Review

Immune Checkpoint Inhibition as a Strategy in the Neoadjuvant Treatment of Locally Advanced Rectal Cancer

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ABSTRACT

The treatment of locally advanced rectal cancer (LARC) has seen major advances over the past 3 decades, with multimodality treatment now standard of care. Combining surgical resection with radiotherapy and/or chemotherapy can reduce local recurrence from around 20% to approximately 5%. Despite improvements in local control, distant recurrence and subsequent survival rates have not changed. Immune checkpoint inhibitors have improved patient outcomes in several solid tumor types in the neoadjuvant, adjuvant, and advanced disease setting; however, in colorectal cancer, most clinical trials have been performed in the metastatic setting and the benefits confined to microsatellite instability–high tumors. In this article, we review the current preclinical and clinical evidence for using immune checkpoint inhibition in the treatment of LARC and discuss the rationale for specifically exploring the use of this therapy in the neoadjuvant setting. We summarize and discuss relevant clinical trials that are currently in setup and recruiting to test this treatment strategy and reflect on unanswered questions that still need to be addressed within future research efforts.

Keywords: neoplasm, rectum, radiotherapy, chemotherapy, immunotherapy, immunology, clinical trials, immune checkpoint inhibitor, colorectal cancer

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer globally,[1] and approximately one-third of CRC tumors are located in the rectum.[2] Nearly three-quarters of patients diagnosed with rectal cancer present with localized, nonmetastatic disease.[2] Although surgery alone for early-stage tumors is often curative, in locally advanced disease, this approach has historically resulted in unacceptably high rates of local recurrence (16% in stage II and 29% in stage III).[3] Consequently, neoadjuvant treatment is recommended before surgery, based on high-quality magnetic resonance imaging (MRI) staging,[4,5] with the aim of reducing local recurrence rates to approximately 5%.[6] Although very effective from an oncological standpoint, the functional and psychological long-term sequelae of combined therapy incorporating radical surgery (± permanent stoma) are well recognized.[7]

The use of multimodality neoadjuvant treatment for locally advanced rectal cancer (LARC) has resulted in three primary response patterns: pathological complete response (pCR) with rates of approximately 14%, some tumor regression in approximately 65%, and no macroscopic regression in approximately 20% of patients.[8–10] Thus, in a small proportion of well-selected patients who achieve complete clinical response (cCR), treatment de-escalation and "organ preservation" are possible, and these patients can potentially avoid the risks and sequelae of surgery.[11,12] Published series have demonstrated similar disease free survival (DFS) and overall survival (OS) for patients who have organ preservation compared with those who undergo surgery,[13,14] with 3-year colostomy-free survival of up to 74%.[14] For patients who respond poorly to neoadjuvant treatment, mortality is driven by distant relapse and up to 30% will die from distant disease.[15] Therefore, increasing the

proportion of patients who achieve cCR and reducing distant recurrence have both been identified as top research priorities.[16]

Over the past 2 decades, an improved understanding of the role of the immune system in response to malignant cells has spurred the development of targeted therapies to shape this response for therapeutic benefit.[17] Naturally occurring checkpoint molecules within the host immune system, for example PD-1 and CTLA-4, exist, which prevent immune overactivation. These T-cell surface proteins, which bind to PDL-1 on antigen-presenting cells (APCs) and tumor cells (PD-1), or B7-1 and B7-2 on APCs, downregulate immune activation.[18] Therapies that inhibit the binding of these checkpoint molecules therefore increase T-cell–mediated antitumor immunity. In clinical practice, immune checkpoint inhibitors (ICIs) have been particularly successful in tumor types such as melanoma, renal cancer, and non–small-cell lung cancer (NSCLC); however, for other solid organ tumors their use has lagged.[19]

In CRC, extremely encouraging response rates with immunotherapy treatment have been observed in a select group of patients, predicted in a large part on the presence of microsatellite instability (MSI) within the tumor. Errors in DNA base pairing introduced during cell replication are usually repaired via the mismatch repair (MMR) system and microsatellites are short DNA sequences that are markers of deficient MMR. Disease with MSI is defined by high mutational burden and very low or absent copy number variation, whereas microsatellite stable (MSS) disease is characterized by low mutation burden and high copy number variation.[20] MSI-High (MSI-H) disease is enriched in earlier CRC disease stages with rates of 10 to 15% for stage II-III,[20] falling to approximately 4% for patients with stage IV CRC.[21] Overall, MSI confers improved prognosis in nonmetastatic CRC,[22] but there is a trend to worse prognosis for those with stage IV disease.[23] An improved understanding of processes underlying MSI response/nonresponse, as well as identification of additional biomarkers, may be the key to unlocking the potential for more widespread treatment of CRC with immune checkpoint therapy by potentiating this beneficial biological effect in MSS CRC. This is particularly true in rectal cancer in which MSI has a low prevalence.[24]

This concise review aims to explain the rationale for using immune checkpoint inhibition as part of the neoadjuvant management of LARC. We first describe the current landscape of neoadjuvant therapy for LARC, followed by the clinical evidence for immune checkpoint inhibition in CRC and the biological rationale underpinning the combination of immune checkpoint inhibitors with treatments such as radiotherapy and chemotherapy. Finally, we summarize current clinical studies that are assessing this strategy and reflect on unanswered gaps in knowledge that still need to be addressed.

CONTEMPORARY NEOADJUVANT TREATMENT STRATEGIES FOR LOCALLY ADVANCED RECTAL CANCER

There are two principal radiotherapy strategies in widespread use for LARC: a short-course radiotherapy (SCRT) regimen (25 Gy in 5 fractions) and a long-course chemoradiotherapy (LCRT) regimen (45.0–50.4 Gy in 25–28 fractions) with concurrent fluoropyrimidine chemotherapy[4,5] (Fig. 1). Attempts to intensify neoadjuvant radiation sensitivity using additional or alternative cytotoxics, such as oxaliplatin,[25–30] have had inconsistent results and no additional agents are widely used concomitantly with radiotherapy as standard of care. The recently reported toxicity results from the phase III ARISTOTLE trial, which has tested LCRT intensification with irinotecan, have shown a modest increase in G3 toxicity, but with no significant difference in pCR rates (20.2 versus 17.4%; $p = 0.45$)[31] and the full primary outcome of DFS results from this trial are awaited.

Traditionally, SCRT treatment was followed immediately (within 1 week) by surgical resection; however, this approach had inferior tumor downstaging outcomes compared with LCRT,[9,32] albeit similar local control. It has since been demonstrated that leaving a gap of 4 to 8 weeks before surgery (SCRT-delay) offers equivalent oncological outcomes to LCRT and delay, or SCRT and immediate surgery, with less postoperative morbidity.[33] The SCRT-delay strategy also achieves higher rates of complete response not usually appreciated with SCRT-immediate surgery (pCR 11.8 versus 1.7%).[33] LCRT has traditionally been followed by surgery at approximately 6 weeks, as this interval demonstrated improved tumor downstaging compared with an interval of 2 weeks. [34] Despite data indicating that a longer interval from LCRT to surgery does not affect outcomes, [35,36] there is now a large evidence base to suggest that lengthening the interval between LCRT completion and surgery may enhance response. [8,37–41] In a recent analysis of pooled clinical trial data, Gambacorta et al[42] demonstrated that cumulative pCR rates were higher in a group of patients with a median interval of 6 weeks or more (18.8%) compared with those with an interval of less than 6 weeks (11.6%; $p < 0.01$), with no significant difference between DFS or OS. There was a significant correlation between pCR and time from LCRT to surgery as a continuous variable ($p < 0.01$, linear regression coefficient 2.24) with a significant increase in cumulative pCR rate between week 4 (1%) and 11 (13%), plateauing at week 16 (pCR rate of 14%). Landmark studies in the neoadjuvant management of LARC are summarized in Figure 1. [9,32,33,37,43–58]

In addition to the maturation of tumor regression, exploiting the gap between completing SCRT/LCRT and subsequent resection is attractive for a number of reasons. Clinical studies administering adjuvant chemotherapy for rectal cancer have been negative,[59–61] closed

