chemoradiotherapy [90, 91]. Lack of activity might be due to the fact that after neoadjuvant treatment and the surgically complex TME, which often has prolonged recovery period, there is a chemotherapy-free period of approximately 20 weeks until adjuvant systemic treatment can be administered. Furthermore, adjuvant chemotherapy following neoadjuvant chemoradiation and TME can, at times, only be administered at a reduced dose [74]. The long-term follow-up of the European Organization for Research and Treatment of Cancer 22921 randomized study in rectal cancer (EORTC 22921; n = 1,011) found no survival benefit for adjuvant bolus 5-FU/leucovorin following neoadjuvant radiotherapy or chemoradiotherapy in T3 or T4 disease, including node-positive patients [92]. Ten-year disease-free survival was 47.0% in the adjuvantly treated arm and 43.7% in the surveillance arm (HR 0.91, 95% CI 0.77-1.08, P = 0.29). The 10-year overall survival was 51.8% in the arm with adjuvant treatment and 48.4% in the arm without (HR 0.91, 95% CI 0.77-1.09, P = 0.32). Of the 506 patients who received adjuvant chemotherapy, 57% did not receive the intended 4 cycles as scheduled, and 27% could not start adjuvant treatment at all [7]. In another randomized trial, 635 rectal cancer patients with clinical stage T3-T4 disease were given long-course 5-FU-based neoadjuvant chemoradiotherapy, followed by adjuvant bolus 5-FU/leucovorin or observation [93]. The 10-year overall survival rates did not differ, with 63.4% in the adjuvant treatment group and 63.0% in the observation group. Similarly, the randomized PROCTOR/SCRIPT trial of adjuvant 5-FU/leucovorin or capecitabine vs observation (n = 470), showed no long-term survival benefit for adjuvant systemic chemotherapy in stage II-III rectal cancer following neoadjuvant (chemo)radiotherapy [94]. In that study, 75% of the included patients received

the assigned adjuvant chemotherapy. Furthermore, the CHRONICLE trial, consisting of adjuvant oxaliplatin/capecitabine treatment after neoadjuvant chemoradiation, had to be terminated prematurely due to scanty accrual [95]. Of the 113 patients enrolled, only 48% completed the assigned 6 cycles of adjuvant chemotherapy.

A recent phase 2 randomized study in 321 patients with postoperative pathologic stage (yp) II or stage III rectal cancer following preoperative fluoropyrimidine-based chemoradiotherapy found that adjuvant oxaliplatin/5-FU/leucovorin improved 3-year disease-free survival (71.6%) compared to 5-FU/leucovorin (62.9%; HR 0.657, 95% CI 0.434-0.994, P = 0.047) [96]. A main strengths of this study is that 96% of the patients completed the intended 4 cycles of adjuvant chemotherapy. A limitation is the inherent lower statistical power of a phase 2 study. Furthermore, there are no exact data provided on the interval from surgery to the start of adjuvant chemotherapy. Hence, there could be an imbalance here between arms that might explain the lower-than-expected 3-years disease-free survival in the bolus 5-FU/leucovorin arm, and favor the FOLFOX arm. Overall, while these results could suggest that patients with yp II-III rectal cancer following fluoropyrimidine-based neoadjuvant chemoradiotherapy might benefit from the addition of oxaliplatin to adjuvant chemotherapy, long-term survival data are warranted to confirm it.

Due to the shortcomings of these trials, adjuvant systemic therapy for rectal cancer is currently recommended by the ESMO [10] and NCCN [11] guidelines, but not by the Dutch [16] clinical practice guidelines.

Systemic treatment of metastatic disease

In metastatic rectal and colon cancer, there are no differences in indications for systemic chemotherapy or targeted treatment with EGFR inhibitors and antiangiogenic drugs [11-13, 97]. However, it may be questionable whether a rectal carcinoma with a different metastatic

pattern, composition of drug targets, often previous local treatment, and metastasizing after radiotherapy has the same sensitivity for systemic treatments as a colon carcinoma.

A retrospective analysis of 399 chemorefractory metastatic CRC patients that received cetuximab monotherapy vs best supportive care in a randomized phase 3 study (NCIC-CTC-CO.17) observed that the efficacy of cetuximab was modulated by the location of the wild type *KRAS* primary tumors [98]. The median progression-free survival was 5.4 months in the cetuximab-treated patients with a primary tumor located distally (from the splenic flexure to the rectosigmoid), and 1.9 months (P = 0.002) in the cetuximab-treated patients with a proximal primary tumor (from cecum to the transverse colon). A prospective analysis of metastatic CRC patients whom received first-line cetuximab in combination with chemotherapy in a randomized phase 2 study (AIO KRK-0104) reported that patients with a distal *KRAS* codon 12/13 wild type primary tumor (n = 68; tumors of the splenic flexure, descending and sigmoid colon, and rectum) had a better median progression-free survival (HR 0.54) and median overall survival (HR 0.42) compared to patients with a proximal *KRAS* codon 12/13 wild type primary tumor (n = 27; tumors from the cecum to the distal part of the transverse colon) [99]. A retrospective analysis of a cohort of 435 chemorefractory metastatic colon cancer patients treated with cetuximab in combination with chemotherapy found that patients with a distal *KRAS* and *BRAF* wild type primary tumor (n = 158; splenic flexure, descending and sigmoid colon) had a longer median progression-free survival (30 weeks, 95% CI 26-34 week, univariate P = 0.02) than those with a proximal (n = 45; cecum, ascending colon, and hepatic flexure) *KRAS* and *BRAF* wild type tumor (18 weeks, 95% CI 11-31 weeks) [64]. These studies also showed that *KRAS* or *BRAF* mutant CRCs of metastatic stage showed no difference in overall or progression-free survival according to the primary tumor location [64, 98, 99]. The higher frequency of human epidermal receptor (HER) family members amplification, of epiregulin overexpression, and the stronger EGFR signaling in

