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Review Article

A Review of Scheduling Strategies for Radiotherapy and Immune Checkpoint Inhibition in Locally Advanced Rectal Cancer

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ABSTRACT

Colorectal cancer (CRC) is the third most common malignancy across the globe and, despite advances in treatment strategies, survival rates remain low. Rectal cancer (RC) accounts for most of these cases, and traditional management strategies for advanced disease include total neoadjuvant therapy (TNT) with chemoradiotherapy followed by curative surgery. Unfortunately, approximately 10–15% of patients have no response to treatment or have recurrence at a short interval following radiotherapy. The introduction of immunotherapy in the form of immune checkpoint blockade (ICB) in metastatic colorectal cancer has improved clinical outcomes, yet most patients with RC present with microsatellite stable disease, which lacks the immune-rich microenvironment where ICB is most effective. There is evidence that combining radiotherapy with ICB can unlock the mechanisms that drive resistance in patients; however, the sequencing of these therapies is still debated. This review offers a comprehensive overview of clinical trials and preclinical models that use radiotherapy–immunotherapy combinations in RC in an attempt to extrapolate the ideal sequencing of the two treatment modalities. The results highlight the dearth of evidence to answer the question of whether ICB should be given before, during, or after radiotherapy, yet it is suggested that improving the relevance of our preclinical models will provide a platform with higher translational value and will lead to appropriate clinical trial designs.

Keywords: neoplasm, rectal cancer, radiotherapy, chemotherapy, immunotherapy, immunology, clinical trials, radiotherapy-immunotherapy combinations, immune checkpoint inhibitor, colorectal cancer, treatment schedule, locally advanced rectal cancer

INTRODUCTION

Globally, the incidence of colorectal cancer (CRC) reached 10% in 2020, while almost 900,000 deaths were recorded in the same year.[1] In most CRC cases (~ 80%), tumorigenesis occurs with the mutation of the adenomatous polyposis coli gene (*APC*) and then progresses owing to the ensuing incremental mutational burden that follows the activation of oncogenic KRAS and the loss of the tumor suppressor TP53.[2]

Patients with CRC can present with high microsatellite instability (MSI-H) or with microsatellite stable (MSS) disease depending on the number of genetic mutations found in the DNA mismatch repair (MMR) machinery, which includes the following genes: *MSH2*, *MLH1*, *PMS2*, *MSH3*, *MSH6*, and *MLH3*.[3] Around 15% of stage II and III CRC diagnoses demonstrate MSI.[4] Rectal cancer (RC) is almost exclusively MMR proficient (95%) and therefore MSS.[5] This, however, is not the only difference between RC and colon cancer as they present at different incidental rates, with differing roots of origin, spread, and response to treatment, making them two distinct tumor entities.[6]

Locally advanced rectal cancer (LARC) is defined as stage III-IV rectal tumors with extramural vascular invasion; it is treated with preoperative radiotherapy or chemoradiotherapy (CRT) followed by surgery in the form of total mesorectal excision (TME).[7,8] Radiotherapy can be prescribed as either long-course (LCRT) (45 Gray [Gy] in 25 fractions over 5 weeks) or short-course (SCRT) radiotherapy (25 Gy in 5 fractions over 1 week) with the current literature not providing definitive evidence on the superiority of one regimen over the other.[9,10] Of the patients with RC who have received neoadjuvant therapy followed by surgical management, most achieve significant reduction in tumor size (~ 65%),[11] while ~ 20% show a pathologic complete response (pCR).[12,13] Considering the high rates of salvageable tumor relapses (~ 88%),[12] it is important to pursue nonsurgical treatment options that allow organ preservation, improve clinical outcomes, and offer a good quality of life for patients with RC.[5]

In recent years, novel immune-modulating treatment methods directed at MSI RC have demonstrated unprecedented improvement in the clinical outcomes of this subgroup,[5] with modest benefit also observed in patients with metastatic MSI colon cancer.[14–16] The use of immunotherapy in the larger MSS subgroup has not been as successful to date, but strategies to improve clinical responses to immunotherapy-based treatment regimens in patients with MSS CRC are currently being pursued.[17] Options include combining immune checkpoint blockade (ICB) with immunostimulatory treatments, such as radiotherapy, or bringing immunotherapy treatment into earlier, nonmetastatic disease settings (e.g., neoadjuvant treatment) where tumor immune escape mechanisms may not be as well developed.[17] This narrative review appraises the dosing and scheduling strategies for radiotherapy and ICB in the clinical and preclinical settings in an attempt to extrapolate the ideal sequencing of the two treatment modalities. An online search was conducted on Jun 30, 2023, to identify articles of clinical trials (Jan 2017 to Jun 2023) and preclinical studies (Jan 2000 to Jun 2023) that have published results on the combinations of radiotherapy and ICB (Supplemental Tables S1 and S2, available online).

IMMUNE CHECKPOINT BLOCKADE IN CRC

In the largest dMMR (MMR deficient) and MSI-H metastatic CRC (mCRC) cohort to be treated with a checkpoint inhibitor at the time, the CheckMate-142 study reported disease control for almost 69% of its patients, with 31.1% of all patients achieving an objective response by 12 months.[18] Pembrolizumab was assessed as a second or further line of treatment in patients with dMMR and MSI-H mCRC, where the KEYNOTE-164 trial achieved an objective response rate of 33% with a minority of its patients experiencing grade 3-4 adverse events (AEs) (13–16%).[19] Finally, the KEYNOTE-177 study tested pembrolizumab against fluorouracil-based chemotherapy in the first-line setting of mCRC, where duration of progression-free survival (PFS) improved by almost 100% (16.5 vs 8.2 months) and the incidence of grade 3 or higher treatment-related side effects dropped to one-third (22% vs 66%).[20] Overall, the results of these studies established a clear role for programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) inhibitors in advanced stages of CRC; however, it was still unclear whether their role could be extended to earlier settings or RC alone.

