Tumour Review

The optimal timing for the interval to surgery after short course preoperative radiotherapy (5×5 Gy) in rectal cancer - are we too eager for surgery?R. Glynne-Jones^{a,*}, M. Hall^b, I.D. Nagtegaal^c^a Radiotherapy Department, Mount Vernon Centre for Cancer Treatment, Mount Vernon Hospital, Northwood HA6 2RN, United Kingdom^b Department of Medical Oncology, Mount Vernon Centre for Cancer Treatment, Mount Vernon Hospital, Northwood HA6 2RN, United Kingdom^c Department of Pathology, Radboudumc, PO BOX 9101, 6500 HB Nijmegen, the Netherlands

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ABSTRACT

Background: The improved overall survival (OS) after short course preoperative radiotherapy (SCPRT) using 5×5 Gy reported in the early rectal cancer trials could not be replicated in subsequent phase III trials. This original survival advantage is attributed to poor quality of surgery and the large differential in local recurrence rates, with and without SCPRT. Immuno-modulation during and after SCPRT and its clinical implications have been poorly investigated. We propose an alternative explanation for this survival benefit in terms of immunological mechanisms induced by SCPRT and the timing of surgery, which may validate the concept of consolidation chemotherapy.

Material and methods: We reviewed randomized controlled trials (RCTs) and studies of SCPRT from 1985 to 2019. We aimed to examine the precise timing of surgery in days following SCPRT and identify evidence for immune modulation, neo-antigens and memory cell induction by radiation.

Results: Considerable variability is reported in randomised trials for median overall treatment time (OTT) from start of SCPRT to surgery (8–14 days). Only three early trials showed a benefit in terms of OS from SCPRT, although the level of benefit in preventing local recurrence was consistent across all trials. Different patterns of immune effects are observed within days after SCPRT depending on the OTT, but human leukocyte antigen (HLA)-1 expression was not upregulated.

Conclusions: SCPRT has a substantial immune-stimulatory potential. The importance of the timing of surgery after SCPRT may have been underestimated. An optimal interval for surgery after 5×5 Gy may lead to better outcomes, which is possibly exploited in total neoadjuvant therapy schedules using consolidation chemotherapy. Individual patient meta-analyses from appropriate SCPRT trials examining outcomes for each day and prospective trials are needed to clarify the validity of this hypothesis. The interaction of SCPRT with tumour adaptive immunology, in particular the kinetics and timing, should be examined further.

Introduction

Short course preoperative radiotherapy (SCPRT) reduced the 5-year local recurrence (LR) rate in patients with rectal cancer from 27% to 11%, from 11% to 5%, and from 12% to 5% in the Swedish [1], Dutch [2–4], and Medical Research Council (MRC) CR07 trial [5,6] respectively, compared to surgery alone or surgery and selective postoperative radiotherapy or chemoradiotherapy. Earlier trials of SCPRT often used larger fraction sizes, larger fields and sometimes 2-field delivery [7–15].

SCPRT reduced the LR rate both before and after the introduction of total mesorectal excision (TME) [1,2,5,11,13,15] but the impact on overall survival (OS) is less consistent (Fig. 1). Only early trials, which

compared SCPRT and immediate surgery with surgery alone demonstrated an improvement in OS [1,11,13,15,16,17], which is maintained long-term [18], but no benefit in OS is reported in subsequent trials [3,4,5]. A Cochrane meta-analysis confirmed better local control after SCPRT versus surgery alone, but reports no reduction in distant metastases and similar OS [19]. Some suggest the improvement in OS in early trials reflects the contemporary poor surgical techniques and the absolute reduction in LR of 17% achieved by SCPRT [20] in the SRCT [18], compared with only 6% in both the Dutch TME [3] and CR07 trials [5].

As we show in (Fig. 1) OS has improved over time from Stockholm I in the 1980s to Stockholm III completed in 2013. The quality of surgery improved, but also imaging. Local staging in terms of MRI has come

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Fig. 1. The overall survival reported in phase III randomised trials of SCPRT prior to surgery compared with TME surgery alone with or without postoperative radiotherapy/chemoradiotherapy.

into play over the past 12 years. CT was not readily available in many units until the mid-1990s, and so many patients in the early trials would have had undetected small volume metastatic disease at presentation.

Our hypothesis is that, in the Stockholm II and Swedish Rectal cancer trial, the slightly later timing of surgery allowed memory cells against the cancer to be formed by the effects of SCPRT and for these to move not only into loco-regional lymph nodes but more systemically.

SCPRT using 5×5 Gy is usually delivered over five consecutive days with immediate surgery within 1–7 days of completing RT, hence there is usually insufficient time for down-staging [21]. Almost the only variables between these trials are the overall treatment time (OTT) from the start of radiotherapy to the time of surgery, the field arrangements and the field size. If SCPRT starts on a Monday, the OTT (Fig. 2A) from the start of radiotherapy to surgery is usually between 7 and 12 days, with a median of 9 days. Trials showed considerable variability in the median OTT (Fig. 2B).

Radiation is an effective treatment to achieve local control in many cancers. Deeper understanding of the molecular processes involved in the cytotoxicity from radiation and technological advances have improved outcomes. The tumor microenvironment, historically in terms of the degree of oxygenation, and more recently immune effects have provided a fruitful source of radiation research, particularly since the development of checkpoint inhibitory treatments. Understanding the interaction between the immune system, radiation and cancer has become increasingly relevant. Immune cell infiltration based on the numbers of CD3+ and CD8+ lymphocytes within and at the periphery of a primary rectal cancer, using the immune-score methodology, appears both prognostic and predictive [22].