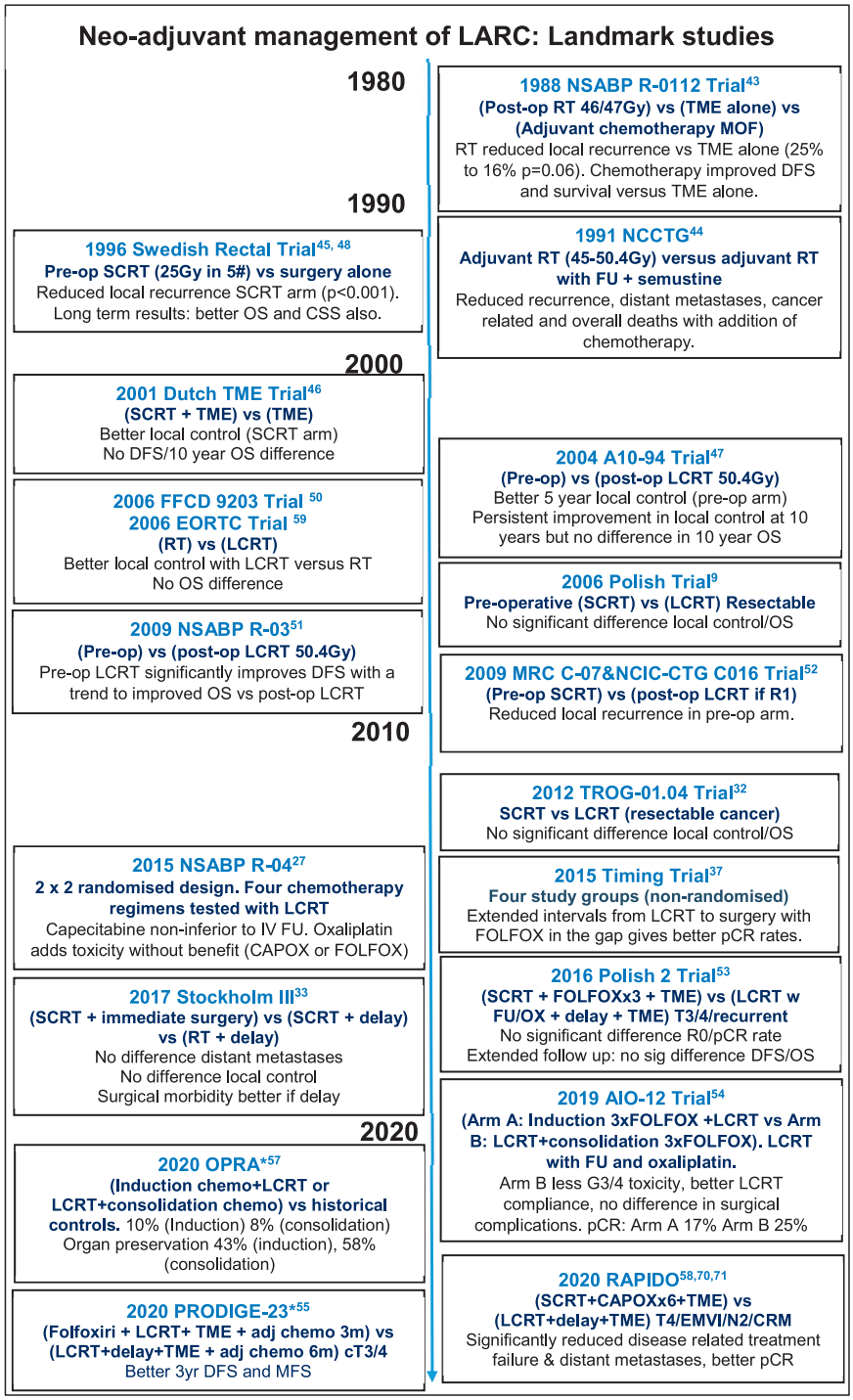

Figure 1.—Landmark studies in the treatment of locally advanced rectal cancer. Asterisk (*) indicates awaiting full results. AIO: Arbesitsgemeinschaft Internistische Onkologie; CAPOX: capecitabine and oxaliplatin; CRM: circumferential resection margin; CSS: cancer-specific survival; DFS: disease-free survival; EMVI: extramural vascular invasion; EORTC: European Organisation for Research and Treatment of Cancer; FFCD: Federation Francophone de Cancerologie; FOLFOX: 5-fluorouracil and oxaliplatin; FU: fluorouracil; LCRT: long-course chemoradiation; MFS: metastasis-free survival; MOF: 5-fluorouracil, vincristine and semustine; MRC: Medical Research Council; NCCTG: North Central Cancer Treatment Group; NCIC-CTG: National Cancer Institute of Canada Clinical Trials Group; NSABP: National Surgical Adjuvant Breast and Bowel Project; OPRA: organ preservation in rectal adenocarcinoma; OS: overall survival; OX: oxaliplatin; pCR: pathological complete response; RAPIDO: rectal cancer and preoperative induction therapy followed by dedicated operation; RT: radiotherapy; SCRT: short course radiotherapy; TME: total mesorectal excision; TROG: Trans-Tasman Radiation Oncology Group.

early due to poor recruitment,[62,63] or were underpowered to define the benefit of adjuvant chemotherapy in patients with LARC treated with radiation.[64] Neoadjuvant chemotherapy appears to have better compliance,[65,66] and earlier introduction of systemic therapy may impact on distant relapse. Last, there is the opportunity to further downstage the in situ disease and better understand the tumor biology. The results of a recent meta-analysis of outcomes from trials assessing total neoadjuvant strategies are detailed elsewhere (n = 28 studies).[67] In summary, this study showed the average pCR achieved with the addition of systemic chemotherapy to LCRT across all included studies was 22.4% and this was increased by 39% (p = 0.01) compared with comparative studies (n = 10) using LCRT alone.

Adding FOLFOX (5-fluorouracil and oxaliplatin) chemotherapy sequentially with LCRT produces encouraging pCR rates compared with LCRT alone,[38,68] and 2 randomized phase II trials have indicated that the

sequencing of neoadjuvant treatments may be important.[54,57] Specifically, a German phase II trial (CAO/ARO/AIO-12) that compared giving chemotherapy before LCRT versus after radiotherapy, showed better treatment compliance and pCR rates for the latter approach.[54] The preliminary results from the Organ Preservation in Rectal Adenocarcinoma (OPRA) trial have shown than offering consolidation chemotherapy after LCRT improves the 3-year organ preservation rate (59%; 95% CI 50–69%; versus 43%; 95% CI 35–54%; log rank $p = 0.007$) compared with induction chemotherapy before LCRT.[57] For SCRT, radiotherapy followed by three cycles of FOLFOX produces similar response rates to oxaliplatin-based LCRT for cT3 and cT4 tumors.[69]

Even more encouraging are the results from a recent Dutch/Scandinavian Rectal cancer And Preoperative Induction therapy followed by Dedicated Operation (RAPIDO) trial, which demonstrated that SCRT followed by chemotherapy (18 weeks of CAPOX [capecitabine plus oxaliplatin]) before surgery improved disease-related treatment failure and pCR rates compared with LCRT with optional adjuvant chemotherapy (standard arm).[58,70,71] Importantly, this is one of the first trials to achieve improvement in distant recurrence rates, with a reduction of almost 7% for the experimental arm (hazard ratio [HR] 0.69; 0.53–0.89; $p = 0.04$). A similar finding in improving the rate of distant metastases was reported in the PRODIGE-23 trial,[55] which compared the addition of a triple chemotherapy combination (FOLFIRINOX: irinotecan 150 mg/m^2 day 1 (D1), oxaliplatin 85 mg/m^2 D1, 5-fluorouracil 400 mg/m^2 bolus plus 2400 mg/m^2 infusion over 46 hours) with LCRT in the neoadjuvant setting to LCRT alone, with high compliance rates with chemotherapy (> 90%) and radiotherapy (> 98%) being maintained, despite the intensive approach. In RAPIDO there was similarly good compliance when chemotherapy was delivered preoperatively (and improved compared with postoperatively). Although maturing data will be further interrogated, these data continue to support the argument that using systemic treatment in the neoadjuvant setting, rather than postoperatively, is beneficial when used with SCRT or LCRT.[58,70,71]

Other systemic anticancer agents have been trialed in this space but with minimal success. For example, the multicenter phase II AVACROSS study added anti–vascular endothelial growth factor (bevacizumab) therapy to oxaliplatin-based chemotherapy followed by LCRT.[72] Although feasible, with an encouraging pCR rate of 36% (95% CI 22.3%–51.3%), postoperative complications were unexpectedly high, with two deaths (4%), five anastomotic leaks (11%), and 11 patients requiring second operations (23%). The addition of epidermal growth factor receptor–directed therapy (cetuximab)[73,74] also failed to meet pre-specified improvements in pCR-related endpoints. The outcomes from the parallel arm phase II platform trial NRG-GI002, which is testing the addition of radiosensitizers to neoadjuvant radiation as part of a total neoadjuvant approach, are eagerly awaited.[75] A summary of the treatments used and the pCR rates attained from the trials investigating intensification of neoadjuvant treatment is presented in Table 1.

Despite the limited success of targeted agents, the underlying clinical rationale for treatment intensification in the neoadjuvant setting for patients with LARC remains. For example, in the RAPIDO trial, despite an almost doubling of pCR rates in the experimental arm, approximately 10% of patients in both arms had an R1/2 resection, and despite the reduction in distant relapse, more than a fifth of patients developed distant metastatic disease. This underscores an ongoing need to find treatments that improve downstaging and decrease systemic relapse. Biomarkers to identify patients who are likely to be poor responders to radiation and cytotoxic chemotherapy alone are particularly pressing. The molecular landscape of CRC suggests that immunomodulation with ICIs is a logical next step.

CURRENT ROLE OF CHECKPOINT INHIBITION IMMUNOTHERAPY IN COLORECTAL CANCER

Until recently, documented responses to ICIs have largely been confined to colorectal tumors with deficiency in MMR. The MMR status of colorectal tumors can be tested using DNA-based methods, which look for the number of microsatellite markers (2–5: MSI-H, 1: MSI-Low [MSI-L], or 0: MSS), or immunohistochemistry can be used to look for deficiency in one of the MMR proteins (MLH-1, MSH-2, MSH-6, PMS-2). Tumors displaying loss of MMR are known as dMMR and are considered MSI-H, and those without loss of these proteins are known as MMR proficient (pMMR) and are considered MSI-L or MSS. [76] One of the reasons underpinning impressive responses of dMMR tumors to immunotherapy relates to the high mutational burden in MSI-H tumors, which leads to numerous tumor neoantigens that are recognized and targeted by the host immune system, reflected by high levels of tumor-infiltrating lymphocytes (TILs).[77] The marked differences in tumor responses based on MMR status, in CRC and other cancer types, led to the tumor agnostic US Food and Drug Administration approval of immunotherapy for any MSI-H cancers.[78]

Several trials have demonstrated extremely encouraging response rates to immune checkpoint inhibition in patients with metastatic dMMR CRC, even those who were heavily pretreated (Table 2).[79–86] Moving treatment into the first-line setting has improved responses and using doublet immunotherapy treatment has increased response rates even further. For example, recent results from the Checkmate-142 trial reported an objective response rate of 69% in the first-line setting using dual-checkpoint inhibition for patients with dMMR CRC.