The NICHE trial (NCT03026140) investigated the safety and feasibility of neoadjuvant ipilimumab and nivolumab before surgery for patients with early-stage pMMR (MMR proficient) and dMMR colon cancer.[21] The investigators noted either complete or major pathologic responses in 100% of the dMMR patients (n = 20/20) and 27% (n = 4/15) of the pMMR group, such that use of the CTLA-4 and PD-1 inhibitors is promising in the neoadjuvant setting of early-stage colon cancer.[21] In RC, a phase II clinical trial published early results from its investigation of dorstalimab (anti–PD-1) in a dMMR LARC patient cohort and reported a 100% treatment response rate in 12 consecutive patients.[5] These findings highlight the potential benefit of immunotherapy in the management of RC, yet so far only MSI patients seem to reap the benefits of ICB.[18–20]

To devise strategies to optimize ICB in CRC patients, it is important, as a first step, to understand how ICB works. Effector T cells carry surface proteins, known as checkpoint molecules, that hinder the cytotoxic action of T cells upon binding to their ligands on the surface of antigen-presenting cells (APCs) and cancer cells. Perhaps the most well-known example of such an interaction in cancer immunology is the binding of PD-1, found on T cells, to its ligands, programmed cell death ligand-1 and 2 (PD-L1/2).[22] This immunologic "brake" is a reliable, protumor survival mechanism that allows tumors to remain untouched by cytotoxic T cells. This is where the checkpoint blockade's value lies. The important effect of ICB is that it blocks the receptors of the checkpoint molecules, which releases the "brake" from the cytotoxic T cells.[22] Yet, a large number of RC patients have no response to ICB, indicating the pertinent need to develop strategies that will allow MSS primary RC to display and maintain a durable response to checkpoint inhibition.

Radiotherapy induces cell lysis by causing DNA double-strand breaks and free radical damage.[4] The death of cancer cells is followed by a substantial release of neoantigen proteins that can be detected by APCs and prime the cytotoxic T cells.[23] This was clearly shown in two preclinical mouse models of melanoma and breast cancer, where the stereotactic ablation of the tumors led to significant antigen presentation and increased CD4+ and CD8+ cell infiltration into the tumors, a phenomenon which was further augmented when PD-1 inhibition was administered 1 day before radiotherapy.[24] It is

therefore possible that the combination of the antigen-releasing effects of radiotherapy with the immune cell–boosting qualities of ICB will allow restoration of the low immune cell infiltration in MSS RC, which could improve the clinical outcomes of patients with this disease phenotype.[25,26]

Before a clear benefit can be established, however, it is essential to delve into a practical question regarding the combination of the two treatment modalities: what is the optimal treatment schedule for radiotherapy and ICB? This review presents the results of clinical and preclinical studies that have explored RT-ICB combinations in LARC in an attempt to answer this important question.

CLINICAL TRIALS USING RT-ICB COMBINATIONS IN LARC

The literature review identified 13 studies that met the search criteria. Most trials ($n = 7$) scheduled ICB after radiotherapy.[27–33] Four prescribed immunotherapy during the radiation treatment,[34–37] one prescribed it before,[38] and one included two arms that would incorporate it either before or after radiotherapy.[39] The sections below aim to offer an overview of the studies that have yielded results on the effects of combination ICB and radiotherapy in RC. It should be noted that because some of the investigations are ongoing, data on the long-term outcomes of the patients were not available in most trials at the time of writing. Four trials have already published results in journal articles and are presented first in this review,[27,28,36,39] followed by the studies that showcased their results in the form of abstracts in international conferences.[29–35,37,38] A summary of the studies can be found in Table 1.

In the VOLTAGE study (NCT02948348), the investigators aimed to evaluate the combination of preoperative CRT with adjuvant anti–PD-1 treatment followed by surgery in patients with MSS (n = 39) and MSI ($n = 5$) T3-4 lymph node–negative (LN−) (N0) or lymph node–positive (LN+) (N1-2) LARC.[27] CRT included capecitabine at 1650 mg/m^2 daily and LCRT (50.4 Gy in 28 fractions). Nivolumab monotherapy was administered at 240 mg every 2 weeks for five cycles starting 2 weeks post CRT, followed by surgery 10 weeks later. The study's primary outcome was the pCR rate as measured by using the tumor regression grade (TRG). The authors reported pCR rates (TRG 0) of 30% for MSS ($n = 37$) and 60% for MSI ($n = 5$) patients, with the percentage reaching 38% for the MSS group when including the patients who achieved TRG 1. Recurrence was observed in six MSS cases, whereas none of the MSI patients had relapse. In terms of the treatment safety, AEs relating to PD-1 treatment were noted in 21/39 patients, 10.3% (4/39) of whom experienced grade 3 or 4. Finally, the 3-year overall survival (OS) and relapse-free survival in the MSS patients reached the very promising rates of 79.5% and 97.4%, respectively, and 100% in the MSI cohort.[40]

The phase-II, single-center NCT04231552 study recruited 30 patients with T3-4 N0 or T1-4 LN+ pMMR or dMMR primary RC.[28] The treatment course consisted of SCRT (25 Gy in 5 fractions) followed by two 21-day cycles of capecitabine and oxaliplatin (CAPOX) chemotherapy and the PD-1 antibody camrelizumab (200-mg intravenous [IV] drip) on day 1 of each chemotherapy cycle starting 1 week post radiotherapy. The tumors were surgically resected 7 days from the end of chemotherapy, and the resected specimens were analyzed for residual disease according to the International Union Against Cancer TNM staging system (8th edition). Of 27 evaluable patients, the authors reported pCR rates (ypT0N0) of 46.2% (12/26) for patients with pMMR and 100% (1/1) for those with dMMR disease, with a total pCR rate of 48.1% for all 27 patients. Similar to the VOLTAGE trial (ClinicalTrials.gov identifier NCT02948348),[27] the patients in this study started treatment with the PD-1 inhibitor at a median time point of 12 days post SCRT, which the authors justified as being in line with previous evidence warranting the induction of checkpoint inhibition no later than 14 days after irradiation.[41,42]