It is generally accepted that human tumours are somewhat both antigenic and immunogenic to cytotoxic T-lymphocytes, which recognise peptides on the tumour cell surface presented by major histocompatibility complex (MHC-I) antigens – the human leukocyte antigen (HLA-I) in humans. There are no validated biomarkers for activity or resistance to radiotherapy – let alone such markers for the utility /actionability of RT in combination with immunotherapy – despite the almost universally accepted tenet that radiation can make ‘cold’ tumours ‘hot’ [23,24].

The question is whether the cytotoxic effects from radiotherapy can be enhanced by secondary immune effects, and whether these immune effects are clinically relevant. If SCPRT can exert both regional and systemic immune-mediated effects besides the cytotoxic activity within the treatment target volume [25], then what is the optimal interval to achieve these effects? – and hence what is the ideal timing for adding

Fig. 2. A and B show how overall treatment time (OTT) is defined and the median OTT in the randomised large trials of SCPRT.

immune checkpoint inhibition to capitalise on these effects.

Host immunity is activated by antigens on human leukocyte antigen (HLA) class I molecules, which protect against foreign antigens at many different sites in the body [26]. Usually peripheral blood is sampled in T-cell studies, since repeated biopsy of tumour, lymph nodes and distant tumour or normal tissue sites is difficult, but blood contains few of the total T-cells in the body [27] – although circulating T-cells may be the most activated. T-cell immune selection pressure favours tumour sub-clones, which have low HLA expression and attract less tumour infiltrative lymphocytes (TILs). Hence early stage tumours are predominantly HLA-I positive, but become more HLA-I negative as the cancer progresses [28]. Radiation can potentially re-establish HLA

expression within a few days [29].

Memory cells can persist in peripheral tissues after removal of the tumour [30], and can circulate via the blood but retain the capacity to re-enter lymph nodes. In contrast, tissue-resident memory T cells appear confined to parenchymal tissues [31]. Their protective capacity may be compartmentalized in tissue sites favouring loco-regional effects initially and distant effects later. Hence, the timing of surgery may affect migration and distribution [32]. We hypothesize that a delay 5–12 days after the completion of SCPRT before surgery might produce more favourable immune effects, and a longer OTT might allow memory cells to proliferate and disseminate to lymph nodes and distant organs - accounting for improved OS.

In this review we examine the timing of surgery after SCPRT, and investigate immune effects and systemic oncological outcomes.

Search strategy and selection criteria

We searched PubMed, Medline, Embase, Web of Science and the Cochrane Library from January 1985 until June 2020 for randomized, controlled, phase II and III trials comparing SCPRT using 5×5 Gy and surgery with either surgery alone or CRT for patients with non-metastatic rectal cancer. We also searched for relevant retrospective population and institutional analyses of SCPRT. Articles were identified through hand-searching relevant papers and abstracts from international meetings within 5 years. We used MeSH or combined free terms using the key words: “rectal cancer, neoadjuvant, preoperative treatment, radiation, short course radiotherapy”. We restricted our searches to reports published in English. Studies meeting the inclusion criteria and relevant to the broad scope were selected for full-text review. The primary endpoint of interest was OS.

Results

Study selection and characteristics of included studies

After reviewing 542 relevant titles and abstracts, 297 were excluded due to duplication or irrelevancy. The full-text articles of 109 articles were obtained to clarify eligibility. We excluded another 61 articles (reviews or inadequate data). Finally, 13 randomised trials and 31 other SCPRT studies [1–18,33–49] were included into the review with pre-clinical data on the timing of immune effects. The literature screening process is shown in Fig. 3. 316 patients enrolled in Stockholm II up to 1990 are also included in the SRCT and hence double counted, but not patients enrolled thereafter.

Variability in timing between start of SCPRT and surgery

Most trials in their protocol specified SCPRT be delivered over 5–7 days and surgery take place within 7 days. It is difficult to identify the actual duration of SCPRT, the interval following surgery and hence the OTT, as trials tend to present limited data in early preliminary reports [2,11,16,33] rather than with the definitive outcome data. The trials report a median OTT from start of SCPRT to surgery in randomised trials ranging between 8 [34–36] and 14 days [37], with the Dutch TME and CR07 trials [5,38] showing an OTT of 9 days. Some trials provide no data [39–43] or only randomised to a longer interval (4–8 weeks) [44–46]. The relevant randomised trials are tabulated (Table 1).

In the SRCT, the median OTT was 11 days. The duration of SCPRT was ≥ 7 days in 131/566 (23%). The interval after SCPRT to surgery was ≥ 8 days in 66/566 (12%) of patients i.e. a substantial proportion would have an OTT of 13–16 days [16,17]. In the Dutch TME trial 38% had an OTT of ≤ 8 days with a median 9 days, and exceeded 10 days (a minor protocol violation) in only 110/846 eligible patients (13%) [2]. A subsequent report, from patients recruited in the Netherlands, excluded 78 patients because they violated the protocol (i.e. OTT exceeded 10 days) [47]. In this group 489/642 (76%) of the irradiated

patients received SCPRT within 5 days and/or surgery with 5 days of completion of SCPRT (OTT of 10 days). In the CR07, the median OTT was 9 days, with an interval to surgery of only 4 days [5].

Population data from 5888 patients in the Netherlands shows a median OTT of 9 days [48]. In other population studies median OTT varies from 9 [49] – 10 days [50,51]. Single centre retrospective studies report median OTT ranges from 7 days [52] – 9 days [53] and observational studies report longer OTT [54].

