Histopathology

Histopathology 2025, **87**, 44–57. DOI: 10.1111/his.15423

The tumour–stroma ratio as predictive aid towards a biopsy-based treatment strategy in rectal carcinoma

Meaghan Polack,[1] Gabi W van Pelt,[1] Davita H van den Heuvel,[1] Elma Meershoek Klein-Kranenbarg,[2] Annet G H Roodvoets,[2] Hein Putter,[3] Augustinus S L P Crobach,[4] Iris D Nagtegaal,[5] Koen C M J Peeters,[1] Rob A E M Tollenaar,[1] J Han J M van Krieken[5] & Wilma E Mesker[1]

[1]*Department of Surgery,* [2]*Clinical Research Center, Department of Surgery,* [3]*Department of Biomedical Data Sciences,* [4]*Department of Pathology, Leiden University Medical Center, Leiden and* [5]*Department of Pathology, Radboudumc, Nijmegen, the Netherlands*

Date of submission 24 October 2024
Accepted for publication 25 January 2025

Polack M, van Pelt G W, van den Heuvel D H, Klein-Kranenbarg E M, Roodvoets A G H, Putter H, Crobach A S L P, Nagtegaal I D, Peeters K C M J, Tollenaar R A E M, van Krieken J H J M & Mesker W E (2025) *Histopathology* **87**, 44–57. https://doi.org/10.1111/his.15423

The tumour–stroma ratio as predictive aid towards a biopsy-based treatment strategy in rectal carcinoma

Aims: Tumour–stroma ratio (TSR) scores of biopsy material in rectal carcinoma (RC) could aid a biomarker-based, upfront and personalised treatment strategy selection for RC patients. In a large retrospective, multicentre cohort, we aimed to validate the predictive value of biopsy-scored TSR on neoadjuvant therapy response, and secondarily, disease-free and overall survival (DFS, OS).

Methods and results: Scanned haematoxylin and eosin-stained RC biopsy slides were collected from Leiden University Medical Center ($N = 116$) and from the clinical PROCTOR-SCRIPT ($N = 142$) and RAPIDO ($N = 271$) trials. TSR was scored per protocol and categorised as stroma-low (≤ 50%) or stroma-high (> 50%). Major response was defined as tumour regression grade (TRG) 1 + 2 by Mandard, including pathological complete response. Ultimately, a large and varied cohort with 373 RC patients was established. Locally advanced RC was more often stroma-high ($P < 0.001$). We subsequently observed significantly lower major response rates in the stroma-high RC after a neoadjuvant treatment approach (hazard ratio = 0.63, 95% confidence interval = 0.41–0.99; $P = 0.044$). Despite correction for well-known risk factors in Cox hazard regression analysis, such as (y)pTNM substages or residual tumour status, the TSR had no singular significant influence on DFS nor OS in multivariate analysis ($P = 0.438$; $P = 0.934$, respectively).

Conclusions: Biopsy-scored TSR can predict neoadjuvant therapy efficacy, as RC patients with stroma-high biopsies show less major response. However, patient survival is multifactorial, although response is an important predictor, influenced by

Address for correspondence: W E Mesker, Department of Surgery, Leiden University Medical Center, Albinusdreef 2, ZA Leiden 2333, the Netherlands. e-mail: w.e.mesker@lumc.nl

Abbreviations: AT, adjuvant therapy; cCR, clinical complete response; CRT, chemoradiation (25x1.8-2 Gray and capecitabine monotherapy); DFS, disease-free survival; EMVI, extra mural vascular invasion; H&E, haematoxylin and eosin; LARC, locally advanced rectal carcinoma; LUMC, Leiden University Medical Centre; MRF, mesorectal fascia; NAT, neoadjuvant treatment, including the CRT and RAPIDO regimens; OS, overall survival; pCR, pathological complete response; PROCTOR, Preoperative Radiotherapy and/Or adjuvant Chemotherapy combined with TME surgery in Operable Rectal cancer (5-FU + Leucovorin); RAPIDO, Rectal cancer and Preoperative Induction therapy followed by Dedicated Operation (5×5 Gray followed by 6 cycles capecitabine and oxaliplatin); RC, rectal carcinoma; SCRIPT, Simply Capecitabine in Rectal cancer after Irradiation Plus TME (capecitabine monotherapy); SCRT, short course radiotherapy (5×5 Gray); SD, standard deviation; SE, standard error; TME, total mesorectal excision; TNM, tumour-node metastasis; TRG, tumour regression grade; TSR, tumour-stroma ratio; UNITED, Uniform Noting for International application of the Tumour-stroma ratio as Diagnostic tool.

