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Delivery of Personalized Care for Locally Advanced Rectal Cancer: Incorporating Pathological, Molecular Genetic, and Immunological Biomarkers Into the Multimodal Paradigm

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Approximately one-third of all newly diagnosed colorectal cancer (CRC) is composed of rectal cancer, with the incidence rising in younger patients. The principal neoadjuvant treatments consist of neoadjuvant short-course radiotherapy and long-course chemoradiation. Locally advanced rectal cancer (LARC) is particularly challenging to manage given the anatomical constrictions of the pelvis and the risk for local recurrence. In appropriately treated patients, 5- and 10-year overall survival is estimated at 60 and 50%, respectively. The prognosis for LARC has improved in recent years with more access to screening, advances in surgical techniques, and perioperative care. Furthermore, the refinement of the multidisciplinary team with combined-modality management strategies has improved outcomes. These advancements have been augmented by significant improvements in the understanding of the underlying tumor biology. However, there are many instances where patient outcomes do not match those for their tumor stage and accurate prognostic information for individual patients can be difficult to estimate owing to the heterogeneous nature of LARC. Many new combinations of chemotherapy with radiotherapy, including total neoadjuvant therapy with targeted therapies that aim to diminish toxicity and increase survival, are being evaluated in clinical trials. Despite these advances, local recurrence and distant metastasis remain an issue, with one-third of LARC patients dying within 5 years of initial treatment. Although much of the new pathological, molecular genetics, and immunological biomarkers allow refinement in the classification and prognostication of CRC, the relative importance of each of these factors with regards to the development and progression of LARC remains incompletely understood. These factors are often insufficiently validated and seldom consider the individual characteristics of the host, the tumor and its location, the local available expertise, or the probable location of recurrence. Appreciating the mechanisms behind these differences will allow for a more comprehensive, personalized approach and more informed treatment options, leading to ultimately superior outcomes. This review aims to first outline the current multidisciplinary context in which LARC care

should be delivered and then discuss how some key prognosticators, including novel histopathological, molecular genetics, and immunological biomarkers, might fit into the wider context of personalized LARC management in the coming years.

Keywords: chemotherapy, radiation, rectal cancer, mesorectal excision, prognostic markers, personalized medicine, survival

INTRODUCTION

Colorectal cancer (CRC) is the second highest cause of cancer-related mortality in Europe with an estimated 500,000 cases in 2018 (1). Approximately one-third of all newly diagnosed CRC is composed of rectal cancer with the incidence rising in younger patients throughout the western world (2). The principal neoadjuvant treatments consist of neoadjuvant short-course radiotherapy and long-course chemoradiation (nCRT) (3–6). The latter is mainly used in the treatment of locally advanced rectal cancer (LARC), defined for the purpose of this review as clinical stage T3–4 or any clinical T stage with node-positive disease ($\geq cT_{3-4}$ or any cT with $cN_{1/2}$). LARC is particularly challenging to manage given the anatomical constrictions of the pelvis and the risk for local recurrence. Neoadjuvant therapy followed by total mesorectal excision (TME) with either low anterior resection (LAR) or abdominoperineal excision is associated with improved survival (3–6). In appropriately treated patients, 5- and 10-year overall survival (OS) is estimated at 60 and 50%, respectively (5). Aside from this survival benefit, nCRT may reduce local recurrence (LR) rates, downsize the tumor, and facilitate subsequent successful R0 resection with sphincter preservation (7).

The prognosis for LARC has improved in recent years with more access to screening, advances in surgical technique and perioperative care, along with the refinement of the multidisciplinary team with combined-modality management strategies. These advances have been augmented by significant improvements in the understanding of the underlying tumor biology reflected by new pathological, molecular genetics, and immunological biomarkers (8, 9). However, there are many patients whose outcomes do not match those typical for their tumor stage and accurate prognostic information for individual patients can be difficult to estimate owing to the heterogeneous nature of the disease. Many new combinations of chemotherapy with radiotherapy, including total neoadjuvant therapy (TNT) with targeted therapies that aim to diminish toxicity and increase survival, are being evaluated in clinical trials (10, 11). Despite these advances, local recurrence and distant metastasis remain an issue, with one-third of LARC patients dying within 5 years of initial treatment (10).