Table 1.—Summary of pathological complete response rates

Trial or Author Name	Year	Type of Study	No. of Patients	Type of Neoadjuvant Treatment	pCR (%)
Lyon R90–01[34]	1999	Randomized trial	201	Radiotherapy with surgery after 2 weeks vs radiotherapy with surgery after 6–8 weeks	pCR not reported
Stein[35]	2003	Prospective cohort	40	LCRT with irinotecan and 5-fluorouracil. Group A: Surgery 4–8 weeks post LCRT. Group B: Surgery 10–14 weeks post LCRT.	21% (4–8 weeks) 14% (10–14 weeks), $p = 0.97$
CAO/ARO/AI0–94[47]	2004	Phase III RCT	823	Preoperative vs postoperative LCRT	9% in preoperative LCRT arm
Polish Trial (Bujko)[9]	2006	Phase III RCT	312	Preoperative SCRT vs LCRT	0.7% (SCRT) 16.1% (LCRT) (Significance not reported)
Tulchinsky[40]	2008	Retrospective cohort study	132	LCRT followed by surgery at 7 weeks or less vs > 7 weeks	17% (7 weeks or less) 35% (> 7 weeks), $p = 0.03$
ACCORD-12/0405-Prodige 2[25]	2010	Phase III RCT	598	LCRT vs LCRT with oxaliplatin	13.9% (LCRT) 19.2% (LCRT with oxaliplatin), $p = 0.09$
Kim[74]	2010	Single-arm phase II trial	40	LCRT with concurrent capecitabine, cetuximab and irinotecan	23.10%
GCR-3[65]	2010	Phase II randomized trial	108	LCRT with neoadjuvant vs postoperative chemotherapy (CAPOX)	13% (postoperative) 14% (preoperative), $p = 0.94$
STAR-01[29]	2011	Phase III RCT	747	LCRT vs LCRT with oxaliplatin	16% both arms
AVACROSS[74]	2011	Phase II single arm trial	47	Induction XELOX and bevacizumab followed by radiotherapy with concomitant XELOX and bevacizumab	36%
TROG 01.04[32]	2012	Phase III RCT	326	Preoperative SCRT vs LCRT	15% (LCRT) 1% (SCRT) (Significance not reported)
CAO/ARO/A10–04[28]	2012	Phase III RCT	1236	LCRT with adjuvant 5-fluorouracil vs LCRT with concomitant and adjuvant FOLFOX	13% (LCRT + adjuvant 5-fluorouarcil) 17% (LCRT with FOLFOX), $p = 0.038$
EXPERT-C[73]	2012	Randomized phase II trial	165	Induction CAPOX followed by LCRT with adjuvant CAPOX vs the same regimen with CAPOX-cetuximab (CAPOX-C)	9% (CAPOX) 11% (CAPOX-C), $p = 1.0$
Sloothaak[39]	2013	Retrospective cohort study	1593	LCRT followed by surgery at < 13 weeks, 13–14 weeks, 15–16 weeks, > 16 weeks after the start of LCRT	10.3% (< 13 weeks) 13.1% (13–14 weeks) 18.0% (15–16 weeks) 11.0% (> 16 weeks), $p = 0.013$
Probst[8]	2015	Retrospective cohort study	17,255	Exact treatment unknown. Time from LCRT to surgery < 6 weeks, 6–8 weeks or > 8 weeks.	8.7% (< 8 weeks) 11.7% (6–8 weeks) 13.2% (> 8 weeks), $p < 0.001$
Garcia-Aguilar[38]	2015	Phase III RCT	259	LCRT with surgery at 6–8 weeks vs LCRT + 2 × mFOLFOX6 vs LCRT + 4 × mFOLFOX6 vs LCRT + 6 × mFOLFOX6	18% (LCRT alone) 25% (LCRT + 2 mFOLFOX6) 30% (LCRT + 4 mFOLFOX6) 38% (LCRT + 6 mFOLFOX6), $p = 0.0036$
FOWARC[30]	2016	Phase III RCT	495	Fluorouracil-radiotherapy vs mFOLFOX6-radiotherapy vs mFOLFOX6	14% (Fluorouracil-radiotherapy) 27.5% (mFOLFOX6-radiotherapy) 6.6% (mFOLFOX6)
PETACC-6[26]	2016	Phase III RCT	1094	LCRT with capecitabine adjuvant chemotherapy vs LCRT with neoadjuvant and adjuvant CAPOX	pCR not reported
GRECCAR-6[36]	2016	Phase III RCT	265	LCRT followed by surgery at 7 vs 11 weeks	15% (7 week arm) 17.4% (11 week arm), $p = 0.5983$
Polish 2 Trial (Bujko) [53]	2016	Phase III RCT	541	SCRT + FOLFOXx3 (Group A) vs LCRT w FU/OX + delay (Group B)	16% (Group A) 12% (Group B), $p = 0.17$
Stockholm III[33]	2017	Phase III RCT	840 overall trial, 697 for pCR substudy	SCRT vs SCRT + delay vs LCRT + delay	0.3% (SCRT) 10.4% (SCRT + delay) 2.2% (LCRT + delay)

Table 1.—Continued

Trial or Author Name	Year	Type of Study	No. of Patients	Type of Neoadjuvant Treatment	pCR (%)
Cercek[66]	2018	Retrospective cohort study	628	LCRT + adjuvant chemotherapy vs total neoadjuvant treatment (TNT, LCRT with sequential neoadjuvant chemotherapy)	21% (LCRT + adjuvant chemotherapy) 36% (TNT). $p < 0.001$
AIO-12[54]	2019	Phase II RCT	311 (306 evaluable)	Induction chemotherapy (FOLFOX) + LCRT vs LCRT + consolidation chemotherapy	17% (Induction chemotherapy) 25% (consolidation chemotherapy). Not compared statistically (pick the winner trial design).
ARISTOTLE[31]*	2020	Phase III RCT	564	LCRT vs LCRT with irinotecan	17.4% (LCRT) 20.2% (LCRT with irinotecan), $p = 0.45$
OPRA[57]*	2020	Phase III RCT	324 (307 evaluable)	Induction chemotherapy + LCRT vs LCRT + consolidation chemotherapy (CAPOX or FOLFOX)	10% (induction chemotherapy) 8% (consolidation chemotherapy). Rates of organ preservation 43% (induction), 58% (consolidation), $p = 0.01$
RAPIDO[58,70,71]	2020	Phase III RCT	920	SCRT + CAPOX/FOLFOX vs LCRT and optional adjuvant chemotherapy	27.7% (SCRT + CAPOX/FOLFOX) 13.3% (LCRT + optional adjuvant chemotherapy), $p < 0.001$
PRODIGE 23[55]*	2020	Phase III RCT	231	LCRT + 6 months adjuvant chemotherapy vs mFOLFIRINOX + LCRT + 3 months adjuvant chemotherapy	11.7% (LCRT + adjuvant chemotherapy), 27.5% (mFOLFIRINOX + LCRT + adjuvant chemotherapy), $p < 0.001$

AIO: Arbesitsgemeinschaft Internistische Onkologie; CAPOX: capecitabine and oxaliplatin; FOLFOX: 5-fluorouracil and oxaliplatin; LCRT: long course chemoradiation; mFOLFIRINOX: fluorouracil, irinotecan and oxaliplatin; OPRA: organ preservation in rectal adenocarcinoma; pCR: pathological complete response; RAPIDO: rectal cancer and pre-operative induction therapy followed by dedicated operation; RCT: randomized controlled trial; SCRT: short course radiotherapy; TNT: total neoadjuvant treatment; TROG: Trans-Tasman Radiation Oncology Group.
*Await full results.

Although these responses in both treatment naïve and heavily pretreated patients with metastatic dMMR CRC are exciting, approximately 95% of patients with metastatic CRC have pMMR disease. Traditionally, responses to ICIs in patients with metastatic CRC unselected for MMR status have been very low (< 10%),[19,87–90] and the proposed mechanisms of resistance of pMMR colorectal tumors to ICIs are discussed elsewhere.[91] Nevertheless, some encouraging data have recently been presented in abstract form from a trial that recruited patients with pMMR CRC (Table 2).[85,92] Patients with heavily pretreated metastatic CRC (n = 179) were randomized to receive either a combination of durvalumab (anti-PDL-1) 1500 mg 4 weekly with tremelimumab (anti-CTLA 4) 75 mg on day 1, or best supportive care. Patients in the former group survived longer compared with the control arm (6.6 months median OS versus 4.1 months, $p = 0.07$, HR 0.72, 95% CI 0.54–0.97). In patients with pMMR tumors, the HR for death was 0.66 (90% CI 0.48–0.89; $p = 0.02$) and those with a tumor mutational burden achieved significantly longer survival when treated with immunotherapy compared with best supportive care alone (median OS 5.5 versus 3.0 months; $p = 0.004$). The results of this trial show that using combination treatment is one way in which resistance of pMMR disease to ICIs may be overcome.

Another strategy for overcoming this resistance is to use immunotherapy earlier in the disease trajectory. Recently published evidence from an early-phase trial (NICHE NCT03026140) in the neoadjuvant setting in colon cancer, has shown improved response rates to immunotherapy compared with those previously seen for metastatic patients, and in particular for those with pMMR disease. Patients with dMMR and pMMR colon cancer were treated with concurrent anti-PD1 and anti-CTLA4 therapy.[86] All of those with dMMR disease displayed a pathological response, but, encouragingly, so did 27% (95% exact CI 8%–55%) of those with pMMR disease (Table 2). Although provocative, these data require validation in a larger cohort, but certainly provide optimism regarding the benefit that may be gained from using immunotherapy to treat CRCs with traditionally resistant molecular profiles. The use of immunotherapy both for patients with pMMR and those with dMMR CRC is an area of intense interest, and a contemporary list of ongoing clinical trials is available elsewhere.[93]

RATIONALE FOR USE OF IMMUNOTHERAPY AS A NEOADJUVANT STRATEGY FOR RECTAL CANCER

There are important distinctions between the metastatic and neoadjuvant setting that provide a strong

Table 2.—Selection of clinical trials that have tested immune checkpoint inhibition for dMMR and pMMR CRC

Trial	No. of Patients	Disease Setting	MMR Status	Previous Treatment	Trial Treatment	Trial Outcomes
Checkmate-142[79]	119	mCRC	dMMR	≥1 line of previous treatment	Nivolumab alone Nivolumab + ipilimumab	ORR 31% nivolumab ORR 55% nivolumab + ipilimumab
Checkmate-142[80]	45	mCRC	dMMR	No previous treatment for mCRC	Nivolumab + ipilimumab	ORR 69%
Keynote-016[81]	41	Cohort A: dMMR mCRC ($n = 11$) Cohort B: pMMR mCRC ($n = 21$) Cohort C: dMMR non-CRC ($n = 9$)	dMMR and pMMR	≥2 prior lines of treatment	Pembrolizumab	dMMR CRC 40% immune-related ORR pMMR 0% immune related ORR
Keynote-164[82]	63	mCRC	dMMR	≥1 line of previous treatment	Pembrolizumab	ORR 33%
Keynote-164[82]	61	mCRC	dMMR	≥2 prior lines of treatment	Pembrolizumab	ORR 33%
Keynote-177[83]	307	mCRC	dMMR	No previous treatment for mCRC	Pembrolizumab versus chemotherapy ± cetuximab or bevacizumab)	Overall response 44% (pembrolizumab) Overall response 33% chemotherapy
NCT01693562[84]	36	mCRC	dMMR	Unknown	Durvalumab	ORR 22%
NCT02227667[84]	11	mCRC	dMMR	Unknown	Durvalumab	ORR 27%
Canadian Cancer Trials Group CO.26 study[85]	179	mCRC	dMMR ($n = 2$), pMMR ($n = 166$), unknown ($n = 12$)	Patients had received all available standard therapies	Durvalumab + tremelimumab + best supportive care versus best supportive care	OS 6.6 months versus 4.1 months (HR 0.72, 90% CI 0.54–0.97, $p = 0.07$) Disease control* 22.7% versus 6.6%. (HR 4.16, 90% CI 1.4–12.3, $p = 0.006$)
NICHE[86]	35 evaluable	Neo-adjuvant colon cancer	dMMR ($n = 15$) and pMMR ($n = 20$)	Nil (neo-adjuvant setting)	Nivolumab + ipilimumab (with or without celecoxib for pMMR patients)	100% pathological response dMMR disease, 27% pMMR disease

dMMR: mismatch repair deficient; HR: hazard ratio; mCRC, metastatic colorectal cancer; ORR: objective response rate; pMMR: mismatch repair proficient.
*Disease control consists of complete response/partial response or stable disease.

rationale to further investigate the use of immune checkpoint inhibition neoadjuvantly for LARC. With the primary disease in situ, there are differences in the intrinsic tumor biology, as well as extrinsic microenvironment. Also, the combination of immunotherapy with current neoadjuvant treatments of radiation and chemotherapy potentially offer a therapeutic advantage compared with using immune checkpoint inhibition alone.