distal colon and rectal tumors versus proximal colon tumors might explain these results [56, 64, 67]. Whether the HER pathway enrichment findings of stage II-III CRC mentioned above also hold in metastatic disease could be evaluated in patients presenting with synchronous primary tumor and visceral metastatic lesions. Preferential assignment of EGFR inhibitors to metastatic patients with (*K*)*RAS* wild type primary tumors located in the distal colon or rectum would require reanalysis by primary tumor site of major CRC trials such as CRYSTAL, PRIME, and FIRE 3 [99].

Drug targets in the microenvironment of colorectal cancer

Sustained angiogenesis is a key feature of the tumor microenvironment that drives cancer growth and metastasis [100]. VEGFA is the main regulator of angiogenesis that binds to the VEGF receptor VEGFR2, which is present on endothelial cells. Bevacizumab is a humanized monoclonal antibody against VEGFA. A retrospective analysis of two independent, non-randomized cohorts of metastatic CRC patients that received first-line chemotherapy with (*n* = 667) or without bevacizumab (*n* = 213) suggested that the addition of bevacizumab may primarily benefit patients with primary tumors located in the sigmoid colon and rectum vs patients with primary tumors arising from the cecum to the descending colon [101]. Another retrospective analysis of metastatic CRC patients from three independent datasets including two randomized phase 3 trials (AVF2107 and NO16966), whom received either first-line chemotherapy (*n* = 1209) or chemotherapy plus bevacizumab (*n* = 818), found that the effect of bevacizumab is independent of the primary tumor location in the proximal colon or the distal colorectum, as divided at the splenic flexure [102]. However, several studies have reported survival differences between patients with rectal or colon cancer treated with bevacizumab-containing regimens. In the seminal phase 3 study that demonstrated survival benefit by adding bevacizumab to 5-FU and irinotecan [103], metastatic rectal cancer patients

(*n* = 92) had a 24.2 months median overall survival compared to 19.5 months for metastatic colon cancer patients (*n* = 310) in the bevacizumab arm [104]. In a randomized phase 3 study comparing bevacizumab in combination with capecitabine (*n* = 140) vs capecitabine alone (*n* = 140) as first line treatment of metastatic CRC patients aged 70 years and older (AVEX trial), median progression-free survival of rectal cancer patients was better than that of colon cancer patients in the bevacizumab arm (accordingly HR 0.41 vs 0.67) [105]. The Investigation of Treatment Efficacy and Safety (BRiTE) trial showed a better median overall survival for metastatic rectal cancer patients compared to metastatic colon cancer patients [106]. In this observational study (*n* = 1,445) with bevacizumab added to the first line chemotherapy, a median survival of 29.2 months was found in metastatic rectal cancer (*n* = 293) compared to 21.9 months in metastatic colon cancer (multivariate $P < 0.02$). Overall, these findings are hypothesis-generating and need to be validated by data relating precise primary tumor location to the efficacy of antiangiogenic drugs in additional randomized metastatic CRC studies.

The fact that the primary rectal tumor is often irradiated, while the colon tumor is not, might lead to differences in the microenvironment of cancer cells. For example, chemokine receptor 4 (CXCR4) and its corresponding chemokine ligand 12 (CXCL12), which are expressed by both cancer and microenvironment cells such as stromal and immune cells, form an important communication network between cancer cells and their microenvironment [107]. Binding of the ligand to its receptor activates downstream signaling that leads to the promotion of cancer cell migration and metastasis, and protects cancer cells from genotoxic stresses such as chemotherapy. Hypoxia-inducing cancer treatments such as radiotherapy can increase CXCR4 and CXCL12 protein expression levels in the tumor, as demonstrated in a glioblastoma mouse model [108] and in irradiated human nasopharyngeal tumors [109]. Moreover, paired tumor tissue analysis showed that pelvic radiotherapy followed by systemic

treatment with bevacizumab, capecitabine and oxaliplatin upregulated nuclear CXCL12 expression in cancer cells of the primary tumors of 50 de novo metastatic rectal cancer patients [110]. These data suggest that disrupting the interaction of cancer cells with their microenvironment might be of interest to study in rectal cancer. Phase 1 trials in patients with advanced solid cancers with the CXCR4 inhibitor CTCE-9908 and CXCR4 peptide antagonist LY2510924 were recently completed and showed that these drugs were well tolerated [111,112]. The CXCR4 antagonist AMD3100 is currently being tested in combination with bevacizumab as treatment for patients presenting with recurrent high-grade glioma (ClinicalTrials.gov Identifier: NCT01339039). Further studies are warranted to determine the precise role of the CXCR4/CXCL12 axis in rectal cancer, and whether its inhibition could increase the efficacy of conventional therapies.