Although much of the new molecular and immunological data allows refinement in the classification and prognostication of CRC, the relative importance of each of these factors with regards to the development and progression of LARC remains incompletely understood. Current excitement about novel prognostic markers, such as tumor budding, The Cancer Genome Atlas' Consensus Molecular Subtypes (CMS), and the Oncotype DX (Genomic Health, Redwood, California, USA), and Immunoscore tests (Integrative Cancer Immunology Laboratory, INSERM, Paris, France) underestimate the complexity of the disease (8). These factors are often insufficiently validated and seldom consider the individual characteristics of the host, the tumor and its location, the local available expertise, or the probable location of recurrence (9). Appreciating the mechanisms behind these differences will allow for a more comprehensive, personalized approach and more informed treatment options, leading to ultimately superior outcomes.

This review aims to first outline the current multidisciplinary context in which rectal cancer care should be delivered and then discuss how some key prognosticators of LARC, including novel histopathological, molecular genetics, and immunological biomarkers, might fit into the wider context of personalized LARC management in the coming years.

MULTIDISCIPLINARY ASSESSMENT, HISTOPATHOLOGY, AND STAGING

The Multidisciplinary Team

A multidisciplinary approach to rectal cancer is crucial owing to the complexity of the disease. Improved radiological staging, mesorectal grading, and oncological outcomes can be mainly attributed to the development of the multidisciplinary approach to LARC management, incorporating surgeons, pathologists, radiologists, and oncologists. Therefore, a multimodality approach is crucial in providing personalized and effective treatment to rectal cancer patients (12).

Radiological Staging and Assessment

MRI is the preferred imaging modality for pelvic staging in LARC before treatment, aiding in deciding the need for neoadjuvant therapy while also potentially predicting patients who have worse outcomes. Good response to neoadjuvant therapy is seen in node negative patients with more superficially located tumors. Free resection margins (CRM) and the absence of adverse pathological features on MRI are all predictors of good response to treatment (13, 14). The combination of extramural venous invasion (EMVI), involvement of regional lymph nodes, and higher T stages on MRI are associated with synchronous metastatic disease and worse outcomes (15). MRI maintains a high specificity and moderate sensitivity for the detection of EMVI (16), and MRI-detected EMVI (mrEMVI) in particular may represent an independent adverse feature whose prognostic and predictive importance (to nCRT response) is somewhat underestimated (14, 15). MRI, however, is limited when restaging patients after neoadjuvant therapy owing to its failure to accurately differentiate residual tumor from post–nCRT-related desmoplastic reactions, inflammation, and fibrosis

(17). The accuracy of correctly staging the tumor and nodes is roughly 45–67% and 76–93% when standard MRI modalities are used (18). However, for CRM, mean sensitivity and mean specificity in a meta-analysis of over 1,500 patients were 76.3 and 85.9%, respectively (19). Furthermore, sensitivities of 21–55% and specificities of 76–93% are reported when MRI is used to accurately identify response to neoadjuvant therapy (18, 20). Interestingly, the diagnostic performance of MRI for mrEMVI evaluated after the nCRT is good and may have additional prognostic impact (21–23). Diffusion weighting is one newer imaging method that has been shown to improve re-staging accuracy overall (19).

Histopathology and Staging

The recent fifth edition of the WHO Classification of Digestive System Tumours reflects important recent advancements in our understanding of digestive system cancers. For the first time, certain digestive tumor types including CRC are defined as much by their molecular phenotype as their histological characteristics (24). However, histopathologic assessment remains the "gold standard" for diagnosis of LARC while the extent of the disease anatomically, as assessed by clinicopathological staging, remains the most useful prognosticator. Macroscopic evaluation of the resected specimen is still an extremely important aspect in histopathological staging. Both the grade and distance from the circumferential resection margin (CRM) impact survival, LR, and adjuvant therapy decisions. An intact mesorectum is the gold standard as it has been shown to reduce local recurrences to $<$10% (25). Therefore, its correct evaluation has huge implications for patients, especially in the era of minimally invasive surgery (26). A clear CRM is associated with significantly improved local recurrences and oncological outcomes, although consensus on the distance from this margin is still controversial (27).