Recently, the TORCH trial (ClinicalTrials.gov identifier NCT04518280) investigated the addition of ICB in the TNT phase in patients with LARC.[39] In the phase II multicenter study, 130 treatment-naïve patients with T3 and T4 LN+/− disease and no metastases were recruited across four hospitals. They were randomly assigned to receive either two cycles of CAPOX and toripalimab (PD-1 inhibitor) followed by SCRT and four additional cycles of CT+ICB and then surgery or watch and wait (WW) (group A) or SCRT followed by six cycles of induction CAPOX with toripalimab and surgery or WW (group B). The primary endpoint was complete response—either clinical or pathologic. The investigators reported the preliminary findings from one center, where most patients had MSS LARC (59/32), and three patients had MSI disease. The pCR was 56.2%, with no distinction made between microsatellite status or treatment arm. The most common AE was grade 3 or 4 thrombocytopenia, which was reported in 22/62 (35.5%) patients.

The single-arm phase II BFH-NGRTPD (NCT04911517) trial aimed to assess the rates of complete response achieved with the addition of tislelizumab (PD-1 antibody) to long-course chemoradiotherapy (LCCRT) for patients with cT3-4a N0M0 or cT1-4a N1-2M0 MSS LARC.[36] Patients ($n = 26$) received LCCRT with capecitabine and concurrent tislelizumab starting on day 8 of radiotherapy, followed by TME 6–8 weeks post irradiation. The primary endpoint was pCR, which in the interim results was reported to be 50% (13/26), while the objective response rate reached 73.1%. Five (19.2%) patients reported immune-related AEs—including one grade 3 ICB-related colitis—with no grade 4 or 5 AEs being noted.

The phase II PANDORA trial (NCT04083365) investigated the addition of a PD-L1 inhibitor, durvalumab,

Table 1. Results from clinical trials on radiotherapy and immune checkpoint inhibition in colorectal cancer

Trial	Intervention	Target	No. of Participants	Microsatellite/ MMR Status	Time to Surgery/Pathologic Assessment	pCR
NCT02948348 (VOLTAGE)[27]	LCCRT followed by nivolumab and radical surgery 12 weeks post CRT induction	PD-1	42 (evaluable)	MSS and MSI	12 weeks post RT	30% (MSS) and 60% (MSI)
NCT04231552[28]	SCRT (25 Gy in 5 fractions) with subsequent CAPOX plus camrelizumab 1 week after SCRT, followed by radical surgery 1 week later	PD-1	27 (evaluable)	pMMR and dMMR	9 weeks post RT	46.2% (pMMR); 100% (dMMR); 48.1% (pMMR + dMMR)
NCT04083365 (PANDORA)[29]	LCCRT followed by durvalumab 1 week post CRT. Surgery performed 10–12 weeks post CRT	PD-L1	55	Not available	10–12 weeks post RT	32.70%
NCT03127007 (R-IMMUNE)[30]	Neoadjuvant concurrent LCCRT and 5-FU with atezolizumab on day 1 of weeks 3, 6, 9, and 12. Surgery scheduled at week 15	PD-L1	26	Not available	10 weeks post RT	24%
NCT03503630 (AVERECTAL)[31]	SCRT (25 Gy in 5 fractions) followed by mFOLFOX-6 and avelumab 1 week post RT	PD-L1	40	Not available	14–15 weeks post RT	37.50%
NCT05216653 (PRECAM)[32]	Neoadjuvant SCRT followed by envafolimab and CAPOX with TME 2 weeks later	PD-L1	12	pMMR	up to 10 weeks post RT	66.60%
NCT03102047 (NSABP FR-2)[33]	Neoadjuvant CRT (no regimen) followed by durvalumab starting 3–7 days post RT. TME 8–12 weeks post RT	PD-L1	45	MSS	8–12 weeks post RT	22.20%
NCT03854799 (AVANA)[34]	Neoadjuvant LCCRT with capecitabine and avelumab starting on day 1. Surgery 8–10 weeks post CRT	PD-L1	100 (evaluable)	pMMR and dMMR	8–10 weeks post RT	23%
NCT02921256 (NRG-GI002)[35]	Experimental arm III: mFOLFOX-6 (IV) every 2 weeks followed by concurrent capecitabine and LCRT with or without pembrolizumab every 3 weeks beginning on day 1 of RT	PD-1	137	Not available	8–12 weeks post RT	31.9% (PA) vs 29.4% (CA) (ns)
NCT04911517 (BFH-NCRTPD)[36]	LCCRT with capecitabine and concurrent tislelizumab (IV) followed by TME and optional adjuvant therapy	PD-1	26	pMMR	6–8 weeks post RT	50%
NCT05215379[37]	LCCRT + sintilimab followed by CAPOX/ capecitabine + 2 cycles consolidation sintilimab. WW or surgery	PD-1	23	pMMR	Not available	30.40%
NCT04340401 (PKUCH 04)[38]	Neoadjuvant camrelizumab + CAPOX followed by LCCRT. Consolidation CAPOX if no disease progression. TME or WW	PD-1	21 evaluable for pCR	Unclear	Not available	33.30%
NCT04518280 (TORCH)[39]	Group A: induction CAPOX with toripalimab followed by SCRT and consolidation CAPOX + ICB. Group B: SCRT followed by consolidation CAPOX + ICB. WW or TME 2–4 weeks later	PD-1	62 (evaluable)	MSS and MSI	23–25 weeks post neoadjuvant treatment	56.20%