Does the timing of surgery after SCPRT influence down-staging?

The early trials of SCPRT and surgery did not individually report any down-staging [10,11]. A combined analysis of 2 early Swedish trials showed a significant association between the OTT and tumour pathological size. The interval after SCPRT also significantly decreased the risk for nodal involvement (OR 0.73, 95%hCL 0.58–0.92) – particularly when the OTT was ≥ 10 days [37]. Later studies confirm down-staging is only observed if surgery is delayed > 10 days [21,38]. SCPRT leads to smaller tumour size ($p < 0.001$) [38], a reduction in FDG-uptake (maximum standardized uptake value - SUVmax) of 39% [55] and a 50% reduction in tumour-cell-density, which continues to diminish over 15 days [56].

Does the timing of surgery after SCPRT affect surgical mortality?

An increased postoperative mortality was reported in early trials [7,11,12]. Treatment volumes then reduced from L2 to mid L4 [15,16], and age limited to ≤ 80 . A non-significant excess mortality was noted if two radiotherapy parallel-opposed beams were used (compared with protocol-mandated 3 or 4 beams) [1].

Subgroup analyses of Stockholm I + II showed higher post-operative mortality after an OTT of ≥ 10 days ($P < 0.001$) [57]. In Stockholm II 13/272 (5%) of the SCPRT patients died of inter-current disease within 6 months of surgery versus 4/285 (1%) of the non-irradiated group ($P = 0.02$ [15]. Despite this early mortality, patients who underwent SCPRT and curative surgery had better OS (46%) versus (39%; $P = 0.03$). Subsequent international SCPRT trials [2,5] mandating a brief interval of 2–5 days from completion of RT.

In the Dutch study postoperative mortality was 8.4% after an interval of > 3 days following SCPRT, compared to 4.1% in those with a shorter interval ($p = 0.02$) [58,59,60]. A subsequent analysis aligned the timing with the Stockholm III analysis [61] i.e. surgery 0–3 days, 4–7 days, 8–27 days and 28 days or more [62]. Patients aged ≥ 75 years operated 4–7 days after the last fraction of radiotherapy had a higher chance of dying due to non-cancer-related causes compared to patients with an interval of 0–3 days [61].

Initially, the Stockholm III trial [63] recommended surgery within 11 days from the start, or delayed for some weeks. An impaired post-operative leucocyte response after a short interval appeared associated with postoperative complications [64]. A subsequent analysis, showed delaying 4–8 weeks was associated with fewer postoperative complications ($p = 0.001$) [36]. In the last analysis, significantly more surgical complications were reported after 8–13 days from the start of SCPRT until surgery, although 30-day and 90-day mortality were equivalent [62]. Long-term cancer-specific-survival and OS were not examined according to the individual OTT, and no trial has actually randomized the interval between SCPRT and immediate surgery. UK population data has not shown differences in postoperative outcomes in relation to mortality according to length of interval to surgery [49].

Does the timing of surgery after SCPRT influence overall survival?

In Stockholm II patients who underwent SCPRT and curative surgery had better OS (46%) versus (39%; $P = 0.03$). Death due to rectal carcinoma showed a relative reduction of 25% ($P = 0.02$) with SCPRT in all included patients and 40% ($P = 0.001$) after SCPRT and curative surgery. The major difference in OS derives from patients with Dukes B [15]. In the SRCT, with a 13-year median follow-up, the OS rate in the SCPRT group was 38% versus 30% in the non-irradiated group

Fig. 3. Flow chart of selection process of articles included in the review.

($P = 0.008$). The cancer-specific survival rate was 72% versus 62% in the non-irradiated group ($P = 0.03$), and the LR rate was 9% v 26% ($P < 0.001$), respectively [18]. Sub-analysis showed OS in stage I was 54% in the irradiated patients versus 45% in the non-irradiated patients, and stage II 38% versus 30% and stage III 18% versus 16% respectively. It seems difficult to accept that these differences for the earlier stages rather than stage III simply reflect poor surgical technique. We hypothesize that a 9% difference on survival for stage I and an 8% for stage II in part reflects immune effects from the SCPRT optimized by slightly later surgery.

Later trials employed TME and so SCPRT had less effect on LR and in turn on OS?

Later trials used optimal quality TME (complete and within the mesorectal plane) in 50% of patients [2,5,6], have fewer events and may be underpowered to show differences in OS. In the Dutch TME trial a benefit in cancer-specific-survival was reported only in patients with stage III and a negative CRM (i.e. likely to be better quality surgery) [2]. The CRO7 trial initially reported better DFS for patients who received SCPRT (HR 0.76, 95% CI 0.62–0.94, $p = 0.013$), with 6% difference at 3 years (95% CI 5.3–6.8) (77.5% vs 71.5%), but no OS benefit [5]. Only surgery in the mesorectal plane (i.e. optimal quality) conferred a significantly improved DFS, and patients with surgery in the intra-mesorectal or intramuscular plane did not benefit. (Fig. 4). Subsequent reports with 8 years follow-up showed no statistical difference in DFS or OS [65]. In CRO7, those who underwent non-optimal surgery

(i.e. within the muscularis propria plane and intra-mesorectal plane) had less events in terms of LR in the SCPRT arm 14/261 (5.4%) compared with the selective CRT arm 28/291 (9.6%) [6]. This difference was substantially less than that reported in the SRCT, where there was almost a threefold reduction from 27% to 11% [1].