Tumor Budding

Tumor budding is a histopathological feature of epithelial cancers where tumor cells or cell clusters of less than five cells detach from the invasive margin and migrate into the peritumoral stroma (28–30). It is thought to correspond to the initial phase of tumor invasion and has been reported to be relevant to metastatic activity and prognostic outcome in CRC (28–30). The process is hypothesized to involve the epithelial–mesenchymal transition resulting in resistance to apoptosis, potentially impacting negatively on radiotherapy response (29). The widespread implementation of tumor budding as an adjunct to the TNM classification has been hindered by a lack of consensus definition, reproducibility, and method of assessing budding. Recent consensus recommendations for assessing budding include protocols for incorporating tumor budding into CRC pathological reporting (31). The evaluation of tumor budding in pre-treatment biopsies is also possible and may help identify rectal cancer patients with worse outcomes or who will not respond to therapy, thus providing the opportunity to deliver individualized care for these patients (29).

Lymphovascular, Venous, and Perineural Invasion

Lymphovascular invasion (LVI) is defined as tumor cell invasion into the lymphatic and/or blood vessels. It is regarded as an adverse pathological finding and has a crucial part in the development of metastatic disease (32). The presence of LVI is significantly associated with worse OS and disease-free survival (DFS) (33). EMVI is defined pathologically as tumor cells present in the vasculature outside the muscularis propria. When present, it produces more locally advanced tumors that invade the mesorectum, ultimately impacting negatively on survival and recurrence rates (34). Pathological assessment of EMVI has resulted in under reporting of cases mainly caused by a lack of pathological definition and staining methods used (35). Perineural invasion (PNI) is defined as the neoplastic invasion of nerves or nerve sheaths. Although it is a significant pathological finding, it is under reported in the majority of cases (0.05%) (36). PNI is a key pathological feature in a number of cancers outside of CRC. The presence of PNI represents a process for neoplastic invasion and spread outside the traditional lymph and blood vessel route. Worse OS and DFS is encountered in patients with PNI (37).

Tumor Deposits

Tumor deposits were first described in 1935, although their characterization and clinical significance have changed over the years. Tumor deposits are defined as isolated tumor foci found in the pericolic or perirectal fat or in the mesocolic fat/adjacent mesentery away from the invasive margin of the tumor without evidence of residual lymphatic tissue (38). In the absence of $\geq$1 positive lymph node, they are documented as N1c (i.e., not in T stage category, but rather in the higher N stage category) (39). The presence of tumor deposits is associated with a higher tumor stage, metastatic disease, and a poorer prognosis (40).

MULTIMODAL THERAPY, PATHOLOGIC COMPLETE RESPONSE, AND ORGAN PRESERVATION

The standard multimodality therapy for LARC generally includes nCRT (usually 50–54 Gy and 5-fluorouracil) followed by surgery in the TME plane with or without adjuvant chemotherapy (5, 6). With this treatment paradigm, the incidence of local recurrence is $<$10% and, in approximately 15% of patients, a pathologic complete response (pCR) may be observed (3–6). In patients achieving a pCR, improved LR rates have been reported. Consequently, pCR has become a well-established surrogate for DFS and interest in achieving this outcome has grown (41).

It is now understood that tumor regression is time dependent and a longer waiting time between nCRT and surgery has been one method proposed to increase rates of pCR (42, 43). Intensification of the chemotherapy regimen has also been suggested (44). Of the six large phase-III RCTs comparing nCRT with or without oxaliplatin, only the CAO/ARO/AIO-04 study reported an improved survival with additional chemotherapy and no phase-III RCT of concurrent irinotecan has been reported

(10). Consequently, single-agent 5-fluouracil/capecitabine-based nCRT remains the standard of care in LARC. A new variation of this approach aimed at reducing the instance of micrometastatic disease and local recurrence rates is TNT. This consists of chemotherapy administered either before (induction chemotherapy) or after (consolidation chemotherapy) nCRT and before surgery (45). Weighted mean local recurrence and distant failure rates of 3.5 and 20.6%, respectively, have been reported (11). Consequently, this new strategy is increasingly being considered in patients with high-risk LARC owing to improved compliance with chemotherapy and disease control.