The literature search included trials that had published results as of Jun 30, 2023.
CA: control arm; CAPOX: capecitabine and oxaliplatin; CRT: chemoradiotherapy; dMMR: mismatch repair deficient; ICB: immune checkpoint blockade; IV: intravenously; LCCRT: long-course chemoradiotherapy; LCRT: long-course radiotherapy; mFOLFOX-6: oxaliplatin, 5-FU, and leucovorin; MMR: mismatch repair; MSI: microsatellite instability; MSS: microsatellite stability; ns: nonsignificant; PA: pembrolizumab arm; pCR: pathologic complete response; PD-1: programmed death-1; PD-L1: programmed death ligand-1; pMMR: mismatch repair proficient; RT: radiotherapy; SCRT: short-course radiotherapy; TME: tumor microenvironment; WW: watch and wait; 5-FU: 5-fluorouracil.

after CRT.[29] The patients received LCRT with 50.4 Gy for 5 days per week for 5 weeks with concurrent capecitabine (825 mg/m^2 orally twice daily) followed by durvalumab (1500 mg IV) 1 week post CRT every 4 weeks for three cycles. Surgery was performed 10–12 weeks post CRT induction. The study set out to measure pCR as the primary outcome, with complete clinical response (cCR) and disease-free survival as secondary outcome measures. Of the 55 evaluable patients, 18 (32.7%) achieved a pCR. Some form of tumor regression was reported in almost 62% of patients, with the rate of ICB-related AEs at 41.8% (23/55). No grade 4 AEs were noted and the investigators highlighted durvalumab's promising activity in conjunction with neoadjuvant CRT.

The muticenter phase Ib/II R-IMMUNE study (NCT03127007) included 26 T2 and T3 patients receiving LCRT (45–50 Gy in 25 fractions) with 5-fluorouracil (5-FU; 225 mg/m^2 IV) with or without atezolizumab (anti–PD-L1; 1200 mg IV) on day 1 of weeks 3, 6, 9, and 12 followed by surgical management on week 15.[30] The investigators aimed to determine the safety and efficacy of the treatment combination and reported that 9/26 (13%) patients had a grade 3-4 AE and 6 of the 25 evaluable patients (24%) achieved a pCR. The safety and efficacy profile of the treatment combination supported further investigation and the trial is ongoing.

In the phase II AVERECTAL (NCT03503630) study, which evaluated the addition of the PD-L1 inhibitor avelumab to the neoadjuvant treatment of LARC, patients with T2 N1-3 and T3 RC received SCRT (25 Gy in 5 fractions), followed by six cycles of chemotherapy (mFOLFOX) and avelumab 1 week later (10 mg/kg every 2 weeks before surgery).[31] The primary outcome—pCR (TRG 0) rate—was reached by 37.5% (15/40) of the patients, a significant improvement from the historical control group (16% pCR). In total, 27/40 (67.5%) patients had a major pathologic response rate (mpRR) (TRG 0 and 1), and the investigators reported a 17.7% rate of severe AEs (grade 3-5), none of which related to avelumab. The schedule of checkpoint inhibition in relation to radiotherapy in this study shadows those described above; although the response rates are similar, the comparison of the outcomes should be undertaken with caution because the AVERECTAL study does not disclose the MMR status of its patients and the investigators used a different chemotherapy combination from the rest of the groups.

The phase II PRECAM trial (NCT05216653) included patients with cT2-4a N+ or cT3-4a N0 pMMR LARC who received neoadjuvant SCRT followed by six cycles of a PD-L1 antibody (envafolimab) and two concurrent cycles of CAPOX, with surgery (TME) scheduled 2 weeks after the end of the full neoadjuvant course.[32] The study assessed the pCR in 12 evaluable patients and reported rates of 76.6%; the investigators noted a favorable safety profile, with 85.7% (18/21) of patients reporting grade 1-2 AEs and only two patients (9.5%) experiencing grade 3 or 4 thrombocytopenia.

Another study that scheduled ICB after the course of radiotherapy was NSABP FR-2 (NCT03102047), which included 45 patients with stage II-IV MSS LARC.[33] The phase II trial was a single-arm study assessing the sequential addition of durvalumab (PD-L1 antibody) to neoadjuvant CRT (no specific regimen reported). ICB commenced 3–7 days post radiotherapy for four cycles every 2 weeks and surgery was performed 8–12 weeks following the completion of radiotherapy. The primary endpoint, modified NAR (neoadjuvant rectal) score, was 12.03 compared to the historical control of 15.6 (p = 0.06); pCR and cCR rates were 22.2% and 31.1%, respectively. Sphincter preservation was achieved in 71.4% of the cases and the most common grade 3 AEs were diarrhea, lymphopenia, and back pain. Elevated amylase and lipase was the sole grade 4 AE. The trial is ongoing to assess the survival outcomes of the patients and identify predictive biomarkers of response.

In the phase II trial (AVANA–NCT03854799) that also used avelumab to measure its additive effect on neoadjuvant CRT on the pCR of patients with resectable LARC, the investigators recruited 101 patients from 10 centers between 2019 and 2020.[34] Most patients (89%) had cT3-4 RC, with cancer detected in the LNs in 94% of cases. The treatment involved preoperative LCRT (50.4 Gy in 28 fractions over 5.5 weeks) and capecitabine (825 mg/m^2 twice daily for 5 days/week), with avelumab starting on day 1 of CRT at 10 mg/kg every 2 weeks. Surgery would be performed 8–10 weeks following the end of CRT. The results showed a favorable profile for the regimen, with 60% (60 of 100) of the evaluable patients achieving a major pathologic response, and a pCR reported in 23% (23/100) of the participants. The investigators noticed that the treatment exerted a downstaging effect on the tumors, with only 36% of the patients remaining at T3 and T4 disease (down from the initial 89%). There was also an observed reduction in the rates of LN+ disease from 94% down to 21%. The microsatellite status was available for 62 patients, most of whom (60/62) were pMMR patients; 8% achieved a pCR and 69% had a major response to treatment. Of the two MSI-H patients, one (50%) had a complete tumor response. The investigators also reported a promising safety profile on the treatment combination; the main grade 3 or higher immune-related AEs were skin reactions noted in 4% of the patients, while general grade 3 or 4 AEs were reported in 8/100 patients, who mainly had diarrhea. After passing the safety and feasibility stage, the trial is ongoing, with the investigators evaluating the effects of avelumab plus neoadjuvant CRT on the long-term PFS and OS rates of the patients.