The greatest benefit of SCPRT in these latter trials employing was achieved with high quality TME without an involved CRM [4,6]. In addition the Dutch TME and CRO7 trials specifically showed that SCPRT could not compensate for an involved circumferential margin (CRM) associated with poor surgery [6,66].

These results are therefore inconsistent with the explanation of poor surgical technique to account for differences in results the Stockholm II and Swedish Rectal cancer trial and those within the Dutch TME trial and CRO7.

Linking local recurrence and survival

The absolute difference in LR in CRO7 was 11% v 4% (HR 0.38) compared to the SCRT 27% v 13% (HR 0.46) [5], but the rate of metastatic disease appears more constant. Population data show OS has improved modestly over time for rectal cancer [67]. Later rectal cancer trials may have been underpowered to show a statistically significant difference in OS, but both the reduction in LR and the improvement in OS should have demonstrated the same relative differences with SCPRT –which are not seen in subsequent trials (Fig. 1).

Table 1
Reported Randomised studies of SCPRT (5 x5 Gy) in rectal cancer. No = number, RT = radiotherapy, * Combined with SRCT # 57% also in SRCT. @authors information.

Trial names (authors)	No. of pts	Protocol duration for SCPRT (days)	Protocol Interval to surgery after start of SCPRT	Protocol Interval to surgery after end of SCPRT	Down-staging reported	Reported Interval from start of SCPRT to surgery	Reported Interval in days from end of SCPRT (Range)	% with surgery > 7 days after fin of SCPRT	OTT > 10 days	Overall survival difference
Uppsala (1980-1985) Pahlman 1985	360 182 SCPRT Total 471	5-7 days	Not stated	Within 7 days	NO	Reports 172/182 (95%) Received RT within 5-7 days	213/234 (91%)	14/182 (8%) 7 at 8 days 7 > 8 days	Not stated	No
Pahlman 1990 ¹¹ , Frykholm 1993 ¹²	236 SCPRT and 235 post-op RT				Dukes A 15(6%) versus 13(6%) Dukes B 123(52%) vs 120 (51%) Dukes C 71 (30%) vs 71 (30%)	RT within 5-7 days Median 10.5 days *	Within 7 days	range 8-22 days	Not stated	Reduced local recurrence only 21% vs 12%
Stockholm I ^{8,7} (1980-1987) Cedemark 1985	849 424 SCPRT 425 surgery alone	5-7 days	Not stated	1-7 days	NO Dukes A 117 (28%) vs 113 (26%) Dukes B 123 (30%) vs 141 (33%) Dukes C 117 (28%) vs 117 (28%)	Not stated	Not stated	Not stated	Not stated	No
Stockholm 1987 Stockholm 1990 Cedemark 1995					Dukes B 69 (25%) vs 79 (28%) Dukes C 63 (23%) vs 92 (32%)	Not stated	Not stated	Not stated	Not stated	Yes
Stockholm II ^{9, 13} (1987-1993) Anon. Ann Surg Oncol 1996 Martling 2001	577 272 SCPRT 285 surgery alone	5-7 days	Not stated	Within 7 days	YES (p = 0.02) Dukes A 98 (36%) vs 78 (27%) Dukes B 69 (25%) vs 79 (28%) Dukes C 63 (23%) vs 92 (32%)	Not stated 'Vast majority' Received RT within 5 days Mon-Friday	Not stated	Not stated	Not stated	Yes
*316/577 included in Swedish Rectal Cancer trial ^{14, 1}										
Swedish Rectal Cancer trial ^{14, 1} (1987-1990) SRCT 1993 SRCT 1996 SRCT 1997	566*	5 days	Not stated	Within 7 days	YES (p = 0.008). Dukes A 181 (33%) vs 154 (27%) Dukes B 195 (35%) vs 173 (31%) Dukes C 177 (32%) vs 230 (41%)	Median 11 38% at 8 days Median 9 days	Not stated	66/566 (12%)	Not stated	Yes
*The Swedish RCT trial contained 316 patients previously included in Stockholm II (of whom 272 received SCPRT and 285 surgery alone)										
Dutch TIME ^{2,3,4}	1805	5-7 days	3-5 days	not > 10 days	NO	Median 9 days	Median 3 days	9/642 (1.5%) -	110/846 (13%) (Kaptein 2001)	No
CR0 ^{5,6} (1998-2005)	1350 674 SCPRT 676 selective postop CRT	5-7 days	Not stated	Within 7 days	Minimal T down- staging only	Median 9 days	Mean 4 days	9% only	no	no
Polish ^{15,16}	155	5-7 days	Within 7 days	Not stated	Not assessable	Median 8 days	Not stated	Not stated	Not stated	OS similar to CRT arm
TROG ¹⁷	163		Not stated	3-7 days	Not assessable	Not stated	Not stated	Not stated	Not stated	OS similar to CRT arm
Pach 2012 ¹⁸ Pach 2012 ¹⁸	77 77	5 days 5 days	Not stated Not stated	7-10 days 4-5 weeks	Yes Yes	Median 14 days Not stated	Median 9 days@ Median 33 days@	75% 100%	No No	No No
Salmenkylä 2012 ¹⁹ Finnish Trial	278	5 days	< 7 days	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated
Stockholm III ^{20,21, 22} Stockholm III ²²	357 355	5 days 5 days	Within 1 week 4-5 weeks	Within 1 week 4-5 weeks	Not evaluable YES	Median 8 days Median 47 days		75% 100%	No No	No No

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Table 1 (continued)