In patients with a clinical complete response (cCR) to nCRT on endoscopic and radiological examination, organ preservation, via local excision or active surveillance, has emerged in an effort to avoid the morbidity and quality of life issues associated with TME (46, 47). The long-term outcomes of a large, international, multi-institutional expectant "watch and wait" database with more than a thousand patients recently demonstrated that LR occurs mostly in the first 2 years, primarily in the bowel wall, underscoring the necessity of endoluminal examination to detect local regrowth early and at a salvageable stage. Local unsalvageable disease after this strategy was infrequent (12%), with a 5-year OS of 85% (47).

However, the majority of patients have less favorable responses to treatment, and local recurrence and distant metastasis remain an issue. Some patients may benefit from "straight up" surgery or alternate neoadjuvant and adjuvant therapies to increase response rates and survival, as well as to avoid the morbidity associated with standard treatment (48–50). For these reasons, personalizing chemotherapy regimens with surgery or organ preservation likely represents the future of rectal cancer management. Molecular, genetic, and immunological biomarkers are required to help guide patient selection for these novel therapeutic strategies in an efficacious and cost-effective manner, while minimizing treatment-related toxicities.

MOLECULAR PATHOGENESIS OF RECTAL CANCER

While the successive genomic alterations that underlie the adenoma to carcinoma sequence remain the framework for our understanding the process of tumorigenesis in CRC (51, 52), recent improvements in sequencing technology have recognized genetic differences between colon and rectal cancers.

Genomic Instability

It has been appreciated for some time that genomic instability facilitates the multistep progression of CRC (53), and LARC is remarkable for its association with genomic instability. This process results from three different molecular pathways: chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) (54, 55). Understanding the pathway to tumorigenesis has significant clinical ramifications for LARC management, with potential implications for screening and surveillance, response to neoadjuvant and adjuvant therapies, and the selection for targeted therapies.

Chromosomal Instability Pathway

CIN is the most frequent cause of genomic instability in CRC, occurring in 70% of sporadic CRC, with increasing prevalence in left-sided CRC, including rectal cancer. CIN refers to the gains and losses of whole chromosomes with resultant to aneuploidy, amplifications and loss of heterozygosity (56). This phenotype is usually secondary to alterations in mechanisms that ensure the fidelity of chromosomal segregation (57). These karyotypic abnormalities occur in combination with the accumulation of the "classic" driver mutations in CRC such as APC, KRAS, and SMAD4 (57). CIN in particular has been associated with early-onset CRC, whose incidence has been climbing in recent decades, predominantly in the left colon and rectum (2, 58).

Microsatellite Instability

In between 10 and 20% of CRC patients, defects in the mismatch repair (MMR) proteins lead to CRC displaying MSI (59). Microsatellites are repetitive sequences distributed throughout the genome that consist of nucleotide repeats that are more frequently copied incorrectly in the presence of a deficient MMR system (dMMR) (60). The resultant MSI phenotype results in numerous frameshift mutations in coding and non-coding microsatellites with neoantigen formation, a "hypermutator phenotype," and an enhanced local immune response. Consequently, MSI or dMMR in this paper refers interchangeably to MSI CRC by PCR or dMMR tumors by immunohistochemistry. Diagnosis of MSI is via PCR amplification. Alternatively, immunohistochemistry can confirm the presence or absence of MMR proteins (59). MSI is less frequently found in rectal cancer. It primarily occurs in right-sided CRC and is associated with poorly differentiated tumors, a high mucinous component, numerous tumor-infiltrating lymphocytes (TILs), and with the presence of a "Crohn's-like" host response (61). A subset of MSI CRC is caused by autosomal dominant mutations in the DNA MMR system termed Lynch syndrome (59). Lynch syndrome is the most common heritable cancer predisposition syndrome (62).

CpG Island Methylator Phenotype

CIMP occurs due to the "serrated pathway." It is characterized by DNA hypermethylation at specific regulatory sites, enriched in CpG motifs (CpG islands) in the promoter regions of tumor suppressor genes, thus leading to transcriptional silencing (63). There is some overlap between CIMP and sporadic MSI cancers owing to their association with methylation of the MLH1 promoter and an activating *BRAF* mutation (64). It is observed in about 15% of tumors (51), particularly right-sided CRC, and is infrequent in rectal tumors (65).