TSR. Scoring TSR on RC biopsy material is a reliable histological parameter, implementation of which in treatment guidelines could improve upfront selection for a watch-and-wait strategy.

Keywords: neoadjuvant treatment, prediction, rectal cancer, tumour microenvironment, tumour regression grade, tumour response, tumour–stroma ratio

Introduction

Optimalisation of therapeutic strategies in rectal carcinoma (RC) has been subject to many clinical trials over the years.[1] Management of RC has evolved, shifting the paradigm from initially aiming for enhanced locoregional control to whole-organ preservation, rapidly improving patient-related outcomes such as disease-free and overall survival (DFS and OS, respectively).[1,2] Currently, the cornerstone of international treatment guidelines encompasses risk stratification, based on disease extent as defined by the tumour–node–metastasis (TNM) classification[3] and clinical imaging factors such as mesorectal fascia (MRF) involvement and/or extramural vascular invasion (EMVI).[4,5] Implementation of total mesorectal excision (TME) surgery[6] and pre-operative treatment regimens[7–11] have led to optimal local control.

Beginning with short-course radiotherapy (SCRT) for improvement of locoregional control and survival,[7] regimens that now include pre-operative radiotherapy and chemotherapy, i.e. neoadjuvant therapy,[10–12] have given rise to the watch-and-wait strategy,[13] delaying and even potentially sparing patients burdensome surgery. However, response rates are prone to variation.[14] Moreover, heterogeneity is observed in reached clinical and pathological complete response (cCR and pCR, respectively).[10,15–17] With increasing RC incidence[4,18] and high rates of treatment complications,[4,19] it is thus pivotal to improve upfront treatment selection. Current pathological risk factor parameters, however, focus mainly upon the tumour epithelial compartment, i.e. neoplastic cells alone.[4]

Convincing evidence is emerging that elements of the tumour microenvironment, especially the tumour stroma, are a detrimental influence on tumour behaviour, promoting tumour invasion and metastasis.[20,21] The tumour–stroma ratio (TSR) is a robust and cost-effective histopathological parameter based on intratumoural stromal percentages.[22] The TSR has been validated as an independent biomarker in multiple tumour types: indeed, stroma-high (> 50% stroma) gastrointestinal tract carcinomas not only have worse OS and DFS,[23–25] as observed in colon carcinoma in the recently published prospective international UNITED study,[26,27] but also predict worse response to (neo)adjuvant therapy.[28–31] Although often collectively termed, RC is a different entity from colon carcinoma.[4] Literature on TSR in RC specifically is scarce and consists mainly of relatively older, single-centred RC series with limited patients and/or treatment types.[25,32–34]

To address this knowledge gap, we integrated two prominent clinical trials, i.e. PROCTOR-SCRIPT[9] and RAPIDO,[10] with our local cohort. This collaboration enabled us to create an extensive overview of the TSR in varied RC patient populations. A more biomarker-based approach on biopsies is crucial to improve future selection of responders, and the TSR could aid in this prediction, as previously shown.[32,33] As a primary endpoint, we assessed the correlation between TSR and neoadjuvant therapy response. This study analysed the predictive potential of biopsy-scored TSR on DFS and OS as secondary endpoints. We hypothesised that the more aggressive and resistant stroma-high tumours would reach a response less frequently, and would have worse DFS and OS compared to their stroma-low counterparts, potentially influencing patient selection for a watch-and-wait strategy in the future.

Methods

PATIENT COHORTS

Our local cohort [Leiden University Medical Centre (LUMC); $N = 116$] was combined with available material from two well-established, independent clinical validation cohorts, i.e. subgroups of the PROCTOR-SCRIPT[9] ($N = 142$) and RAPIDO[10] ($N = 271$) trials. Hence, a large series comprising various TNM stages and treatment regimens was analysed. All cohorts included patients aged ≥ 18 years with given informed consent. Additional inclusion and exclusion criteria for the clinical trials are mentioned in previous reports.[9,10] Summarising, the PROCTOR-SCRIPT was a combined study

assessing the role of adjuvant chemotherapy compared to observation in RC patients treated with neoadjuvant therapy consisting of (chemo)radiotherapy and TME, whereas the RAPIDO analysed different neoadjuvant regimes in locally advanced RC (LARC). The LUMC cohort consisted of consecutive patients with a variety of (neo)adjuvant therapy types, available material of which was collected from patients operated after 2000 with stages I–III RC and no previous malignancy < 10 years prior to current RC.