Trial names (authors)	No. of pts	Protocol duration for SCPRT (days)	Protocol Interval to surgery after start of SCPRT	Protocol Interval to surgery after end of SCPRT	Down-staging reported	Reported Interval from start of SCPRT to surgery	Reported Interval in days from end of SCPRT (Range)	% with surgery > 7 days after fin of SCPRT	OTT > 10 days	Overall survival difference
Berlin cancer Society Trial ^{23, 24}	399	5 days	Not stated	Within 7 days	No data	No data	No data	No data		No data
Eitta 2010 ²⁵	16	Not stated	Not stated	Within 7 days	N/A compared with CRT	No data	No data	No data		no

Short term effects of radiotherapy on tumour and normal tissues

Few data apart from size of the primary tumour and nodal status derive from comparisons of irradiated and un-irradiated histological specimens in early trials [38]. The Dutch TME trial examined the intrinsic tumor characteristics and histopathological and molecular changes in terms of mucin production, inflammatory responses and immunological changes [47,68], apoptotic response [69,70] p53 up-regulation [71,72], gene-expression [73,74] and HLA expression [75,76]. There is little evidence that SCPRT altered these characteristics in a substantial proportion of patients – in particular HLA-DR was induced by IFN γ in rectal cancer cell lines, but not in human tumours after irradiation. Using 1198 samples from Dutch patients within the trial, more apoptotic cells were observed after SCPRT compared to surgery alone (23 versus 11 apoptotic cells/mm² tumor epithelium respectively) $P < 0.001$). However, the level of apoptosis did not increase over the subsequent 2–6 days following SCPRT [70].

Does the timing of surgery after SCPRT lead to more favourable immune effects?

The neo-antigens produced by irradiation and dying cancer cells need to be presented by antigen-presenting cells (APCs) to form antigenic peptide-MHC-1/HLA-1 complexes, which in turn induce a sub-population of memory CD8+ T cells. The optimal timing of this process is unknown [77]. There are a number of molecules of interest as potential indicators of neoantigens, which can influence immune responses and facilitate both presentation and recognition of MHC class I peptides. These include but are not limited to: ICAM-1, high-mobility group box-1 (HMGB1) protein, Integrins, cadherins, CD80, Fas, TRAIL, Calreticulin.

Virtually all the available clinical information derives from the Dutch TME trial, and is therefore limited to a narrow window of 4–5 days post SCPRT as patients with an OTT of > 10 days were considered a protocol violation and not assessed [47]. A study based on a subset of 160 Dutch patients within the trial evaluated the host response against rectal cancer cells and subsequent outcomes. Patients with an extensive peri-tumoral inflammatory infiltrate had half the LR rates (3.4% versus 6.9%, $p = 0.03$) [68] and intra-tumoral T-cells was associated with lower rates of distant metastases (32% versus 76%, $p < 0.0001$) [47]. High numbers of peri-tumoral and intra-tumoral cytotoxic T cells (CD8+) correlated with a lower risk of distant metastases ($p = 0.0005$) [68].

Using the whole 1530 Dutch population, a second study excluded patients with an overall treatment time > 10 days ($n = 78$) from the analysis. The presence of extensive peri-tumoral inflammatory infiltrate was again associated with better OS ($p < 0.0001$), lower rates of distant recurrence ($p < 0.0001$) and LR ($p = 0.03$).

SCPRT does modify this immune response. The timing of surgery appears critical. Patients in the control arm, who underwent surgery alone had an extensive inflammatory reaction at the edge of the tumour in 126/706 (18%). This response was reduced to only 40/598 (6.7%) in patients, who underwent 5×5 Gy [47]. In a subset of 160 patients operated immediately on the day after radiotherapy (OTT 6) showed no inflammatory reaction at all [68]. The average extensive inflammatory reaction was 7% ($n = 570$) from day 2 until day 5 after radiotherapy (OTT 7–10), rising to 15% and 18% baseline, respectively ($n = 46$ and $n = 17$) at 6 and 7 days after irradiation (OTT 11 and 12). Thus there is a rapid initial decrease in an inflammatory reaction ($p < 0.001$) after SCPRT compared to patients undergoing TME alone, with a subsequent rebound within 4–5 days [47]. (Fig. 5).

Any advantage extending the interval to surgery further to 4–8 weeks?

In the Stockholm III trial, extending the interval to surgery to 4–8 weeks, increased pathological complete response (pCR) rates and significantly reduced postoperative complications (HR 0.61, $p = 0.001$) [78,79] with a non-significant reduction in inter-current deaths (57/357 versus 47/355) ($p = 0.13$) [36]. The longer interval did not

Fig. 4. Forrest plot showing results of SCPRT versus selective postoperative CRT in the CR07 trial according to the quality of surgery. For local recurrence and more particularly for DFS, only patients having surgery in the mesorectal failed to cross unity. Patients with surgery on the intramesorectal or intramuscular plane do not appear to benefit in DFS.

Effect of treatment on circumferential resection margin and plane of surgery subgroups

CRM=circumferential resection margin. HR=hazard ratio. +ve=positive. -ve=negative.

significantly reduce distant metastases, or improve OS (HR 0.90 (0.70–1.15) $p = 0.46$).

A small randomised trial (154 patients) investigated the time interval between SCPRT and surgery and 5-year OS and recurrence rates comparing an interval after surgery of 7–10 days and 4–6 weeks. [37]. The median was 9 days in the short interval compared to 33 days in the delayed surgery arm. Significantly more systemic recurrences occurred in patients treated with a short interval – 12.3% versus 2.8% ($P = 0.035$) although when local and distant recurrences were combined there was no difference – 9/65 (13.8%) versus 9/71 (12.7%) respectively. 5-year OS was lower in the short interval (63% versus 73%)—but the difference was not statistically significant.