CMS Subtypes

In an effort to refine the molecular genetic classification of CRC, an international consortium of experts have outlined a CMS classification based on results from six independent transcriptomic-based studies (66). Here, the CIN phenotype was

subdivided into three further CMSs, each with distinguishing features: CMS2 ("canonical"), epithelial, marked WNT and MYC signaling activation; CMS3 ("metabolic"), epithelial and evident metabolic dysregulation; and CMS4 ("mesenchymal"), prominent transforming growth factor-β activation, stromal invasion, and angiogenesis (66). MSI or mismatch repair deficient (dMMR) tumors, on the other hand, represented in the CMS1 ("microsatellite instability, hypermutated, immune") subtype, occurs when there is deficiency in MMR proteins, generally caused by sporadic epigenetic silencing (e.g., by hypermethylation) or by constitutional mutations (e.g., in Lynch syndrome). Samples with mixed features (13%) are proposed to represent a transition phenotype or intratumoral heterogeneity. Although the CMS classification does not yet impact on colon or rectal cancer management, it is hoped that this robust classification system may provide the basis for future clinical stratification and subtype-based targeted interventions.

CLINICAL IMPLICATIONS OF RECTAL CANCER MOLECULAR PATHOGENESIS

Microsatellite Instability

Although MSI is a well-established biomarker in CRC, its significance in rectal cancer specifically was until recently uncertain. This is mainly a result of the relative infrequency of MSI rectal cancer, accounting for just 2–15% of all MSI CRC (67, 68). However, it has long been appreciated that the occurrence of MSI in LARC is highly predictive of Lynch syndrome (65, 67, 69). Consequently, whenever MSI or dMMR in LARC is recognized, diagnostic constitutional mutation analysis should be undertaken, either via Sanger sequencing of MMR genes (70) or as part of a next-generation sequencing multiplex gene panel, regardless of BRAF mutation status (69, 71).

Even once the diagnosis has been made, the optimal treatment of Lynch-associated LARC remains unclear. For instance, unlike in MSI colon cancer, deficiency in the MSH2/MSH6 heterodimer is the most common pattern of MMR protein loss (69). Mutations in these MMR genes have been associated with increased frequencies of extracolonic cancers (72). In the biggest analysis of MSI rectal cancer to date, 23% of participants developed an extracolonic malignancy, contributing to 45% of mortalities (69). Thus, although evidence of benefit is limited (73–75), surveillance for multiorgan cancer development, ideally as part of clinical trials, is important. The extent of bowel resection also remains controversial. Optimal oncologic outcome must be balanced against sphincter preservation and quality of life (76, 77). The incidence of metachronous CRC was observed to be 19% at 10 years (78–81) with an associated up to six-fold excess mortality (78). For this reason, an aggressive management approach is suggested in recurrent and/or metachronous disease.

There remains a scarceness of long-term oncological outcome data for MSI rectal cancer specifically; this is despite evidence that MSI rectal and colon cancers have distinct biology (82). Thus, the prognosis for LARC with MSI treated with conventional multimodal therapy was until recently unclear (83). Unlike MSI colon cancers, where there is a preponderance of right-sided cancers in aging females, over a third of MSI CRC in Asian men developed in the rectum (68). MSI rectal cancer displays other distinctive features, with a significant mucinous component (84) and a decreased occurrence of MLH1 promoter hypermethylation and expression of BRAF mutations unlike Lynch dMMR colon cancer, which has no hMLH1promoter hypermethylation (85, 86). The reduced incidence of BRAF mutations may in part explain the improved prognosis of MSI LARC, with 5-year OS and DFS being reported as 90.6 (69) and 70% (87), and 5-year OS of 50% for disease with distant metastatic spread (88).

The development of predictive biomarkers to appropriately select patients for the various treatment modalities for LARC has proved challenging. Despite the general agreement that MSI predicts poor response to adjuvant 5-fluouracil–based chemotherapy (89), its impact on response to 5-fluouracil given as part of nCRT is controversial (90). In MSI with LARC, an increased sensitivity to radiotherapy has been observed in preclinical studies (91–93). Initial underpowered series with little clinicopathologic detail and considerable heterogeneity have described pCR rates of between 0 and 60% (59). More recently, in the largest ever clinical series, tumor regression was demonstrated to be excellent, with a pCR rate of 27.6 vs. 18% in patients with MSI and MSS LARC, respectively (69, 87).