Favorable Tumor and Host Immune Environment

In vivo and early trial evidence suggest that augmented host antitumor immune responses are triggered when immune checkpoint inhibition is given with the primary tumor in situ. Liu et al[94] demonstrated stronger expansion of tumor-specific CD8+ T cells in blood and peripheral organs after neoadjuvant treatment with anti-PD-1 and anti-CD137 therapies in a mouse breast cancer model, compared with post-surgery. Half of mice treated with this combination neoadjuvantly displayed long-term survival, versus none in the adjuvant group. In the clinical setting, Blank et al[95] reported results of a small, early-phase, but translationally rich study, which treated patients with stage III melanoma with combination immune checkpoint inhibition in the neoadjuvant versus adjuvant setting. Again, an increased expansion of T-cell clones was observed preoperatively. Similar results have also been observed in patients with NSCLC[96] and bladder cancer.[97]

In the study by Blank et al,[95] the rate of grade 3/4 immune-related adverse events was much higher (90% in both arms) compared with the expected incidence of toxicity from studies using the same combination in patients with stage IV melanoma.[98] This high level of toxicity combined with the high level of pathological responses seen in this study imply that, whether given neoadjuvantly or adjuvantly, in patients with earlier-stage disease there is less intrinsic suppression of the host immune system. When using immune checkpoint inhibition earlier in the disease trajectory, a lower dose may therefore achieve the same efficacy as in the metastatic setting and may be required to reduce unacceptable levels of adverse events related to immune stimulation.

In summary, this preclinical evidence and clinical results from patients with colon cancer and other cancer types provide an impetus to test neoadjuvant immunotherapy more extensively for treatment of LARC.

Immune Checkpoint Inhibition and Radiation Treatment

Local effects

Traditional radiobiological dogma dictates that radiation-induced tumor cell death is mediated via direct DNA damage, free radical damage, and subsequent DNA double-strand breaks, which if not repaired leads to catastrophic death.[99,100] But it is increasingly recognized that the tumor response also may be a result of the effects on the immune microenvironment. Radiation stimulates an antitumor immune response via several mechanisms, such as increased antigen release and novel peptide antigen expression on tumor cells, production of interferon type 1,[101,102] complement activation, translocation of calretinin to the tumor cell surface, increased expression of MHC class I,[103] expression of RAE-1,[104] and release of damage-associated molecular patterns such as HMGB1 and ATP leading to dendritic cell recruitment.[105] The aggregate effect is to induce local, tumor-specific T-cell responses. In turn, this may prime the local tumor environment for treatment with immunotherapy.[106] Combination therapy has already achieved success in the clinic for some solid tumor types, such as NSCLC, bladder cancer, and melanoma.[107–111]

It remains unclear which radiotherapy fractionation offers most synergy in combination with checkpoint inhibition. Dovedi and colleagues[112] found similar combinatorial activity from anti-PD-1 treatment and radiotherapy across a range of radiotherapy fractionation schedules. Others[113] demonstrated that a higher dose per fraction (5 Gy per fraction) increased calreticulin release, stimulating an increase in immune recognition compared with more fractionated regimens. In contrast, they showed that the optimum ratio of effector to T regulatory cells occurs when a dose of 2 Gy per fraction is used.

Several other variables, such as host, tumor, and treatment-related factors influence responses,[114] in particular, the scheduling of immunotherapy delivery in relation to radiotherapy. For example, clinical trial evidence from lung cancer has shown that survival is increased when immune checkpoint inhibition is delivered after radiation in the stage III setting.[110] Preliminary findings from the VOLTAGE-A trial in LARC[115] also showed encouraging results with sequential treatment. In contrast, preclinical evidence from Dovedi et al[101] implies that earlier use of immunotherapy and specifically its combination with radiation may lead to improved responses. Dovedi et al[101] demonstrated that in vivo treatment with radiation and concurrent anti-PD-L1 inhibition contributed to overcome radiation-induced immune suppression, and correlated with improved survival versus radiotherapy alone. This improvement in survival was not observed when sequential treatment (immune-checkpoint inhibition at day 7 post-radiotherapy) was used. Analysis of tumor-infiltrating CD8+/CD4+ T cells at 24 hours after the last dose of radiotherapy demonstrated an acute increase in PD-1 expression, which was almost eliminated by day 7. This suggests deletion or anergy of tumor-specific CD8+ T cells by the later time point as the mechanism for the differential response dependent on the timing at which immune checkpoint inhibition is delivered.

In summary, radiotherapy alters the local tumor immune microenvironment in a way that could be exploited with immune checkpoint inhibition to produce a synergistic therapeutic response. More work is required in both the preclinical and clinical settings to understand the optimal timing for immune-checkpoint delivery. Clinical evidence implies that improved responses are seen with sequential treatment, but preclinical studies suggest that concomitant treatment may be able to enhance responses further. Last, the binary concept of "immune activation" may need to be supplanted for a more qualitative measure of the type of immune response elicited by different doses, fractionations, and schedules.

Distant effects

The effects of immunotherapy-radiotherapy combinations may not only occur at the local tumor level. Although the immune-stimulatory effects of radiotherapy are predominantly in the radiation field, several case reports on tumor types such as melanoma[116] and NSCLC[117] have demonstrated tumor regression distant to the irradiated site when radiotherapy is combined with immune checkpoint inhibition. These abscopal effects (shrinkage of untreated metastases concurrently with shrinkage of tumors within the area the localized radiotherapy treatment) are rarely seen with radiotherapy in isolation.[118] This can be explained in part by considering how the immune system shapes cancer growth. It is hypothesized that the host immune system acts to control malignant growth initially via elimination, in which immunity functions as an extrinsic tumor suppressor or later on, via an equilibrium state, whereby the immune system holds remaining tumor cells in a state of functional dormancy. Finally, tumors become clinically apparent when they escape immune control, a state in which the tumor has learned to evade the host immunity. This three-stage process of elimination, equilibrium, and escape is referred to as immunoediting.[119]

Radiotherapy can elicit a tumor-specific T-cell response that, when combined with systemic immune checkpoint inhibition, switches immunoediting by the host immune system back into the elimination and equilibrium phases. This may explain why disease sites may regress even though they are distant to the primary tumor that is being irradiated. [118] Supporting this hypothesis, Dovedi et al[112] demonstrated that radiotherapy alone increases T cells in an irradiated, but not out-of-field tumor, and that similarly anti-PD-1 therapy alone did not increase T-cell infiltration in either the primary or distant tumor. The combination of radiotherapy and anti-PD-1 therapy led to increased T-cell infiltration in both the irradiated and out-of-field tumor and an increase in T-cell diversity compared with nontreated or anti-PD-1 monotherapy-treated mice. Sequential immune inhibition did not achieve the same level of abscopal effect as concurrent treatment, suggesting that exhaustion of tumor-infiltrating T cells may occur after radiation unless the PD-1/PD-L1 axis is blocked.

In conclusion, combination of radiotherapy and immunotherapy has the potential to improve out-of-field, distant micrometastatic disease by fundamentally resetting the host–tumor immune interaction.

Immune Checkpoint Inhibition and Chemotherapy

There has been concern that cytotoxic chemotherapy, when used concomitantly with immunotherapy in the neoadjuvant setting[120] may kill proliferating T cells and dampen the induction of a broad T-cell response. Despite this, Vincent et al[121] demonstrated that fluoropyrimidine therapy is capable of eliminating immunosuppressive myeloid-derived cells within the tumor microenvironment, thereby enhancing host antitumor immune responses. Furthermore, Tesniere et al[122] showed that oxaliplatin elicits immunogenic cell death in microsatellite stable colon tumors. Preclinical data presented in abstract form (Dosset et al, unpublished data, 2018) demonstrated that the combination of fluoropyrimidine and oxaliplatin is capable of increasing PD-L1 and CD8+ T-cell tumor infiltration in animal models.[123] The combination treatment with anti-PD1 and FOLFOX therapy further improved tumor volume reduction compared with chemotherapy or anti-PD1 monotherapy in CT26 and MC38 mice.[123] Evidence of immune activation in patients with rectal cancer after four cycles of FOLFOX has also been demonstrated and quantified by increases in CD3, CD8, MHC1, and PD-L1 in rectal cancer biopsies,[124] with similar findings reported by Teng et al[125] These data suggest, again, that the received wisdom of "immune activation" as a binary switch that is turned off by cytotoxic treatment needs refining. Indeed, combining chemotherapy and immunotherapy in CRC could lead to a synergistic therapeutic effect at both the local and systemic levels.

Overall, evidence from CRC and other tumor types provides a sound rationale to test immune checkpoint inhibition in combination with radiotherapy and/or chemotherapy. Although questions remain over which dose, fractionation, and scheduling of chemo-radiotherapy will produce optimal clinical responses in combination with immunotherapy, these uncertainties can only be addressed with future, translationally rich clinical studies.

CURRENT EVIDENCE AND ONGOING TRIALS INVESTIGATING CHECKPOINT INHIBITORS FOR RECTAL CANCER IN THE NEODJUVANT SETTING

The intense interest in exploring these questions in vivo has culminated in a wealth of clinical trials that are either currently recruiting, or in set up, that use a named checkpoint inhibitor in this context (Table 3). All of the trials use radiation treatment (6/22 SCRT and 15/22

Table 3.—Current trials testing the use of immune checkpoint blockade in the neoadjuvant setting for treatment of LARC (search of clinicaltrials.gov October 2020 using terms *rectal cancer/adenocarcinoma plus immunotherapy/PD-1/PDL-1/CTLA-4*)

NCT Number (Study Name)	Trial Phase	Study Intervention	Immune Check-point Target	Scheduling of Radiation/ICI	Host Institution	Status	Primary Endpoint	Target Recruit-ment, *n*
NCT03102047	II	Durvalumab for patients with MSS rectal cancer (stages II-IV) after completion of LCRT and before surgery.	PD-L1	Sequential	NSABP Foundation Inc.	Recruiting	Median NAR score (compared with historical controls using Wilcoxon test)	47
NCT02948348 (VOLTAGE)[115]	Ib/II	Nivolumab delivered sequentially after LCRT for patients with LARC.	PD-1	Sequential	Takayuki Yoshino, National Cancer Center Hospital East	Recruiting	pCR. (Phase Ib will determine the RPIID)	50
NCT03299660 (Avec-Rec)	II	Avelumab delivered post LCRT presurgery in patients with resectable LARC.	PD-L1	Sequential	Peter MacCallum Cancer Centre, Australia	Recruiting	pCR rate as assessed in surgical specimen	45
NCT04124601 (CHINOREC)	II	Neoadjuvant LCRT with sequential ipilimumab and nivolumab in patients with rectal cancer.	CTLA-4, PD-1	Sequential	State Hospital Wiener Neustadt, Austria and Medical University Vienna, Austria	Recruiting	Incidence of treatment emergent adverse events	80
NCT04340401	II	3 cycles of CAPOX (capecitabine plus oxaliplatin) and SHR-1210 (anti-PD-1 inhibitor), followed by LCRT, followed by 2 cycles of consolidation CAPOX before surgery for patients with rectal cancer.	PD-1	Concomitant	Beijing Cancer Hospital	Recruiting	pCR rate	25
NCT03921684	II	LCRT followed by 6 cycles of mFOLFOX6 in combination with nivolumab before surgery for patients with LARC	PD-1	Sequential	Baruch Brenner, Rabin Medical Center	Recruiting	pCR and incidence of treatment emergent adverse events	29
NCT04017455 (TARZAN)	II	SCRT (5Gy x 5) followed by treatment with bevacizumab (B) and atezolizumab (A). 1 cycle of B, followed by 2 cycles of B and A combination therapy, followed by 1 cycle of A	PD-L1	Sequential	The Netherlands Cancer Institute	Recruiting	Clinical complete and near-complete response rate as assessed by MRI and endoscopy	38
NCT04293419 (DUREC)	II	Patients with high risk rectal cancer receive 6 cycles of mFOLFOX6, followed by LCRT, followed by 8–12 week rest period before surgery. Durvalumab administered throughout (including in the rest period).	PD-L1	Concomitant	Vall d'Hebron Institute of Oncology (VHIO)	Recruiting	pCR rate	58
NCT04109755 (PEMREC)	II	Patients with T3–4N0M0 or TanyN1M0 rectal cancer treated with SCRT with pembrolizumab (200mg) starting on the first day of RT, followed by surgery.	PD-1	Concomitant	University Hospital, Geneva, Switzerland	Recruiting	Tumor regression grade using Mandard regression grade score	25