A retrospective study in LARC compared CD4+ and CD8+ from initial biopsies with surgical specimens after treatment in 19/52 (37%) patients treated with SCPRT followed by surgery within 7 days, and 33/52 (63%) with long-course CRT followed by surgery 4–6 weeks later [80]. The CD8+ count increased in post-treatment tumour samples in the long-course treatment group ($p = 0.04$), but at surgery 7 days after SCPRT there was no difference.

As well as enhancing effector T cells, radiotherapy can inhibit immunosuppressive tumour-associated macrophages (TAMs), T-regulatory cells (Tregs) and myeloid derived suppressor cells (MDSCs). A small study in rectal cancer performed sequential blood samples at baseline, 14 days, 5 weeks and 8 weeks (pre-surgery) following the start of SCPRT. A substantial decrease in circulating immune-inhibitory peripheral MDSCs and Tregs at 7 days after SCPRT showed a nadir at 6 weeks [81]. This study suggests there may be an optimal interval before the Tregs recover and surviving clonogens/stem cells recreate a protective environment.

A retrospective study of 185 patients with rectal cancer showed the density of CD45RO(+) TILs in tumour was associated with tumor down-staging and ypTNM stage 2–4 weeks following 30 Gy/10 fractions and predicted 3-year DFS [82].

Reports of clinically meaningful ‘abscopal’ regression outside the treatment field are rare [83]. Cancer overcomes early host immune responses by inhibiting the function of CD8(+) and CD4(+) T cells, driving a shift to higher regulatory T-cell to T effector ratio, and up-regulating PD-L1/PD-1 expression. Fractionated radiation with higher individual doses appears to be more effective in triggering anticancer immune responses. Hence, current recommendations for radiation schedules to be integrated with immunotherapy support a hypo-fractionated RT regimen - ideally with 3 to 5 doses of < 10 to 12 Gy i.e. not

dissimilar schedules to our current 5x5Gy [84,85]. Such hypo-fractionated radiation induce DAMPS more effectively [86].

Discussion

We have shown that the timing of early surgery after SCPRT varies greatly in the trials by a few days (3–8 days), and this variation appears to impact on tumour response, down-staging, and to a lesser extent - surgical complications, short term mortality and possibly OS. The Stockholm II and SCRT [1,12] are both the longest outliers in terms of the interval to surgery, statistically significant nodal down-staging and the benefit in terms of OS.

We hypothesize that if an inflammatory infiltrate and peri-tumoral CD8+ infiltration is necessary to produce memory cells, then the timing of surgery could be crucial. The trajectory of immune effects derived from the Dutch TME trial suggest that the optimal time for performing surgery is at least 7 days from the end of RT (Fig. 5C). Extrapolating the trajectory could imply an even later optimal timing may be necessary, before the tumour is surgically resected, but waiting too long [81] allows reversion to the baseline state.

Tumours evade the host immune defences by ineffective antigen presentation or mechanisms which subvert an effector response [87] via long-term persistent antigen presentation. Immunologically ‘hot’ tumours characterised by prominent lymphocytic infiltration are associated with improved survival in rectal cancer treated by surgery alone [88]. This lymphocytic infiltration affects patient outcomes independently of TNM classification [70], and may override conventional prognostic factors [89]. The majority of reports suggest cytotoxic CD8+ T-cells are associated with a good prognosis. T-lymphocytes (CD3+) with cytotoxic (CD8+) and memory (CD45RO+) phenotypes within a rectal tumour and at the invasive edge predict not only the risk of recurrence and survival, but also response to CRT [22]. Others suggest a positive correlation between the presence of CD45RO+ (memory T cells) and OS [90].

Preclinical evidence for the timing of immune response, production of neo-antigens, and the creation of memory cells?

The adaptive immune response to viral infections in humans is rapid. CD8 T-cell numbers show a 10^4 – 10^5 -fold expansion in the acute phase [91]. Antigen-specific CD8 T-cells can divide approximately every 6–8 h [92] and expand into short-lived effector cells and memory

Fig. 5. Kinetics of the immune response. A Kinetics of T cell response after vaccination, based on Kim⁶⁶. B. Kinetics in animal models after irradiation, based on Frey⁶¹ and Filatenkov²⁶. C. Kinetics of the immune response in rectal cancer after SCRT, based on Nagtegaal et al.⁴⁸.

precursor cells - peaking between days 7–14, depending on the vaccine, infection or antigen, and then contract. Only a small percentage of effector T-cells survive as long-lived memory cells see Fig. 5A, but studies suggest this process may require at least 12 days [93,94].

Preclinical data in mice colon cancer models confirm immune responses (e.g. CD8⁺ T cells infiltrate) to antigenic signals elicited by radiotherapy are also rapid - reaching a maximal response within 8–10 days after irradiation and waning quickly back to baseline levels [95–98] (Table 2, Fig. 5B).

In the third model (Table 2), a major CD8⁺ T cell infiltrate was observed by day 14 with depletion of MDSCs [97]. Durable remissions from radiation were associated with these reductions in the infiltration of MDSCs, TAMs and Tregs, and an increase in CD8⁺ T cells [98]. Mice in remission resisted re-challenge with tumour cells, and their T cells transferred this antitumor immunity to adoptive hosts.