Another consideration in rectal cancer undergoing nCRT is that in cases of pCR, no residual tumor may be available for further MMR or MSI testing, and in some instances, nCRT has been shown to alter the MMR protein status of the tumor (94). For this reason, testing of the pre-treatment biopsy should be undertaken to ensure a source of suitable, reliable testing material is available (95). Although pCR may also occur with chemotherapy alone, MSI LARC appears to have more heterogeneous responses to induction chemotherapy, with chemoresistance being reported in some instances (96). However, evidence suggests that advanced dMMR tumors may respond remarkably well to immunotherapy (97, 98). Emerging trials evaluating the combination of immunotherapy and nCRT in patients with MSI LARC (NCT02948348, NCT03038477, and NCT03854799) based on the preliminary results of such an approach in the neoadjuvant and metastatic disease settings are currently underway (99, 100).

Chromosomal Instability Pathway

In contrast to MSI LARC, CIN, which is much more common in rectal tumors, displays much less immunogenicity. However, the copy number alterations that are a feature of CIN LARC may generate possible targets for therapy such as HER2 amplification, outlined as part of the following section on targeted therapies.

Specific Mutations

Both APC and TP53 mutations are more common in rectal cancer (101, 102). Conversely, KRAS mutations are less common, as are mutations in BRAF (102). In clinical practice, these differences in genomic alterations determine suitability for treatment with targeted therapies and may affect response to

multimodality therapy and patterns of metastatic spread (103–105). Aside from increasing the interval from the end of nCRT to surgery (43, 106), there are few pathological, molecular, or immunologic features consistently associated with pCR after nCRT (107). Studies included in a recent systematic review evaluating a number of molecular markers, including gene signatures by microarray, single-nucleotide polymorphisms, TP53 and mutations in KRAS, and proteomic profiles failed to find any single reliable predictor of pCR (107). However, a more recent multicenter study of almost 300 patients with stage II/III rectal cancer observed pCR rates in KRAS wild-type and KRAS mutant tumors of 34 and 15%, respectively (108). In multivariable analysis, KRAS mutation remained independently associated with a lower pCR rate.

When metastases occur, rectal tumors more frequently metastasize to the lungs and bones, and less frequently metastasize to the gynecologic organs or the omentum and peritoneum in comparison with colon cancers (102). The reduced incidence of peritoneal involvement has been hypothesized to be a result of the relative infrequency of MSI and BRAF mutations in LARC. There is an increased likelihood of lung metastasis in distal rectal cancers despite a reduced incidence of KRAS mutations, a molecular feature often associated with lung metastasis (104, 105). This is primarily caused by their associated venous drainage via the inferior rectal vein, thus bypassing the portal venous system, although treatment nCRT further increases the likelihood of lung metastasis (103, 109).

Consensus Molecular Subtypes

Although the CMS classification offers clues into the transcriptional program of CRC, this has not yet had a major impact on clinical management. However, in the original paper, the CMS4 ("mesenchymal") subgroup was associated with worse DFS and OS, whereas CMS1 ("microsatellite instability, hypermutated, immune") was associated with worse survival after disease recurrence (66). These molecular subgroups are distributed unequally between rectal and colon cancers. This raises the possibility that the results of future clinical trials with CMS subtype-based targeted interventions may be disproportionately affected by primary tumor location.

POTENTIAL TARGETED THERAPIES

Rectal cancers generally display wild-type RAS genes and are thus often candidates for targeted therapy with EGFR inhibitors. In contrast, RAS mutations are more common in right-sided tumors and these mutations are associated with resistance to EGFR inhibitors (110). The expression of the EGFR ligands, amphiregulin and epiregulin, varies across by primary tumor location and may also predict response to this type of targeted therapy (111). EGFR inhibitors have also been tested in LARC, but when used as an adjunct to standard therapies have not been able to enhance the pCR rate (10).