Table 3.—Continued

NCT Number (Study Name)	Trial Phase	Study Intervention	Immune Checkpoint Target	Scheduling of Radiation/ICI	Host Institution	Status	Primary Endpoint	Target Recruitment, *n*
NCT04304209	II/III	Cohort A: Patients with dMMR/MSI-H LARC receive 3 weekly sintilimab (anti-PD-1), followed by surgery or watch and wait protocol, followed by 4 cycles of adjuvant sintilimab ± CAPOX chemotherapy according to pCR results. Cohort B: pMMR/MSI-L/MSS LARC. All patients receive LCRT with CAPOX chemotherapy, followed by surgery, followed by 4 cycles of adjuvant CAPOX chemotherapy. Patients will be randomised to determine if they receive sintilimab adjuvantly or not.	PD-1	Sequential	Sun Yat-sen University, China	Recruiting	pCR	195
NCT03854799 (AVANA)	II	Patients with LARC receive LCRT with avelumab followed by TME at 8–10 weeks after the end of LCRT	PD-L1		Gruppo Oncologico del Nord-Ovest, Italy	Recruiting	pCR rate	101
NCT04083365 (PANDORA)	II	Patients with operable rectal cancer treated with LCRT, followed by durvalumab starting 1 week after completion of LCRT. Patients will undergo surgery 10–12 weeks after the end of LCRT.	PD-L1	Sequential	Istituto Scientifico Romagnolo per lo Studio e la cura dei Tumori	Recruiting	pCR rate	60
NCT02921256	II	Patients with LARC randomized to 1/3 arms. Arm I: mFOLFOX6 up to 8 weeks, followed by LCRT, followed by surgery. Arm II: mFOLFOX6 up to 8 cycles, followed by LCRT with veliparib, followed by surgery. Arm III: mFOLFOX6 up to 8 weeks followed by LCRT in combination with pembrolizumab for up to 6 cycles starting on day 1 of RT.	PD-1	Concomitant	National Cancer Institute (NCI)	Temporarily stopped for assessment	Change in NAR score (baseline up to 3 years)	348
NCT03127007 (R-IMMUNE)	Ib/II	Atezolizumab delivered concomitantly with LCRT for stage II/III rectal cancer. Phase Ib safety. Phase II efficacy. Randomisation to Experimental arm: Fixed dose 1200 mg/3 weeks atezolizumab given with standard LCRT and until week 12 and surgery planned at week 15 versus standard arm: LCRT followed by surgery.	PD-L1	Concomitant	Grand Hôpital de Charleroi	Recruiting	Rate of adverse events. Rate of pCR.	54

Table 3.—Continued

NCT Number (Study Name)	Trial Phase	Study Intervention	Immune Check-point Target	Scheduling of Radiation/ICI	Host Institution	Status	Primary Endpoint	Target Recruit-ment, *n*
NCT04518280	II	Patients with LARC randomised to SCRT with either induction or consolidation CAPOX plus anti-PD-1 toripalimab. SCRT followed by 6 cycles CAPOX (1000 mg/m^2 D1–14 3 weekly capecitabine plus 130 mg/m^2 oxaliplatin 3 weekly) with 240mg toripalimab 3 weekly or 2 cycles of CAPOX with anti-PD-1 followed by SCRT followed by a further 4 cycles of CAPOX with anti-PD-1. Both arms will be followed with TME surgery.	PD-1	Sequential	Fudan University, China	Not yet recruiting	pCR rate	130
NCT04231552	I/II	Single arm study recruiting patients with LARC and investigating treatment with SCRT followed by CAPOX (capecitabine 1000 mg/m^2 BD for 14 days with oxaliplatin 130 mg/m^2, both 3 weekly) given concomitantly with anti-PD1 camrelizumab (200 mg D1 every 3 weeks) for two cycles.	PD-1	Sequential	Wuhan Union Hospital, China	Recruiting	pCR rate	30
NCT04558684	I/II	Single arm study recruiting patients with LARC and investigating treatment with SCRT followed by two cycles of CAPOX (capecitabine 1000 mg/m^2 BD for 14 days with oxaliplatin 130 mg/m^2, both 3 weekly) with anti-PD1 camrelizumab (200 mg D1 every 3 weeks) for six cycles. Patients who achieve a pCR will be considered for an organ preservation approach and all others will undergo TME surgery.	PD-1	Sequential	Wuhan Union Hospital, China	Not yet recruiting	Clinical complete response rate	30
NCT04357587	I	Single arm study recruiting patients with dMMR stage II/III rectal adenocarcinoma or patients with oligometastatic stage IV disease who are candidates for surgery. All patients will receive LCRT in combination with pembrolizumab (anti-PD-1).	PD-1	Concomitant	Case Comprehensive Cancer Centre	Recruiting	Rate of adverse events, proportion of patients able to complete planned neoadjuvant treatment protocol and the proportion of patients with any delay in planned surgery more than 30 days.	10

Table 3.—Continued

NCT Number (Study Name)	Trial Phase	Study Intervention	Immune Checkpoint Target	Scheduling of Radiation/ICI	Host Institution	Status	Primary Endpoint	Target Recruitment, *n*
NCT04443543	II	Adaptive design cohort study. Patients with LARC will all receive LCRT with 625 mg/m^2 BD of capecitabine and irinotecan 80mg/m2 (UGT1A1*28 6/6) or 65mg/m2 (UGT1A1*28 6/7). Arm 1: patients with MSI-H/dMMR tumours get 3 cycles of Tislelizumab (BGB-A317) 200mg. Arm 2: patients with MSS/pMMR tumors get consolidation chemotherapy according to response to radiation (either XERLIRI or FOLFIRINOX). Any patient who achieves a complete clinical response will be managed with a watch and wait approach rather than radical surgery. Doses for XELIRI: capecitabine 1000 mg/m^2 BD D1–14 with irinotecan 200 mg/m^2 D1 every 3 weeks. FOLFIRINOX: irinotecan 150 mg/m^2 D1, oxaliplatin 85mg/m2 D1, 5-FU 400 mg/m^2 bolus plus 2400 mg/m^2 infusion over 46 hours.	PD-1	Sequential	Fudan University, China	Not yet recruiting	Clinical complete response rate	222
NCT04165772	II	Single arm study treating patients with dMMR stage II/III rectal adenocarcinoma with TSR-02 (anti-PD-1) 500mg flat dose every 3 weeks for 6 months (9 cycles) before standard chemoradiation and surgery. LCRT with an elective dose of 47 Gy and a simultaneous integrated boost to 54 Gy to the primary tumor. Patients who achieve a CR after TSR-02 treatment will proceed to nonoperative follow-up. Those not achieving a cCR will proceed to LCRT.	PD-1	Sequential	Memorial Sloan Kettering Cancer Center	Recruiting	pCR or cCR at 12 months	30
NCT04621370 PRIME-RT	II	Parallel arm study treating patients with LARC. Arm A: SCRT with concomitant durvalumab followed by mFOLFOX6 with durvalumab. Arm B: LCRT with concomitant durvalumab followed by mFOLFOX6 with durvalumab. Total durvalumab in both arms: 4 × 4 weekly 1500 mg cycles. Safety run-in ($n \geq 6$) may include patients with metastatic disease and primary rectal cancer in situ. Main trial ($n = 42$): LARC patients only.	PD-L1	Concomitant	Glasgow CRUK Clinical Trials Unit	Not yet recruiting	pCR or cCR at 6 months	48

Table 3.—Continued

NCT Number (Study Name)	Trial Phase	Study Intervention	Immune Checkpoint Target	Scheduling of Radiation/ICI	Host Institution	Status	Primary Endpoint	Target Recruitment, *n*
NCT04130854 (INNATE)	II	Patients with LARC randomized 3:2 to receive SCRT followed by mFOLFOX6 with APX005M (anti-CD40) given concurrently with radiation and chemotherapy versus SCRT plus mFOLFOX6 without APX005M.	Anti-CD40	Concomitant	University of Texas Southwestern Medical Center	Recruiting	pCR rate	58

BD: twice daily; CAPOX: capecitabine and oxaliplatin; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; D1: day 1; dMMR: deficient mismatch repair; FOLFOX: 5-fluorouracil and oxaliplatin; FU: fluorouracil; Gy: Gray; ICI: immune checkpoint inhibition; LARC: locally advanced rectal cancer; LCRT: long course chemoradiation; mFOLFOX6: modified FOLFOX; MSI-H: microsatellite instability-high; MSS: microsatellite stable; NAR: Neoadjuvant rectal; NCT: national clinical trial identifier; NSABP: National Surgical Adjuvant Breast and Bowel Project; RT: Radiotherapy; OX: oxaliplatin; pCR: pathological complete response; PD-1: programmed cell death protein-1; PD-L1: programmed cell death ligand-1; pMMR: proficient mismatch repair; SCRT: short course radiotherapy; TME: total mesorectal excision.

LCRT) and one trial (PRIME-RT) investigates SCRT and LCRT in two parallel treatment arms. In total, 11 of these trials (50%) use systemic cytotoxic chemotherapy, excluding concomitant radiosensitizing chemotherapy, and one trial (CHINOREC) uses combination immunotherapy (nivolumab and ipilimumab). The most common endpoint is a measure of pathological or clinical complete response (17/22, 77%) and several trials have translational secondary endpoints. Two trials have the neoadjuvant rectal score as the primary endpoint. Although pCR is an outcome that has been very commonly studied in previous neoadjuvant trials (see Table 1), it has not been confirmed to correlate with longer-term outcomes such as DFS or OS.[56] If these trials (Table 3) demonstrate an improvement in pCR, larger confirmatory studies will be required to understand how this surrogate endpoint correlates with meaningful differences in patient outcomes.