In addition, the human melanoma cell line MelJuSo when irradiated using 4 Gy fractions showed upregulated MHC-1 expression over a 4-day time-frame and thereby enhanced antigen processing [29]. Similarly, radiation with 8 Gy in prostate cancer cells significantly increases surface molecules involved in immune recognition via expression of

HLA-ABC, CEA, MUC-1, and ICAM-1 after 96 h [99]. These recovering cells show enhanced sensitivity to cytotoxic T-lymphocyte (CTL)-mediated killing via increased calreticulin expression.

Clinical evidence for the timing of immune response

Clinical trials of immune checkpoint inhibitors high-light the potential mechanisms of immune escape. The kinetics of immune reinvigoration after immunotherapy show the changes evolve rapidly. In melanoma the reinvigoration of exhausted-phenotype CD8 T-cells is detected in blood, peaking as early as day 7 after a single dose of anti-PD-1 treatment [100], but declining thereafter. These findings imply an already-existing T-cell response in some patients, which is revitalized. Additionally, the *post hoc* analysis of the PACIFIC trial suggests starting durvalumab within 14 days after CRT (rather than ≥ 14 days) is associated with more benefit to OS and PFS [101]. Perhaps in rectal cancer there is a rapid but short-lived reinvigoration after 5 × 5 Gy, which also peaks at 7 days, but declines thereafter.

Hence, these preclinical observations of the timing of the immune response above could account for the OS benefit in the SRCT [1] and

Table 2
Preclinical data showing the rapid onset and fall-off in immune responses to fractionated radiotherapy in colon cancer models.

Study	Mice Model	Dose/fractionation of RT	Time of Peak immune effects	Time to disappearance of immune effects			
Dovedi et al 2014 [67]	CT26 colon adenocarcinoma and 4434 cells isolated from BRAF ^{V600E} p16 ^{-/-} mice	5 × 2 Gy	upregulation of PD-L1 on tumour cells and increased infiltration of T cells (CD8 + > CD4 +) Expression peaks 72 h after the last fraction of RT	declines over the next 7 days but not to baseline levels			
Frey et al 2017 [68]	CT26 colon adenocarcinoma	2 × 5 Gy, delivered at days 8 and 12 after tumor cell injection	increase in CD8 + T cells but only at day 8	Back to control level at day 9			
Filatinkov et al 2015 [69]	CT26 colon adenocarcinoma and MC38 colon tumors	1 × 30 Gy	Rise in infiltrating CD8 + T cells starting around day 6 and reciprocal decrease in tumor-infiltrating CD4 + Tregs and MDSCs.	Sustained responses in some mice			
Retis et al 2006 [70]	MC38 adenocarcinoma cells MeJuSo cells	8, 10 Gy, 20 Gy	Maximum day 14 Enhanced T cell recognition in vitro and boosted the efficacy of adoptive CTL immunotherapy in vivo	MHC class I expression is increased for over 11 days after 8 or 10 Gy radiation. Higher RT doses enhances MHC-I expression days – weeks ? depends on cell proliferation. Slowly dividing cells ? longer half-life of MHC class I molecules explaining why the rapidly dividing MeJuSo cells have a shorter radiation-induced increase in MHC class I expression than MC-38 cells. No timing provided			
Wang X et al., 2018	CT 26 colon tumours	5 × 5 Gy	Promoted CD4 + and CD8 + T lymphocyte infiltration (P < 0.05) in tumor, but no effect on the number of the MDSC cells and DC cells.				
TRIAL	No of patients	RT delivery	Upper border RT field	Post-op adjuvant chemotherapy	Post-post radiotherapy	Differences in early mortality	Duration of SCPRT/ interval
Stockholm I Cedermarck 1985 Stockholm 1987	351 SCPRT 343 surgery alone	2 field	L2	No	No	Yes 7% after SCRT vs 2% after surgery alone p=0.01 (Anon 1987)- mainly over 75 years No 2% vs 1% p=0.2	No data (Cedermarck 1985, Anon 1987) Relative reduction in local recurrence similar Dukes A,B and C
Stockholm II (Martling 2001)	272 SCPRT 285 surgery alone	4 beams (Anon 1996)	Mid -L4 Upper border L5 (Anon 1996)	No	No		SCPRT 5–7 days in Vast majority Mon-Friday (Anon 1996) No data (Martling 2001) OS the same (worse after RT) Local recurrence, distant metastases and death due to rectal cancer S all significantly better in SCPRT. But OS only in curatively resected No improvement in DFS or OS
Swedish Rectal Cancer trial (Glimelius 1982, SRCT 1993,SRCT 1996, SRCT 1997)	553 SCPRT 557 surgery alone	3 or 4 field planning	upper border of L5 (but 316 patients included from Stockholm II using mid L4	No	No		Protocol recommended SCPRT Monday to Friday-but no data (Anon 1996)
Dutch TME trial (Kapiteijn 1999, Kapiteijn 2001)	873 SCPRT 875 surgery alone (RT for CRM +)	Multiple fields	Sacral promontory (in 15% S1/S2 junction)	No	Yes	Not in original report	Median interval 4 days (Kapiteijn 1999) no data (Kapiteijn 2001) Van den Broek Dutch patients only 9 days median OTT Interval to surgery 4 days No improvement in DFS or OS
CR07 (Sebag-Montefiore 2009, Quirke 2009)	1350 674 SCPRT 676 selective postop CRT	CT planning multiple fields	Sacral promontory	Yes 235/585 (40%) in the SCPRT group vs 274/609 (45%) in the selective CRT group	Yes only 53/77 (69%) with involved CRM received CRT	No	No improvement in DFS or OS

* in the group of patients irradiated with two beams, in-hospital mortality was considerably higher (15 percent [7/48 patients]) than in those irradiated as stipulated in the protocol with three or four beams (3 percent [13/507], P < 0.001).

not the Dutch TME trial [2]. Early surgery in the majority of patients may have obliterated or at least reduced the immune reaction. Surgery at this time-point removes the dendritic cells in un-activated or partially activated lymph nodes (i.e. before memory cells can proliferate sufficiently and disseminate) (Fig. 4B). Alternatively delaying surgery > 10 days as in the SCRT may have improved outcomes by allowing a host-favourable immune reaction to be re-established in about 10% of patients and dendritic cells can be activated and memory cells formed [102].