Pembrolizumab is another agent that has been tested as part of TNT in a phase II trial including 185 patients with stage T3-4 N0-2 LARC (NRG-GI002, NTC02921256).[35] Patients were randomly assigned 1-to-1 to FOLFOX followed by capecitabine and RT (50.4 Gy in 25 fractions) 3–4 weeks later, with or without the addition of

pembrolizumab (200 mg IV) on day 1 of radiotherapy every 3 weeks for up to six cycles before surgical management. The study's primary endpoint was change in the NAR score, with pCR, cCR, and sphincter preservation rates as secondary endpoints. Among the 135 patients who were evaluable for NAR score, the investigators did not observe a significant difference between the experimental and control groups (11.53 and 14.08, respectively), which was also true for the secondary endpoints (pCR: 31.9% vs 29.4%, $p = 0.75$; cCR: 16.6% vs 13.9%, $p = 0.95$). The rate of AEs was marginally elevated in the pembrolizumab arm (48.2% vs 37.3%), and two deaths were reported relating to FOLFOX, one in each cohort. Of the 90 patients in the pembrolizumab cohort, three (3.7%) experienced grade 3 AEs, whereas no grade 4 or 5 AEs were reported. The authors concluded that although the addition of pembrolizumab to TNT was deemed safe, the nonsignificant changes in the NAR score did not warrant further investigation.

Sintilimab, a PD-1 antibody, was the main focus of the phase II single-arm NCT05215379 trial, investigating its effect in the concurrent administration of LCCRT with consecutive chemotherapy.[37] The study, which involved patients with stage T1-3a N0M0 MSS ultra-low LARC, required that the lower edge of the tumor be no more than 5 cm from the anal verge. Patients were prescribed LCCRT (no specified chemotherapy regimen) with four cycles of sintilimab followed by six cycles of adjuvant CAPOX or capecitabine and two cycles of consolidation ICB. If cCR was achieved, the patients could then opt to adopt the WW strategy instead of TME. The investigators reported a cCR of 43.5% (10/23), with 7 patients (30.4%) achieving a pCR and objective response rate of 52.2% (12/23). Of the 10 patients who underwent surgery, 2 had a pCR and 6 (60%) had an mpRR. The anal and rectal preservation rates were 95.5% and 59.1%, respectively, and grade 3-4 AEs were reported in 4 patients (17.4%). While the long-term effect of sintilimab plus neoadjuvant LCCRT is still awaited, the investigators report a favorable safety and efficacy profile for patients with MSS LARC.

Another study that explored the efficacy of the PD-1 antibody camrelizumab was the phase II single-arm PKUCH 04 trial (NCT04340401).[38] Patients with stage II-III LARC were scheduled to receive three cycles of a triplet regimen consisting of CAPOX and anti–PD-1, followed by LCCRT and two more sequential cycles of CAPOX in the absence of disease progression. The patients would then decide between WW or TME. The primary endpoint, pCR, was reported in 21/25 patients who underwent surgery (33.3% [7/21]), while 4 patients had a cCR or near-cCR and chose the WW strategy. The most common grade 3 AEs included lymphopenia (24%), diarrhea (8%), and thrombocytopenia (4%), which in combination with the reported efficacy of the regimen, makes this another trial that offers promising results for patients with LARC.

MOUSE MODELS OF CRC EXPLORING THE SCHEDULING OF RT-ICB

The mechanisms behind the action of both radiotherapy and ICB on cancer cells have previously been described in detail[43] and although they have mostly been observed in animal models and warrant further investigation, it is worth highlighting some key biological processes that take place when radiotherapy and checkpoint inhibition work together. In brief, the DNA-damaging qualities of irradiation cause tumor necrosis, which leads to the expression of calreticulin on the cancer cells' surface. This increases the production of cancer-associated antigens that attract phagocytes and APCs, which in turn prime cytotoxic T cells. Radiation also results in an increase in the apoptosis-promoting Fas protein, the antigen-presenting MHC-1 (major histocompatibility complex class I) molecules, as well as PD-L1 on the cell surface of cancer cells, with the latter representing one way of immune evasion. When combined with checkpoint inhibition, the immunologic brake is released from the CD8+ T cells, which are able to detect and attack the cancer cells, underlining the fact that radiotherapy and ICB can work synergistically to augment the biological effect of each monotherapy.

When studying the combinatorial effect of radiotherapy and immune-modulating agents preclinically, it is essential that the models used possess an intact immune system. Through the literature search, only two previous preclinical studies have explored the scheduling of radiotherapy with ICB in CRC[44,45]; however, neither of these studies incorporated anatomically relevant models. The studies are nevertheless considered in this review because they offer evidence on the functional synergy between radiotherapy and ICB and a basis for the development of more sophisticated models to test the importance of sequencing the two modalities correctly.