Given the potential overlapping adverse reactions with combination treatment, the safety data from these trials are eagerly awaited. To date, there have been encouraging results from the VOLTAGE trial (NCT02948348; see Table 2)[115] in which of 39 patients, no grade 4 nivolumab-related adverse events were recorded and all severe immune-related adverse events had fully resolved before surgery. Accounting for the one patient who refused surgical resection following a complete clinical response to treatment, 38 (97%) of 39 patients proceeded to radical surgery.

Biomarkers of Response to Immune-Checkpoint Inhibition

Currently, a potential predictive biomarker for response to ICIs in metastatic CRC is MMR, as shown by the KEYNOTE-016 trial,[81] but if validated, will be relevant to a minority of rectal patients in the LARC setting. Critical to the success of the outlined studies will be the exploration of existing, and discovery of novel, biomarkers of selection and response. The opportunity to perform on-treatment biopsies of the primary tumor and tumor microenvironment, as well as pathological examination of the resection specimen taken at the time of radical surgery must be grasped. Within the dMMR population, many patients could be expected to not respond or progress despite treatment with immune checkpoint inhibitors. Prognostic (TILs, high frameshift mutation load, and tumor mutational burden) and predictive biomarkers (TILs, CD8+ as part of Immunoscore) within this group of patients need exploring. Loupakis et al[126] recently demonstrated, in a retrospective cohort study of patients with dMMR CRC treated with ICIs, that higher levels of TILs correspond to higher response rates and survival. PD-L1 has shown clear utility as a biomarker to predict response to immunotherapy in other tumor types such as NSCLC,[127] but has yet to demonstrate utility for patients with CRC. Neither the KEYNOTE-016 trial, nor the Checkmate-142 trial showed any association between objective response rates

and PD-L expression. Thus, prognostic and predictive biomarkers for pMMR CRC tumors remain elusive. Those under investigation include PD-L1, the Immunoscore, POLE (DNA epsilon polymerase) mutations, major histocompatibility complex class II expression, the consensus molecular subtypes classification, and tumor mutational burden.[92,128–130]

In LARC, there is conflicting retrospective evidence regarding the prognostic value of PD-L1 expression undergoing LCRT, with uncertainty as to whether it represents a marker of improved[131] or poor[132] prognosis. The Immunoscore is a CD3+/CD8+ based method of assessment (performed manually[133] or via image analysis software[134,135]), which groups tumors according to a semiquantitative grade based on presence of high- or low-grade CD3+/CD8+ cell assessments. The Immunoscore has also been retrospectively analyzed in a cohort of LARC[136] and showed that higher scores (I4 versus I0) correlate significantly with improved DFS and OS in patients with operable rectal cancer and may also predict for response to LCRT. The combination of PD-L1 status ($\geq$ 1%) and CD8/Treg ratio ($>$ 2.3) predicting for response to neoadjuvant immune checkpoint inhibitor/chemotherapy/radiotherapy combination treatment in pMMR and dMMR LARC has emerged as an exciting novel biomarker candidate, which requires further exploration in a larger patient cohort.[115]

CONCLUSION

The current priorities for improving the outcomes of LARC include reducing distant relapse rates while simultaneously reducing the morbidity associated with multimodality treatment. In this review, we have summarized the key preclinical and clinical evidence that underpins the potential role of ICIs in achieving both of these goals. Using immune checkpoint inhibitors in the neoadjuvant setting, where treatment compliance is likely to be improved, could increase the proportion of patients who respond and avoid surgical resection. Although the gap between radiation and surgery exists to allow for maturation of tumor response, it also provides an obvious opportunity to deliver additional treatments and an excellent platform for translational research. There is good evidence from the metastatic setting that some rectal cancers are responsive to immune checkpoint inhibition. Moving immune-targeted treatment into the neoadjuvant setting, where there is a higher burden of tumor antigens present to incite a host immune response, is a logical progression. Available preclinical evidence suggests that combining immunotherapy with radiation in particular leads to better responses, but questions remain around the optimal dose fractionation of radiation and scheduling of immune checkpoint treatment. Finally, there is a high unmet need to identify biomarkers of response to radiation alone and radiotherapy/immune checkpoint inhibitor combinations in patients with LARC. These are imperative to understand which patients may or may not benefit from treatment intensification and will be critical to the success of this approach. There are a large number of early clinical trials that are currently investigating immune checkpoint inhibition in the treatment of LARC, the results of which are eagerly awaited and will shape the future landscape of immunotherapy research.

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References

1. World Cancer Research Fund. Colorectal Cancer Statistics. 2020. Accessed Apr 25, 2020. www.wcrf.org/dietandcancer/cancer-trends/colorectal-cancer-statistics
2. Cancer Research UK. Bowel cancer incidence statistics. 2020. Accessed Apr 25, 2020. www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer/incidence#heading-Four
3. McCall JL, Cox MR, Wattchow DA. Analysis of local recurrence rates after surgery alone for rectal cancer. *Int J Colorectal Dis*. 1995;10:126–132.
4. National Institute for Health and Care Excellence. *Colorectal Cancer Guidelines [NG151]*. Published on Jan 29, 2020. www.nice.org.uk/guidance/ng151
5. National Comprehensive Cancer Network. *NCCN Clinical Practice Guidelines in Oncology* (NCCN Guidelines) *Rectal Cancer Version 1.2021*. Published on Dec 22, 2020. www.nccn.org/professionals/physician_gls/pdf/rectal.pdf
6. Ceelen WP, Van Nieuwenhove Y, Fierens K. Preoperative chemoradiation versus radiation alone for stage II and III resectable rectal cancer. *Cochrane Database Syst Rev*. 2009: CD006041.
7. Glynne-Jones R, Hughes R. Critical appraisal of the 'wait and see' approach in rectal cancer for clinical complete responders after chemoradiation. *Br J Surg*. 2012;99:897–909.
8. Probst CP, Becerra AZ, Aquina CT, et al. Extended intervals after neoadjuvant therapy in locally advanced rectal cancer: the key to improved tumor response and potential organ preservation. *J Am Coll Surg*. 2015;221:430–440.
9. Bujko K, Nowacki MP, Nasierowska-Guttmejer A, Michalski W, Bebenek M, Kryj M. Long-term results of a randomized trial comparing preoperative short-course radiotherapy with preoperative conventionally fractionated chemoradiation for rectal cancer. *Br J Surg*. 2006;93:1215–1223.
10. Fokas E, Ströbel P, Fietkau R, et al. Tumor regression grading after preoperative chemoradiotherapy as a prognostic factor and individual-level surrogate for disease-free survival in rectal cancer. *J Natl Cancer Inst*. 2017;109.
11. Maas M, Nelemans PJ, Valentini V, et al. Long-term outcome in patients with a pathological complete response after chemoradiation for rectal cancer: a pooled analysis of individual patient data. *Lancet Oncol*. 2010;11:835–844.

12. van der Valk MJM, Hilling DE, Bastiaannet E, et al. Long-term outcomes of clinical complete responders after neoadjuvant treatment for rectal cancer in the International Watch & Wait Database (IWWD): an international multicentre registry study. *Lancet*. 2018;391:2537–2545.
13. Smith JJ, Strombom P, Chow OS, et al. Assessment of a watch-and-wait strategy for rectal cancer in patients with a complete response after neoadjuvant therapy. *JAMA Oncol*. 2019;5:e185896.
14. Renehan AG, Malcomson L, Emsley R, et al. Watch-and-wait approach versus surgical resection after chemoradiotherapy for patients with rectal cancer (the OnCoRe project): a propensity-score matched cohort analysis. *Lancet Oncol*. 2016;17:174–183.
15. Cunningham D, Atkin W, Lenz HJ, et al. Colorectal cancer. *Lancet*. 2010;375:1030–1047.
16. Tiernan J, Cook A, Geh I, et al. Use of a modified Delphi approach to develop research priorities for the association of coloproctology of Great Britain and Ireland. *Colorectal Dis*. 2014;16:965–970.
17. Koebel CM, Vermi W, Swann JB, et al. Adaptive immunity maintains occult cancer in an equilibrium state. *Nature*. 2007;450:903–907.
18. Wei SC, Duffy CR, Allison JP. Fundamental mechanisms of immune checkpoint blockade therapy. *Cancer Discov*. 2018;8:1069–1086.
19. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366:2443–2454.
20. Muzny DM, Bainbridge MN, Chang K, et al. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487:330–337.
21. Venderbosch S, Nagtegaal ID, Maughan TS, et al. Mismatch repair status and BRAF mutation status in metastatic colorectal cancer patients: a pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS Studies. *Clin Cancer Res*. 2014;20:5322.
22. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol*. 2005;23:609–618.
23. Price TJ, Karapetis CS, Joanne Y, et al. Outcomes for metastatic colorectal cancer (mCRC) based on microsatellite instability. *J Clin Oncol*. 2018;36(4_suppl):759.
24. Loree JM, Pereira AAL, Lam M, et al. Classifying colorectal cancer by tumor location rather than sidedness highlights a continuum in mutation profiles and consensus molecular subtypes. *Clin Cancer Res*. 2018;24:1062–1072.
25. Gerard JP, Azria D, Gourgou-Bourgade S, et al. Clinical outcome of the ACCORD 12/0405 PRODIGE 2 randomized trial in rectal cancer. *J Clin Oncol*. 2012;30:4558–4565.
26. Haustermans K, Schmoll HJ, Price T, et al. OC-0250: First results of the PETACC-6 randomized phase III trial in locally advanced rectal cancer. *Radiother Oncol*. 2014;111:S96.
27. O'Connell MJ, Colangelo LH, Beart RW, et al. Capecitabine and oxaliplatin in the preoperative multimodality treatment of rectal cancer: surgical end points from National Surgical Adjuvant Breast and Bowel Project trial R-04. *J Clin Oncol*. 2014;32:1927–1934.
28. Rodel C, Graeven U, Fietkau R, et al. Oxaliplatin added to fluorouracil-based preoperative chemoradiotherapy and postoperative chemotherapy of locally advanced rectal cancer (the German CAO/ARO/AIO-04 study): final results of the multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol*. 2015;16:979–989.
29. Aschele C, Lonardi S, Cionini L, et al. Final results of STAR-01: A randomized phase III trial comparing preoperative chemoradiation with or without oxaliplatin in locally advanced rectal cancer. *J Clin Oncol*. 2016;34(15_suppl):3521.
30. Deng Y, Chi P, Lan P, et al. Modified FOLFOX6 with or without radiation versus fluorouracil and leucovorin with radiation in neoadjuvant treatment of locally advanced rectal cancer: initial results of the Chinese FOWARC multicenter, open-label, randomized three-arm phase III trial. *J Clin Oncol*. 2016;34:3300–3307.
31. Sebag-Montefiore D, Adams R, Gollins S, et al. ARISTOTLE: A phase III trial comparing concurrent capecitabine with capecitabine and irinotecan (Ir) chemoradiation as preoperative treatment for MRI-defined locally advanced rectal cancer (LARC). *J Clin Oncol*. 2020;38(15_suppl):4101.
32. Ngan SY, Burmeister B, Fisher RJ, et al. Randomized trial of short-course radiotherapy versus long-course chemoradiation comparing rates of local recurrence in patients with T3 rectal cancer: Trans-Tasman Radiation Oncology Group trial 01.04. *J Clin Oncol*. 2012;30:3827–3833.
33. Erlandsson J, Holm T, Pettersson D, et al. Optimal fractionation of preoperative radiotherapy and timing to surgery for rectal cancer (Stockholm III): a multicentre, randomised, non-blinded, phase 3, non-inferiority trial. *Lancet Oncol*. 2017;18:336–346.
34. Glehen O, Chapet O, Adham M, Nemoz JC, Gerard JP. Long-term results of the Lyons R90–01 randomized trial of preoperative radiotherapy with delayed surgery and its effect on sphincter-saving surgery in rectal cancer. *Br J Surg*. 2003;90:996–998.
35. Stein DE, Mahmoud NN, Anné PR, et al. Longer time interval between completion of neoadjuvant chemoradiation and surgical resection does not improve downstaging of rectal carcinoma. *Dis Colon Rectum*. 2003;46:448–453.
36. Lefevre JH, Mineur L, Kotti S, et al. Effect of interval (7 or 11 weeks) between neoadjuvant radiochemotherapy and surgery on complete pathologic response in rectal cancer: a multicenter, randomized, controlled trial (GRECCAR-6). *J Clin Oncol*. 2016;34:3773–3780.
37. Garcia-Aguilar J, Smith DD, Avila K, Bergsland EK, Chu P, Krieg RM. Optimal timing of surgery after chemoradiation for advanced rectal cancer: preliminary results of a multicenter, nonrandomized phase II prospective trial. *Ann Surg*. 2011;254:97–102.
38. Garcia-Aguilar J, Chow OS, Smith DD, et al. Effect of adding mFOLFOX6 after neoadjuvant chemoradiation in locally advanced rectal cancer: a multicentre, phase 2 trial. *Lancet Oncol*. 2015;16:957–966.
39. Sloothaak DA, Geijsen DE, van Leersum NJ, et al. Optimal time interval between neoadjuvant chemoradiotherapy and surgery for rectal cancer. *Br J Surg*. 2013;100:933–939.
40. Tulchinsky H, Shmueli E, Figer A, Klausner JM, Rabau M. An interval >7 weeks between neoadjuvant therapy and surgery improves pathologic complete response and disease–free survival in patients with locally advanced rectal cancer. *Ann Surg Oncol*. 2008;15:2661–2667.
41. Ryan EJ, O'Sullivan DP, Kelly ME, et al. Meta-analysis of the effect of extending the interval after long-course chemoradiotherapy before surgery in locally advanced rectal cancer. *Br J Surg*. 2019;106:1298–1310.