Because of the excessive rate of copy number alterations, gene amplifications are more common in left-sided CRC. The amplification of the receptor tyrosine-protein kinase HER2 is an emerging target. The prevalence of HER2 amplification was demonstrated, in a recent retrospective study, to be significantly increased in rectal cancer (10.4%) compared with left-sided (3.6%) and right-sided CRC (2.9%) (112), while another large retrospective study ($n = 717$) demonstrated that HER2 overexpression by immunohistochemistry was found in 16% of rectal cancers and was associated with worse 5-year OS vs. HER2-negative patients (63.5 vs. 73.9%, $P = 0.013$) (113). Contemporary studies of dual HER2-targeted therapy with the combinations of trastuzumab–pertuzumab and trastuzumab–lapatinib have demonstrated promising initial results in metastatic HER2-amplified CRC, rates of response in the region of approximately 30% (114, 115).

Mutations in BRAF, including the V600E "hotspot," are rare in rectal cancer (<1%). BRAF mutations are related to worse prognosis in CRC and a lack of benefit from EGFR inhibitors (116, 117). Thus, despite its relative infrequency, testing for mutations in BRAF is important to guide the use of EGFR inhibitors and of possibly BRAF-targeted agents. At present, there are no FDA-approved anti-BRAF drugs; however, combinations of selective RAF and EGFR inhibitors have shown encouraging initial results and the National Comprehensive Cancer Network recommend a combination of RAF inhibitor vemurafenib with cetuximab and the chemotherapy agent irinotecan for BRAF V600E-mutated CRC owing to its improved progression-free survival compared with regimens without RAF inhibition (118). Moreover, the combination of the RAF inhibitor encorafenib, the mitogen-activated protein kinase [MEK (the protein target of BRAF)] inhibitor binimetinib, and cetuximab was recently granted FDA approval for the treatment of BRAF V600E-mutated CRC if resistant to initial regimens (119).

As noted previously, up to 5% of rectal cancer displays the CMS1 ("microsatellite instability, hypermutated, immune") subtype, most frequently caused by mutations in the MMR genes. This displays upregulation of various immune checkpoints including programmed death (PD-1) and cytotoxic T lymphocyte–associated protein 4 (CTLA4) that act to downgrade the host inflammatory response in an effort to limit immune-mediated tissue damage. This raises the possibility of immune checkpoint inhibition therapy for MSI LARC. The rates of response to single agent anti-PD1 inhibitors varies between 30 and 50% and combination management with dual immune checkpoint inhibition achieves response rates of over 50% (98, 100). Moreover, these strategies have been associated with durable benefit in responders (105).

IMMUNOLOGICAL LANDSCAPE OF RECTAL CANCER

It is well-recognized that the progression and recurrence of cancer is not just regulated by the genomic mutations inherent to malignant cells but also by host and immunological responses (120). Various mechanisms in the TIME may stimulate a pro-tumorigenic environment and disease progression. The recruitment of immunoregulatory cells and upregulation of inhibitory molecules (e.g., T regulatory cells, natural killer cells, type 2 macrophages, dendritic cells, myeloid-derived suppressor

cells, and other cancer-associated cell types), as well as the downregulation of antigen presentation all facilitate immune evasion (120). Furthermore, the promotion of glycolytic and anabolic pathways often leads to an acidic and anaerobic environment with resultant T-cell exhaustion (121). This may be exacerbated by upregulation of various immune checkpoints PD-1 and CTLA4 that act to downgrade the host inflammatory response in an effort to limit immune-mediated tissue damage (122). Loss of MHC class I and II proteins from cell surfaces that are required for antigen presentation to T cells represents another immunosuppressive mechanism (123, 124).

In contrast, clinicopathological evidence demonstrates that effector T cells in the TIME are key effectors of outcome in CRC. Consequently, patients with higher TIL numbers have improved survival (125). Based on this principle, Galon et al. (126, 127) have developed a TIME-based immunocytochemical score, the Immunoscore (HalioDx, Marseille, France), and this has been shown to be a superior prognosticator to even TNM staging (128). From a systemic perspective, an inflammatory marker that is increasingly used is the neutrophil/lymphocyte ratio (NLR). A recent meta-analysis established that a raised NLR correlated with significantly reduced OS and progression-free survival rates in CRC (129). Markers of systemic inflammation, including NLR, is also a predictor of outcome in patients undergoing nCRT for LARC (130). Furthermore, NLR has been shown to be inversely associated with the vigorous antitumor TIL infiltrate associated with MSI CRC (131). Consequently, NLR may represent a more cost-effective and clinical useful marker than newer and ostensibly more sophisticated immune markers.