In two subcutaneous immunocompetent models of BALB/c and C57BL/6 mice injected with CT26 CRC tumor cells, combining radiotherapy with PD-1 or PD-L1 inhibition significantly extended the survival of the mice, something that each inhibitor did not achieve on its own.[44] Radiotherapy was given as 10 Gy in 5 daily fractions, which reflects a standard fractionation regimen, and checkpoint inhibition was administered three times per week, for 3 weeks, starting on day 1 of radiotherapy, day 5, or 7 days after the completion of the treatment. The investigators found that commencing the PD-1/PD-L1 axis blockade on the same day as radiotherapy significantly extended the survival of the mice, as did the administration of the PD-L1 inhibitor on the last day of radiotherapy (day 5). The survival benefit, however, was lost when the drug was given 7 days post radiotherapy completion. The researchers also observed an elevation in the PD-1 expression in CD8+ cells 24 hours after the last radiotherapy fraction, an effect that was absent 7 days

later, suggesting that early inhibition of the PD1/PD-L1 axis is essential to prevent the T-cell anergy associated with radiotherapy administration alone.

In a subcutaneous immunocompetent model of BALB/c mice injected again with CT26 CRC tumor cells, the addition of anti–CTLA-4 before radiotherapy resulted in greater reduction in the tumor burden and increased survival when compared to induction after radiotherapy.[45] More specifically, compared with CTLA-4 administered 1 or 4 days post radiotherapy, CTLA-4 inhibition 7 days prior to single, high-dose irradiation (20 Gy) led to significantly greater benefit for the mice by indefinitely extending the survival of the animals. The study makes a clear point that the timing of checkpoint therapy induction in relation to radiotherapy plays a role in the treatment's effectiveness, which the authors confirmed by exploring the combination of radiation with OX40, a costimulator that increases antigen cross-presentation. When implementing the same variety of treatment schedules with that mouse model, they observed that 50% of the mice treated with OX40, 1 day after radiotherapy, achieved tumor clearance, an effect that was lost with the administration of the drug prior to or 5 days post radiotherapy. It seems that immediate antigen release following radiotherapy might offer a short therapeutic window, suggesting that the timing of immunotherapy with radiotherapy is a key factor to their synergy. It should be noted, however, that the ideal timing can be affected by variations in the radiotherapy dose and fractionation,[46] as well as the murine models and cell lines, therefore caution should be applied when relating these findings to other mouse models and treatment regimens.

DISCUSSION

This review discussed the dosing and scheduling strategies for radiotherapy and ICB in primary RC, with the results unveiling a dearth of both clinical and preclinical data to identify the ideal sequencing pattern. The literature suggests that the timing and ideal sequencing of radioimmunotherapy approaches in cancer therapy are essential parameters to consider.[41,42] The previous sections present a synopsis of 13 clinical trials that explored the combination of radiotherapy with ICB in patients with LARC. Relevant preclinical work was also identified to shed light, for the first time, on the ideal chronologic sequence of these two treatment strategies.

All 13 protocols of the clinical studies considered radiotherapy always in conjunction with chemotherapy; 7 of 13 trials scheduled the immunotherapy after the induction of radiotherapy,[27–33] with four study protocols prescribing it concurrently,[34–37] one scheduling it before,[38] and one administering it either before or after SCRT.[39] Seven trials implemented PD-1 inhibitor therapy[27,28,35–39] and six used a PD-L1 agent,[29–34] with most (8/13) reporting results on MSS LARC.[27,28,32–34,36,37,39] In those, the pCR ranged from 22.2% to 66.6%, with most of the trials (5/8) using a PD-1 inhibitor.[27,28,36,37,39] Setting aside the heterogeneity of the treatment schedules, some common ground among the trials can be found.

Considering firstly the trials that administered ICB after the end of radiotherapy, the reported pCR rates ranged from 22.2 to 66.6%. The NCT04231552 trial achieved a similar pCR rate with the anti–PD-1 agent camrelizumab as the AVERECTAL study that used the PD-L1 antibody avelumab (46.2% vs 37.5%).[28,31] Both groups of investigators prescribed SCRT to their patients but with different consolidation chemotherapy regimens (CAPOX and FOLFOX, respectively). A similar pCR was reported in the PANDORA trial (32.7%), albeit with capecitabine and LCRT.[29] In the PANDORA and R-IMMUNE trials, the comparison is less challenging; both studies have a similar number of patients (19 and 26, respectively) and prescribe PD-L1 inhibitors (durvalumab and atezolizumab, respectively); however, PANDORA reported higher pCR rates than R-IMMUNE (32.7% and 24%, respectively).[29,30] Neither of those trials reported on the microsatellite status of the patients. With a similar setup but with a higher number of patients ($n = 45$) and a specific focus on MSS disease, the NSABP FR-2 trial finds a similar pCR rate as R-IMMUNE (22.2% vs 24%) with the PD-L1 agent durvalumab.[33] However, an interesting result comes from the PRECAM trial, which includes a small patient cohort size ($n = 12$) with MSS LARC, yet reports the highest pCR rate in this review (66.6%).[32] Compared to the NSABP FR-2 study,[33] which noted a pCR rate of 22.2% and also prescribed PD-L1 and had a similar time to surgery, the difference in response rates is notable. Finally, two studies that included both MSS and MSI patients and both prescribed a PD-1 inhibitor after radiotherapy are the VOLTAGE and NCT04231552 trials,[27,28] with the former reporting 30% (11/35) pCR in MSS patients and the latter finding a higher pCR rate of 46.2% (12/26). The MSI patients in both studies had unsurprisingly better pathologic outcomes with 1/1 (100%) patients achieving a pCR in the NCT04231552 trial vs 3/5 (60%) in the VOLTAGE study.[27,28]