42. Gambacorta MA, Masciocchi C, Chiloiro G, et al. Timing to achieve the highest rate of pCR after preoperative radiochemotherapy in rectal cancer: a pooled analysis of 3085 patients from 7 randomized trials. *Radiother Oncol*. 2021;154:154–160.
43. Fisher B, Wolmark N, Rockette H, et al. Postoperative adjuvant chemotherapy or radiation therapy for rectal cancer: results from NSABP Protocol R-0112. *J Natl Cancer Inst*. 1988;80:21–29.
44. Krook JE, Moertel CG, Gunderson LL, et al. Effective surgical adjuvant therapy for high-risk rectal carcinoma. *N Engl J Med*. 1991;324:709–715.
45. Randomised trial of surgery alone versus radiotherapy followed by surgery for potentially operable locally advanced rectal cancer. *Lancet*. 1996;348:1605–1610.
46. Kapiteijn E, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. *N Engl J Med*. 2001;345:638–646.
47. Sauer R, Becker H, Hohenberger W, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med*. 2004;351:1731–1740.
48. Folkesson J, Birgisson H, Pahlman L, Cedermark B, Glimelius B, Gunnarsson U. Swedish Rectal Cancer Trial: long lasting benefits from radiotherapy on survival and local recurrence rate. *J Clin Oncol*. 2005;23:5644–5650.
49. Bosset JF, Collette L, Calais G, et al. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med*. 2006;355:1114–1123.
50. Gerard JP, Conroy T, Bonnetain F, et al. Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3–4 rectal cancers: results of FFCD 9203. *J Clin Oncol*. 2006;24:4620–4625.
51. Roh MS, Colangelo LH, O'Connell MJ, et al. Preoperative multimodality therapy improves disease-free survival in patients with carcinoma of the rectum: NSABP R-03. *J Clin Oncol*. 2009;27:5124–5130.
52. Sebag-Montefiore D, Stephens RJ, Steele R, et al. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. *Lancet*. 2009;373:811–820.
53. Bujko K, Wyrwicz L, Rutkowski A, et al. Long-course oxaliplatin-based preoperative chemoradiation versus 5 × 5 Gy and consolidation chemotherapy for cT4 or fixed cT3 rectal cancer: results of a randomized phase III study. *Ann Oncol*. 2016;27:834–842.
54. Fokas E, Allgäuer M, Polat B, et al. Randomized Phase II trial of chemoradiotherapy plus induction or consolidation chemotherapy as total neoadjuvant therapy for locally advanced rectal cancer: CAO/ARO/AIO-12. *J Clin Oncol*. 2019;37:3212–3222.
55. Conroy T, Lamfichekh N, Etienne P-L, et al. Total neoadjuvant therapy with mFOLFIRINOX versus preoperative chemoradiation in patients with locally advanced rectal cancer: final results of PRODIGE 23 phase III trial, a UNICANCER GI trial. *J Clin Oncol*. 2020;38(15_suppl): 4007.
56. Fokas E, Glynne-Jones R, Appelt A, et al. Outcome measures in multimodal rectal cancer trials. *Lancet Oncol*. 2020;21:e252–e264.
57. Garcia-Aguilar J, Patil S, Kim JK, et al. Preliminary results of the organ preservation of rectal adenocarcinoma (OPRA) trial. *J Clin Oncol*. 2020;38(15_suppl):4008.
58. Hospers G, Bahadoer RR, Dijkstra EA, et al. Short-course radiotherapy followed by chemotherapy before TME in locally advanced rectal cancer: the randomized RAPIDO trial. *J Clin Oncol*. 2020;38(15_suppl):4006.
59. Bosset JF, Calais G, Mineur L, et al. Fluorouracil-based adjuvant chemotherapy after preoperative chemoradiotherapy in rectal cancer: long-term results of the EORTC 22921 randomised study. *Lancet Oncol*. 2014;15:184–190.
60. Sainato A, Cernusco Luna Nunzia V, Valentini V, et al. No benefit of adjuvant Fluorouracil Leucovorin chemotherapy after neoadjuvant chemoradiotherapy in locally advanced cancer of the rectum (LARC): long term results of a randomized trial (I-CNR-RT). *Radiother Oncol*. 2014;113:223–229.
61. Delbaldo C, Ychou M, Zawadi A, et al. Postoperative irinotecan in resected stage II-III rectal cancer: final analysis of the French R98 Intergroup trialdagger. *Ann Oncol*. 2015;26:1208–1215.
62. Glynne-Jones R, Counsell N, Quirke P, et al. Chronicle: results of a randomised phase III trial in locally advanced rectal cancer after neoadjuvant chemoradiation randomising postoperative adjuvant capecitabine plus oxaliplatin (XELOX) versus control. *Ann Oncol*. 2014;25:1356–1362.
63. Breugom AJ, van Gijn W, Muller EW, et al. Adjuvant chemotherapy for rectal cancer patients treated with preoperative (chemo)radiotherapy and total mesorectal excision: a Dutch Colorectal Cancer Group (DCCG) randomized phase III trial. *Ann Oncol*. 2015;26:696–701.
64. Quasar Collaborative G, Gray R, Barnwell J, et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. *Lancet*. 2007;370:2020–2029.
65. Fernandez-Martos C, Garcia-Albeniz X, Pericay C, et al. Chemoradiation, surgery and adjuvant chemotherapy versus induction chemotherapy followed by chemoradiation and surgery: long-term results of the Spanish GCR-3 phase II randomized trial. *Ann Oncol*. 2015;26:1722–1728.
66. Cercek A, Roxburgh CSD, Strombom P, et al. Adoption of total neoadjuvant therapy for locally advanced rectal cancer. *JAMA Oncol*. 2018;4:e180071.
67. Petrelli F, Trevisan F, Cabiddu M, et al. Total neoadjuvant therapy in rectal cancer: a systematic review and meta-analysis of treatment outcomes. *Ann Surg*. 2020;271:440–448.
68. Maréchal R, Vos B, Polus M, et al. Short course chemotherapy followed by concomitant chemoradiotherapy and surgery in locally advanced rectal cancer: a randomized multicentric phase II study. *Ann Oncol*. 2012;23:1525–1530.
69. Bujko K, for the Polish Colorectal Study G, Wyrwicz L, et al. Long-course oxaliplatin-based preoperative chemoradiation versus 5 × 5 Gy and consolidation chemotherapy for cT4 or fixed cT3 rectal cancer: results of a randomized phase III study. *Ann Oncol*. 2016;27:834–842.
70. Bahadoer RR, Dijkstra EA, van Etten B, et al. Short-course radiotherapy followed by chemotherapy before total mesorectal excision (TME) versus preoperative chemoradiotherapy, TME, and optional adjuvant chemotherapy in locally advanced rectal cancer (RAPIDO): a randomised, open-label, phase 3 trial. *Lancet Oncol*. 2021;22:29–42.
71. van der Valk MJM, Marijnen CAM, van Etten B, et al. Compliance and tolerability of short-course radiotherapy followed by preoperative chemotherapy and surgery for high-risk rectal cancer - Results of the international