Potential Therapeutic Implications of the Different Immune Signatures

Although immune cell quantification approaches such as the Immunoscore give a phenotypic depiction of the TIME, the comparative influences of germline, somatic, and epigenetic variations in CRC immune signatures, including TIL numbers, have yet to be fully elucidated. It is evident that somatic mutational factors in isolation are not adequate to explain this variability. Recent evidence suggests that right- and left-sided CRC, including rectal cancer, have distinctive immunological phenotypes with several biological and clinical distinctions including embryological origin, vascular supply, physiologic function, and local microbiota (132). This may affect somatic mutations and immunological landscapes between the disease locations as well as alter response to therapy.

There is increased infiltration of antitumor $CD56^{bright}$ NK cell subpopulations in rectal cancer and other left-sided CRC (133). This may explain why left-sided CRC with wild-type RAS demonstrates better response to EGFR inhibitors than that which occurs in patients with CRC from other primary tumor locations (134). PD-1 receptors have also been detected on activated NK cells (135, 136), suggesting that these tumors may respond to immune checkpoint inhibition. Therefore, the combination of anti-PD-L1 and EGFR inhibitors may be a potential therapy for RAS wild-type rectal cancer. In contrast, there is increased $CD8^+$ TIL infiltration, cytotoxic activity, interferon-γ signature, and antigen processing machinery in right-sided CRC. This immune phenotype is consistent with the higher tumor mutational burden and incidence of MSI in right-sided CRC (137). However, this favorable $CD8^+$ TIL-mediated antitumor reaction might be impeded by increased concentrations of VEGF-A, which not only mediates immune tolerance but also restricts TIL numbers (133). These results may explain the paradoxical worse prognosis of right-sided CRC despite more efficient $CD8^+$ T-cell infiltration (125) and why right-sided CRC respond better to anti-VEGF therapy than rectal tumors (138). Right-sided CRC also display generally higher levels of PD-1/PD-L1 owing to their immunogenicity (139). Thus, co-targeting angiogenesis and immune checkpoints may represent a promising therapeutic strategy for right-sided CRC but not rectal cancer (140).

The Important Role of the Host Immune System in Radiotherapy Response

Preclinical (141) immunological and genomic (142) studies have demonstrated the critical role of the host immune system in promoting nCRT-induced tumor regression. The presence of TILs in pretreatment biopsies may predict a better response to nCRT in rectal cancer (143, 144). It has been demonstrated that there are important roles for TILs, neoantigens, and PD-1/LAG3 signaling in rectal tumor response to nCRT (145). For this reason, these immune checkpoints may be therapeutic targets to improve nCRT-induced tumor regression in rectal cancer (145, 146). Several clinical trials are ongoing to test whether combining immune checkpoint inhibitors with nCRT may enhance pCR rates and tumor regression (NCT03127007, NCT03102047, and NCT02948348). Preliminary data from a multicenter phase Ib–II study (147) of nivolumab (an anti-PD-1 antibody) monotherapy as an adjunct to conventional nCRT demonstrated a high pCR rate, but the overall participant numbers were small. However, the hypothesis for such a strategy is supported by the evidence that hypermutated rectal cancers, caused by MMR or POLE mutations, have a good prognosis and excellent response to nCRT (69, 145).

CONCLUSION

Rectal cancer has many important clinicopathologic differences from colon cancer and presents a unique challenge requiring careful evaluation and management involving pathologists, radiologists, surgeons, and radiation and medical oncologists. The current TNM classification stratifies rectal cancer patients by stage, ultimately impacting whether patients receive neoadjuvant therapy. However, there is considerable heterogeneity in terms of response rates and outcomes within stages. Therefore, a multimodality approach is crucial in providing appropriate and effective treatment to rectal cancer patients. An understanding of the pathological, molecular genetics, and immunological features underlying CRC tumorigenesis and progression provides a further framework to evaluate the disease and its management. Delivery of personalized multimodal care for LARC in the coming years will require sophisticated prognostic modeling

that accounts for this heterogeneity as well as consideration of the socioeconomic and healthcare context in which this care is delivered.

AUTHOR CONTRIBUTIONS

All authors contributed significantly to all aspects of the paper.

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