The pCR rates reported in the four identified studies that scheduled ICB concurrently with radiotherapy were promising but varied greatly, from 23% to 50%, and most (3/4) used a PD-1 inhibitor.[35–37] The NRG-GI002 trial did not return significantly different results on the pCR, cCR, or NAR score of its cohorts.[35] The investigators scheduled induction mFOLFOX followed by pembrolizumab (anti–PD-1) alongside LCCRT with capecitabine, a different setup to the NCT05215379 trial, which gave anti–PD-1 alongside LCCRT, followed by consolidation ICB and CAPOX or capecitabine.[37] The latter trial reported pCR rates of 30.4% compared to 31.9% in NRG-GI002, but also had a significantly smaller cohort size (23 vs 137). The BFH-NCRTPD trial involved 26 patients with ultra-low RC, followed a similar setup to NCT05215379 but without a chemotherapy-ICB consolidation arm, and reported a higher

(50%) pCR rate.[36] The AVANA study, which reported the microsatellite status in 62/100 patients and was the only one scheduling a PD-L1 antibody on the same day as LCCRT, observed a 23% pCR rate in all its patients (both MSI and MSS), with 5/60 (8%) pMMR patients having a complete tumor response compared to 1/2 (50%) MSI-H patients.[34]

The one trial that scheduled ICB before radiotherapy as part of TNT (PKUCH 04) reported a 33.3% pCR rate,[38] which compares with the VOLTAGE, PANDORA, AVERECTAL, NRG-GI002, and NCT05215379 trials, despite their differences in the agent, chemotherapy regimen, and scheduling approach.[27,29,31,35,37] Finally, the TORCH (NCT04518280) trial, the only one that included two arms to test the efficacy of ICB plus chemotherapy either before or after SCRT, observed a promising safety and efficacy profile on the combination (pCR: 56.2%); however, it failed to report which arm displayed the greatest benefit.[39] It is important to note that these preliminary results came from a small cohort of a single center; therefore, they should be interpreted with caution. A visual summary of the discussed studies in terms of their choice of ICB induction in relation to radiotherapy can be seen in Figure 1.

In the discussed trials, the small sample sizes, as well as the great variability between the schedules and choice of checkpoint inhibitor, render a decision on the optimal ICB-RT schedule challenging. It is expected that the awaited long-term findings of the above trials will aid in the decision-making process, but as the element of safety and clinical practicality of RT-ICB combinations in LARC is being established, a key benefit that could be incurred as a result of this treatment strategy is worth noting: organ preservation (OP). As showcased clearly in two landmark trials, CAO-ARO-AIO-12 (NCT02363374) and OPRA (NCT02008656), both pCR and OP rates can be increased when CRT is prescribed prior to consolidation chemotherapy (pCR: 17% vs 25% in CAO-ARO-AIO-12; OP: 43% vs 58% in OPRA).[47,48] Both trials noted no significant difference in the long-term oncologic or survival outcomes of the patients, suggesting that CRT followed by chemotherapy should be offered to patients with RC. Checkpoint blockade could be introduced alongside TNT, with the ambition to completely avoid surgery. Both the NRG-GI002 and PKUCH 04 trials included ICB as part of a TNT approach with induction chemotherapy and had similar pCR rates (31.9% and 33.3%, respectively).[35,38] Based on the results of the CAO-ARO-AIO-12 and OPRA trials, it is possible that offering CRT and consolidation chemotherapy could unlock the tumor response mechanism that would reflect higher pCR rates.

The significance of scheduling radiotherapy with ICB correctly is supported by preclinical studies; however, whether the inhibitor should be given first or after radiotherapy-induced neoantigen production is an underinvestigated topic and requires animal models that recapitulate rectal cancer in situ and can reflect the clinical setting.[49,50] So far, the mouse models used to answer the question of scheduling radiotherapy with ICB suffer from one limitation that impedes their translational value: they are mostly subcutaneous murine models that use CRC cell lines, which lack the complexity of the tumor microenvironment of rectal tumors when studied at the primary site. In addition, there is evidence of increased tumor immune infiltration in certain cell lines that are used in xenograft studies, like CT26; they reflect a more immunogenic phenotype and display higher sensitivity to CTLA-4 and PD-L1 inhibition than MC38 cells.[51] Both subcutaneous models described in this review used the CT26 CRC cell line on immunocompetent mice and showed that the effect of immunotherapy on the tumors depends on the timing of ICB induction in relation to radiation exposure.[44,45] Similarly, a preclinical study on BALB/c and C57BL/6 mice injected subcutaneously with a mammary and a colon adenocarcinoma cell line, respectively, also observed a synergistic relationship between radiotherapy and ICB with PD-L1, which was absent when either treatment was used on its own.[52] In their study, Deng et al[52] administered a PD-L1 inhibitor on the same day as radiotherapy; they observed tumor regression and extension in survival, also achieved in the experiments of Dovedi et al.[44] However, it seems that different checkpoint inhibitors require different "tuning," as suggested by Buchwald et al.[46] In their model, treated with a CTLA-4 antibody, Young et al[45] claim that giving the antibody 5 days prior to single-dose radiotherapy achieved significantly better responses than concurrent or subsequent induction, whereas both Dovedi et al[44] and Deng et al[52] showed that starting PD-L1 treatment on the same day as fractionated irradiation offered greater survival advantage and immune cell mobilization. A possible explanation is that CTLA-4 seems to exert its function at the early stages of T-cell proliferation in the LNs, whereas the PD-1/PD-L1 axis is involved at a later stage, when T cells have already been activated.[43,46]

The need for more sophisticated and faithful models remains. It is now almost certainly not a question of whether timing matters, but rather of which timing is the most appropriate and how to model it. To retain anatomical relevance and explore clinically applicable radioimmunotherapy combinations, the use of orthotopic mouse models is suggested.[50] In these models, tumor establishment can be achieved via a colonoscopic submucosal injection of murine CRC-derived organoids into the rectal submucosa,[53–55] while there is also a role for carcinogen induction[56] and mechanical disruption.[57] Orthotopic models have reported high take rates and bestow clinical relevance on the preclinical platform in terms of tumorigenesis, local invasion, and metastatic routes, with the added benefit of allowing the tumor microenvironment to be studied in situ.[54] As a result of these characteristics, the orthotopic model's translational value increases, thus helping in the transition of radioimmunotherapy combinations for RC into clinical trials.