Concluding remarks and implications for clinical practice

Rectal cancer is differentiated from colon cancer by the addition of neoadjuvant radiotherapy or chemoradiotherapy and the lack of robust evidence of a role for adjuvant chemotherapy. This later difference might be due to the delayed start of adjuvant treatment following neoadjuvant radiotherapy or chemoradiotherapy, and the complexity of the TME procedure. This delay can be avoided by neoadjuvant administration of systemic chemotherapy, as is currently being tested in the RAPIDO study "Radiotherapy And Preoperative Induction therapy followed by Dedicated Operation" (ClinicalTrials.gov Identifier: NCT01558921) [113]. In the RAPIDO trial, patients with non-metastatic rectal cancer at high risk of local or systemic failure are randomly assigned to either capecitabine-based long-course chemoradiation with TME at 6-8 weeks or short-course radiotherapy followed by 6 cycles of preoperative CAPOX and TME at 2-4 weeks after the last cycle of chemotherapy. The

hypothesis is that a short-course radiotherapy and systemic neoadjuvant chemotherapy would increase disease-free and overall survival without compromising primary tumor control.

The degree of rectal tumor involvement with the mesorectal fascia and the clinical nodal status are particularly important in rectal cancer, as these are two major selection criteria when choosing the optimal neaodjuvant treatment for these patients. High-resolution T2-weighted pelvic MRI is a reliable tool for the preoperative assessment of mesorectal fascia involvement [80, 81, 85]. The morphological nodal status is more difficult to define in clinical practice. Modern functional MRI techniques, such as diffusion and perfusion MRI including MRI with lymph node specific contrast, are currently being tested to further improve the staging and restaging accuracy in rectal cancer [114].

Accurate exclusion of metastatic disease is imperative before taking a TME decision in rectal cancer patients. Rectal cancer is more frequently associated with lung-only metastases than colon cancer. Therefore, given its higher accuracy, staging with chest CT rather than chest X-ray seems more appropriate.

It is still unknown whether different targeted therapy should be considered for colon and rectal cancer. The overall mutational patterns of well-known CRC genes, not only *KRAS*, but also recently identified genes such as *PIK3CA* or F-box/WD repeat-containing 7 (*FBXW7*), show no obvious differences between colon and rectal tumors [56]. However, the molecular characteristics of proximal and distal colon carcinomas are substantially different, and the intrinsic biology and corresponding drug targets of rectal tumors are likely very similar to distal colon tumors [56, 64, 67]. Divergences in the genetic make-up between proximal colon and distal colorectal carcinomas include differences in the mutational status of *BRAF* and the EGFR pathway activation, which can have consequences for treatment with targeted agents such as EGFR and BRAF inhibitors [115]. Furthermore, MSI-high tumors, originating mainly in the proximal colon, are expected to be more sensitive to immune

therapy. This concept is being addressed by a phase 2 clinical trial with the PD-1 inhibitor pembrolizumab, which showed impressive results [69]. Moreover, patients with *BRAF* mutational status- and mucinous histology-independent association of primary tumor location in the proximal colon with resistance to current standard chemotherapy in metastatic CRC, possibly related to their higher level of excision repair cross-complementation group 1 (*ERCC1*) mRNA compared to distal colorectal tumors, substantiates the importance of biological differences in colorectal tumors by site [102]. Stratifying patients according to primary tumor location in the proximal colon vs distal colon vs rectum should be therefore considered in clinical trials testing chemotherapy or targeted agents in colorectal cancer, as it would provide direct molecular comparison between tumors according to site, and it is potentially relevant for therapeutic decision-making.

In both rectal and colon cancer, there can be discordances between the mutational profile of the primary tumor and the metastatic lesions. For instance, there is a relatively high discordance in *KRAS* mutational status between the primary tumor and lung metastases [72]. The clinical significance of this disparity could be greater in rectal cancer, given the higher incidence of lung metastases compared to colon cancer [41]. Furthermore, it might be preferable to determine the *KRAS* status in lung metastasis tissue, since treatment with anti-EGFR antibodies is restricted to patients with tumors that do not harbor the *KRAS* mutation [97]. Additionally, a molecular profiling study of 115 pairs of primary and metastatic tissues of CRC patients found high discordance rates in the mutational profile of *PIK3CA* and *FBXW7* between the primary tumor lesions and corresponding metastases [116]. Moreover, the rate of discordance was augmented by chemotherapy (3.5 fold higher odds for patients that received chemotherapy compared to those that did not).

In conclusion, CRC is not one disease. Future studies on subtyping can contribute to determine the most optimal treatment in the adjuvant and metastatic setting

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