randomized RAPIDO-trial. *Radiother Oncol*. 2020;147:75–83.
72. Nogue M, Salud A, Vicente P, et al. Addition of bevacizumab to XELOX induction therapy plus concomitant capecitabine-based chemoradiotherapy in magnetic resonance imaging-defined poor-prognosis locally advanced rectal cancer: the AVACROSS study. *Oncologist*. 2011;16:614–620.
73. Dewdney A, Cunningham D, Tabernero J, et al. Multicenter randomized phase II clinical trial comparing neoadjuvant oxaliplatin, capecitabine, and preoperative radiotherapy with or without cetuximab followed by total mesorectal excision in patients with high-risk rectal cancer (EXPERT-C). *J Clin Oncol*. 2012;30:1620–1627.
74. Kim SY, Hong YS, Kim DY, et al. Preoperative chemoradiation with cetuximab, irinotecan, and capecitabine in patients with locally advanced resectable rectal cancer: a multicenter Phase II study. *Int J Radiat Oncol Biol Phys*. 2011;81:677–683.
75. George TJ, Yothers G, Hong TS, et al. NRG-GI002: A phase II clinical trial platform for total neoadjuvant therapy (TNT) in rectal cancer. *J Clin Oncol*. 2017;35(15_suppl):TPS3629.
76. Kawakami H, Zaanan A, Sinicrope FA. Microsatellite instability testing and its role in the management of colorectal cancer. *Curr Treat Options Oncol*. 2015;16:30.
77. Fabrizio DA, George TJ Jr, Dunne RF, et al. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. *J Gastrointest Oncol*. 2018;9:610–617.
78. U.S. Food and Drug Administration. FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication. Published May 30, 2017. Accessed Sep 20, 2020. www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-pembrolizumab-first-tissuesite-agnostic-indication
79. Overman MJ, Lonardi S, Wong KYM, et al. Durable clinical benefit with nivolumab plus ipilimumab in dna mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J Clin Oncol*. 2018;36:773–779.
80. Lenz H-J, Lonardi S, Zagonel V, et al. Nivolumab plus low-dose ipilimumab as first-line therapy in microsatellite instability-high/DNA mismatch repair deficient metastatic colorectal cancer: clinical update. *J Clin Oncol*. 2020;38(4_suppl):11.
81. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372:2509–2520.
82. Le DT, Kim TW, Van Cutsem E, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J Clin Oncol*. 2020;38:11–19.
83. André T, Shiu K-K, Kim TW, et al. Pembrolizumab in microsatellite-instability–high advanced colorectal cancer. *N Engl J Med*. 2020;383:2207–2218.
84. Segal NH, Wainberg ZA, Overman MJ, et al. Safety and clinical activity of durvalumab monotherapy in patients with microsatellite instability–high (MSI-H) tumors. *J Clin Oncol*. 2019;37(4_suppl):670.
85. Chen EX, Jonker DJ, Loree JM, et al. Effect of combined immune checkpoint inhibition vs best supportive care alone in patients with advanced colorectal cancer: the Canadian Cancer Trials Group CO.26 Study. *JAMA Oncol*. 2020;6:821–838.
86. Chalabi M, Fanchi LF, Dijkstra KK, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat Med*. 2020;26:566–576.
87. Brahmer JR, Drake CG, Wollner I, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol*. 2010;28:3167–3175.
88. Chung KY, Gore I, Fong L, et al. Phase II study of the anticytotoxic T-lymphocyte-associated antigen 4 monoclonal antibody, tremelimumab, in patients with refractory metastatic colorectal cancer. *J Clin Oncol*. 2010;28:3485–3490.
89. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366:2455–2465.
90. Patnaik A, Kang SP, Rasco D, et al. Phase I study of pembrolizumab (MK-3475; anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *Clin Cancer Res*. 2015;21:4286–4293.
91. Almquist DR, Ahn DH, Bekaii-Saab TS. The role of immune checkpoint inhibitors in colorectal adenocarcinoma. *BioDrugs*. 2020;34:349–362.
92. Chen EX, Jonker DJ, Kennecke HF, et al. CCTG CO.26 trial: A phase II randomized study of durvalumab (D) plus tremelimumab (T) and best supportive care (BSC) versus BSC alone in patients (pts) with advanced refractory colorectal carcinoma (rCRC). Presented at American Society of Clinical Oncology Gastrointestinal Cancer Symposium; January 17, 2019. San Francisco, CA.
93. Cohen R, Rousseau B, Vidal J, Colle R, Diaz LA Jr, Andre T. Immune checkpoint inhibition in colorectal cancer: microsatellite instability and beyond. *Target Oncol*. 2020;15:11–24.
94. Liu J, Blake SJ, Yong MC, et al. Improved efficacy of neoadjuvant compared to adjuvant immunotherapy to eradicate metastatic disease. *Cancer Discov*. 2016;6:1382–1399.
95. Blank CU, Rozeman EA, Fanchi LF, et al. Neoadjuvant versus adjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma. *Nat Med*. 2018;24:1655–1661.
96. Forde PM, Chaft JE, Smith KN, et al. Neoadjuvant PD-1 blockade in resectable lung cancer. *N Engl J Med*. 2018;378:1976–1986.
97. Powles T, Kockx M, Rodriguez-Vida A, et al. Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable urothelial carcinoma in the ABACUS trial. *Nat Med*. 2019;25:1706–1714.
98. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015;373:23–34.
99. Prise KM, Schettino G, Folkard M, Held KD. New insights on cell death from radiation exposure. *Lancet Oncol*. 2005;6:520–528.
100. Eriksson D, Stigbrand T. Radiation-induced cell death mechanisms. *Tumour Biol*. 2010;31:363–372.
101. Dovedi SJ, Adlard AL, Lipowska-Bhalla G, et al. Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. *Cancer Res*. 2014;74:5458.
102. Burnette BC, Liang H, Lee Y, et al. The efficacy of radiotherapy relies upon induction of type i interferon-dependent innate and adaptive immunity. *Cancer Res*. 2011;71:2488–2496.

103. Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med*. 2006;203:1259–1271.
104. Ruocco MG, Pilones KA, Kawashima N, et al. Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves antitumor effects. *J Clin Invest*. 2012;122:3718–3730.
105. Ma Y, Kepp O, Ghiringhelli F, et al. Chemotherapy and radiotherapy: cryptic anticancer vaccines. *Semin Immunol*. 2010;22:113–124.
106. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. *J Natl Cancer Inst*. 2013;105:256–265.
107. Hanna GG, Illidge T. Radiotherapy and immunotherapy combinations in non-small cell lung cancer: a promising future? *Clin Oncol*. 2016;28:726–731.
108. Kang J, Demaria S, Formenti S. Current clinical trials testing the combination of immunotherapy with radiotherapy. *J Immunother Cancer*. 2016;4:51.
109. Schmid SC, Koll FJ, Rödel C, et al. Radiation therapy before radical cystectomy combined with immunotherapy in locally advanced bladder cancer – study protocol of a prospective, single arm, multicenter phase II trial (RACE IT). *BMC Cancer*. 2020;20:8.
110. Antonia SJ, Villegas A, Daniel D, et al. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med*. 2018;379:2342–2350.
111. Koller KM, Mackley HB, Liu J, et al. Improved survival and complete response rates in patients with advanced melanoma treated with concurrent ipilimumab and radiotherapy versus ipilimumab alone. *Cancer Biol Ther*. 2017;18:36–42.
112. Dovedi SJ, Cheadle EJ, Popple AL, et al. Fractionated radiation therapy stimulates antitumor immunity mediated by both resident and infiltrating polyclonal T-cell populations when combined with PD-1 blockade. *Clin Cancer Res*. 2017;23:5514.
113. Golden EB, Frances D, Pellicciotta I, Demaria S, Helen Barcellos-Hoff M, Formenti SC. Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. *Oncoimmunology*. 2014;3:e28518.
114. Golden EB, Apetoh L. Radiotherapy and immunogenic cell death. *Semin Radiat Oncol*. 2015;25:11–17.
115. Yuki S, Bando H, Tsukada Y, et al. Short-term results of VOLTAGE-A: Nivolumab monotherapy and subsequent radical surgery following preoperative chemoradiotherapy in patients with microsatellite stable and microsatellite instability-high locally advanced rectal cancer. *J Clin Oncol*. 2020;38(15_suppl):4100.
116. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. *N Engl J Med*. 2012;366:925–931.
117. Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. *Cancer Immunol Res*. 2013;1:365–372.
118. Reynders K, Illidge T, Siva S, Chang JY, De Ruysscher D. The abscopal effect of local radiotherapy: using immunotherapy to make a rare event clinically relevant. *Cancer Treat Rev*. 2015;41:503–510.
119. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases–elimination, equilibrium and escape. *Curr Opin Immunol*. 2014;27:16–25.
120. Versluis JM, Long GV, Blank CU. Learning from clinical trials of neoadjuvant checkpoint blockade. *Nat Med*. 2020;26:475–484.
121. Vincent J, Mignot G, Chalmin F, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell–dependent antitumor immunity. *Cancer Res*. 2010;70:3052.
122. Tesniere A, Schlemmer F, Boige V, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene*. 2009;29:482.
123. Isambert N, Hervieux A, Zanetta S, et al. Evaluation of the safety and the tolerability of durvalumab plus tremelimumab combined with FOLFOX in mCRC (MEDITREME). Presented at Toulouse Oncoweek; February 5, 2018. Toulouse, France.
124. Roxburgh CS, Shia J, Vakiani E, Daniel T, Weiser MR. Potential immune priming of the tumor microenvironment with FOLFOX chemotherapy in locally advanced rectal cancer. *Oncoimmunology*. 2018;7:e1435227.
125. Teng F, Mu D, Meng X, et al. Tumor infiltrating lymphocytes (TILs) before and after neoadjuvant chemoradiotherapy and its clinical utility for rectal cancer. *Am J Cancer Res*. 2015;5:2064–2074.
126. Loupakis F, Depetris I, Biason P, et al. Prediction of benefit from checkpoint inhibitors in mismatch repair deficient metastatic colorectal cancer: role of tumor infiltrating lymphocytes. *Oncologist*. 2020;25:481–487.
127. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-Positive non-small-cell lung cancer. *N Engl J Med*. 2016;375:1823–1833.
128. Galon J, Pagès F, Marincola FM, et al. The immune score as a new possible approach for the classification of cancer. *J Transl Med*. 2012;10:1.
129. Pagès F, Mlecnik B, Marliot F, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet*. 2018;391:2128–2139.
130. A phase II trial assessing nivolumab in class II expressing microsatellite stable colorectal cancer (ANICCA). 2020. ClinicalTrials.gov.identifier: NCT03981146. Accessed Oct 16, 2020. clinicaltrials.gov/ct2/show/NCT03981146
131. Noh BJ, Kwak JY, Eom DW. Immune classification for the PD-L1 expression and tumour-infiltrating lymphocytes in colorectal adenocarcinoma. *BMC Cancer*. 2020;20:58.
132. Saigusa S, Toiyama Y, Tanaka K, et al. Implication of programmed cell death ligand 1 expression in tumor recurrence and prognosis in rectal cancer with neoadjuvant chemoradiotherapy. *Int J Clin Oncol*. 2016;21:946–952.
133. Park JH, McMillan DC, Edwards J, Horgan PG, Roxburgh CSD. Comparison of the prognostic value of measures of the tumor inflammatory cell infiltrate and tumor-associated stroma in patients with primary operable colorectal cancer. *Oncoimmunology*. 2016;5:e1098801.
134. Vayrynen JP, Vornanen JO, Sajanti S, Bohm JP, Tuomisto A, Makinen MJ. An improved image analysis method for cell counting lends credibility to the prognostic significance of T cells in colorectal cancer. *Virchows Arch*. 2012;460:455–465.
135. Wirta EV, Seppala T, Friman M, et al. Immunoscore in mismatch repair-proficient and -deficient colon cancer. *J Pathol Clin Res*. 2017;3:203–213.
136. Anitei M-G, Zeitoun G, Mlecnik B, et al. Prognostic and predictive values of the immunoscore in patients with rectal cancer. *Clin Cancer Res*. 2014;20:1891.