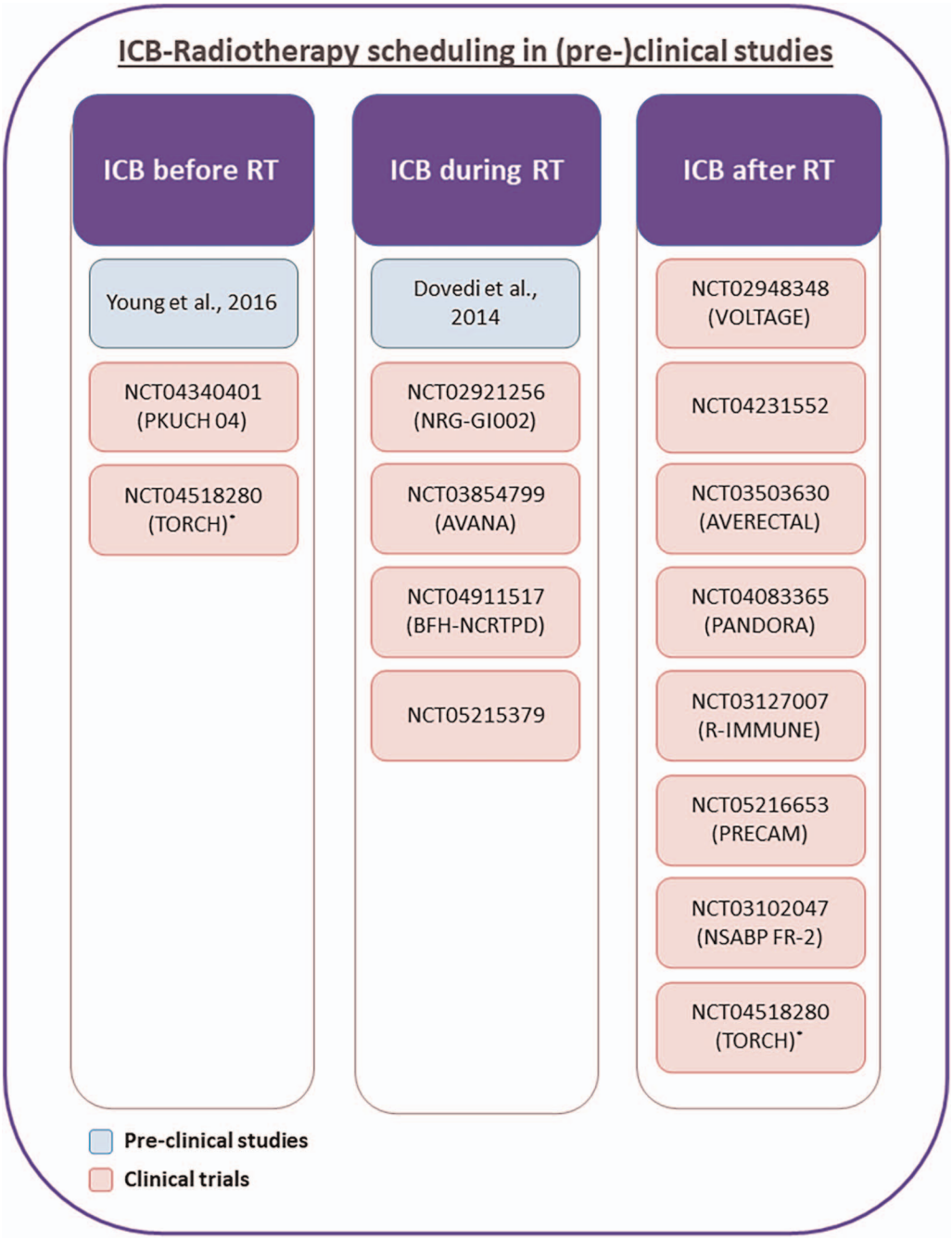

Figure 1. Visual arrangement of studies according to their choice of ICB induction relative to radiotherapy. Studies are allocated according to the schedule that they investigated (clinical trials) or the schedule that they deemed most appropriate (preclinical studies). *The TORCH trial included two scheduling regimens, so it is included both in the "before" and "after" columns. ICB, immune checkpoint blockade; RT: radiotherapy.

Ultimately, when modelling radiotherapy with ICB, whether in the clinical or preclinical setting, it is recommended that three scheduling arms be included to identify whether the action of ICB is optimal neo-adjuvantly, concurrently, or sequentially.

CONCLUSION

This review has explored trials that offer promising results on the role of ICB in the treatment pathway of patients with rectal cancer, especially those who present with MSI disease, with encouraging pCR rates that foreshadow the vital role ICB might play in the first-line treatment of these patients. For now, LARC continues to be an area of clinically unmet need largely because of a lack of effective nonsurgical therapies to target most patients who present with MSS disease. This review undertook an evaluation of the available clinical and preclinical studies that explored the combination of radiotherapy and immu-

notherapy for the treatment of RC. The results revealed that both modalities have an established role in the treatment of CRC as monotherapies, and there is little doubt that the combination of radiotherapy with ICB is a promising care strategy in the primary setting of RC; however, it is still too early to determine the ultimate scheduling regimen of this treatment combination. The development of preclinical platforms that are anatomically appropriate and hold translational value to explore the ideal timing of radioimmunotherapy combinations will be invaluable in the future in guiding clinical trial design. It is suggested that prospective animal studies that seek to explore novel combinations of ICB and radiotherapy use orthotopic mouse models of RC so that both the clinical and anatomical relevance are maintained.

Supplemental Material

Supplemental materials are available online with the article.

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