There are limitations to this review. This hypothesis is speculative and has not been directly tested. However, the only two randomised phase III trials, which show a survival advantage, are the only two studies to show down-staging. In both studies patients were resected a few days later than in all the other trials of 5×5 Gy.

A limitation is that although 2 trials, 316 patients enrolled in Stockholm II are also included in the SRCT and double counted. Also although derived from randomised trials, these post-hoc analyses use retrospectively observational data not based upon individual patient data, and therefore susceptible to bias and confounding.

SCPRT induces tumour T-down-staging and higher pCR rates if surgery is performed after 4–8 weeks [78]. Retrospective series indicate longer intervals are needed to achieve R0 surgery with an optimal cut-off of 20 days [103].

So we acknowledge an optimal timing to allow recovery from the effects of SCPRT to minimise surgical morbidity and at the same time maximise response. Is there also an optimal timing from the perspective of the immune system?

Further applications/exploiting the immune mechanisms

Preclinical data show RT sensitizes refractory tumours to immune checkpoint inhibitors by recruiting and re-invigorating anti-tumour T cells. In the clinic, these changes from RT alone are usually insufficient for major 'abscopal' responses. This rapid time-frame of immune modulation might be exploited if followed by immunotherapy, vaccines or oxaliplatin chemotherapy [104]. High-mobility group box-1 (HMGB1) protein, a measure of immunogenic cell death appears up-regulated after oxaliplatin neoadjuvant chemotherapy and chemoradiation in patients, who subsequently remained free from distant recurrence [105].

High pCR rates (18–35%) have been reported after SCPRT and consolidation chemotherapy [106,107]. The Dutch M1 trial showed a high rate of pCR from SCPRT and consolidation chemotherapy in metastatic disease [106]. A meta-analysis/pooled analysis from 5 retrospective studies in mCRC showed patients, who received pelvic radiotherapy in addition to chemotherapy had significantly better 5-year overall survival (OS) (RR 1.47; 95% CI 1.14–1.89, $p = 0.003$ [107].

The Dutch M1 trial provided the rationale for the Polish 2 [108] and the RAPIDO trial (NCT01558921). In the Polish 2 trial the reported median interval between SCPRT and the start of consolidation chemotherapy in the novel arm was 9 days (IQR 8–11) [109] and there is no benefit in DFS or OS.

The RAPIDO protocol recommended starting chemotherapy later i.e. 11–18 days after the last radiation fraction. Preliminary results of oncological outcomes in RAPIDO [109] show the primary endpoint of disease-related treatment failure was superior in the experimental arm (SCPRT followed by 6 cycles of CAPEOX and then TME) – 23.7% versus 30.4% in the standard arm (HR 0.76 [0.60–0.96]; $p = 0.02$). The probability of distant metastasis was also lower in the experimental arm – 19.8% vs 26.6% (HR 0.69 [0.53–0.89]; $p = 0.004$).

Hence the OTT from start of SCPRT to initiation of chemotherapy in these trials was 14–23 days, which our hypothesis suggests is an appropriate timing to start chemotherapy after SCPRT. Perhaps this different timing explains the success of RAPIDO compared to the Polish 2 trial.

Most clinicians consider delaying some weeks to increase response

and minimise surgical morbidity after SCPRT. Prolonged intervals increase the pCR rate and do not compromise oncological outcomes compared to immediate surgery. However, preclinical data suggest slightly longer intervals after SCPRT may be required for the immune system to be fully activated and incite immune memory cells, which can proliferate and disseminate loco-regionally and to distant organs outside the radiation field to prevent local and distant recurrence. We may in future need to consider an optimum timing from the immune perspective. An individual patient meta-analysis could provide data from appropriate SCPRT trials examining outcomes for each individual day.

The available immune data derives from SCPRT trials with a short interval. These trials show no survival benefit, and suggest that immunological changes from SCPRT alone are either mostly insufficient to produce clinically meaningful benefit, or require longer to mature. Alternatively we may be looking in the wrong place, and the expected immunological activity is evolving in the adjacent draining lymph nodes via dendritic cells and not in the primary tumour.

Our hypothesis proposes that a few days delay might make a crucial difference between achieving an impact on OS in addition to local control. Preclinical and clinical data suggest the most appropriate OTT may be between 11 and 16 days. This interval is exploited by consolidation chemotherapy, which appears to enhance pCR rates after SCPRT and is likely optimum timing for initiation of immune checkpoint inhibition.

Contributors

All authors participated in the development of the manuscript. RGJ, MRH and IDN participated in manuscript conception and provision of data and references. RGJ designed and conducted the literature search and collected data. All authors drafted the manuscript and made critical revisions. All authors approved the final manuscript.

Declaration of Competing Interest

Rob Glynne-Jones has no competing interests to declare,
Marcia Hall has no competing interests to declare,
Iris Nagtegaal has no competing interests to declare

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