Of note, PROCTOR-SCRIPT patients were included postoperatively, hence clinical TNM-stage or clinical risk factors were not registered in the study database, although one inclusion criterion was pathological TNM stages II/III. Stages II/III were given in case no imaging is performed and locoregional extent of disease was uncertain, e.g. in earliest included patients. Moreover, as different versions of the TNM classification were used for optimal grouping and comparison, all variables are converted to TNM version 5 (1997). Age was registered at randomisation (PROCTOR-SCRIPT and RAPIDO) or diagnosis (LUMC). Supporting information, Table S1 gives a detailed overview of treatment types and regimens per cohort.

MATERIALS AND TUMOUR–STROMA ANALYSIS

Scanned haematoxylin and eosin (H&E)-stained slides of diagnostic biopsies were collected at LUMC (PROCTOR-SCRIPT) or requested from Radboud University Medical Centre (Radboud UMC; RAPIDO). At LUMC, slides were scanned with the Panoramic-250 scanner; Radboud UMC used the Panoramic-1000 (3DHistech, Budapest, Hungary; 20× magnification). Analysis was performed with 3DHistech CaseViewer software (version 2.7). Two independent observers (M.P.-G.v.P.: LUMC, PROCTOR-SCRIPT; M.P.-D.H.: RAPIDO) scored the TSR on biopsies according to van Pelt *et al.*,[35] blinded for clinical data. Subsequently, categorisation in stroma-low (≤ 50% stroma) and stroma-high (> 50% stroma) followed (Figure 1, created in BioRender.com). Neoadjuvant therapy response was assessed on resection material through the tumour regression grade (TRG) in five categories as defined by Mandard[36] by local pathologists (LUMC, PROCTOR-SCRIPT) or in three groups (no–partial–complete response; RAPIDO). To ascertain the predictive correlation to TSR, TRG was dichotomised in clinically relevant and previously maintained groups of TRG1 + 2 (including pCR and major responders) and TRG3-5 (non-major responders).[37]

STATISTICAL ANALYSIS

Interobserver agreement using Cohen's kappa was calculated between TSR biopsy scores. Assessment of prediction of TSR to neoadjuvant therapy response was performed subsequently per TSR category and per therapy type. DFS was defined as the period between date of surgery and any first event, i.e. recurrence (locoregional recurrence or distant metastasis), death (any cause) or censoring. OS pertained to the period between date of surgery until death (any cause) or censoring. Censoring occurred when patients were disease-free and/or alive at last registration or after 10 years of follow-up.

Chi-squared tests for nominal, Goodman Kruskal gamma statistics for ordinal and Student's independent *t*-tests for continuous variables were performed. Median follow-up time was calculated with the reversed Kaplan–Meier method; survival analyses were performed with Kaplan–Meier analyses and associated log-rank tests. Cox regression analysis for hazard ratios (HR) with 95% confidence intervals (CI) was calculated in univariate analysis for major response (event defined as pCR/TRG1 + 2) for the period between surgery and diagnosis (LUMC), first radiotherapy dose (PROCTOR-SCRIPT) or randomisation (RAPIDO). The variables of significant influence ($P < 0.05$) in univariate analysis were included in the multivariate analysis.

Continuous variables were expressed in means with standard deviations (SD), whereas nominal and ordinal variables were stated as number of frequencies and corresponding percentages. Two-tailed $P < 0.05$ were considered statistically significant. Statistical analysis was performed using IBM SPSS statistics (version 29.0).

Results

PATIENT COHORTS

Establishing the final patient population, exclusions followed from the three cohorts, e.g. per(i)operative pathological stage IV ($N = 18$) or absence of pathological data ($N = 68$). In total, 373 RC patients were ultimately included in this study (Figure 2). Baseline characteristics of the combined cohort and the separate groups are presented in Table 1. There are differences between cohorts, inherently correlating to the used studies, including treatment type ($P < 0.001$), clinical risk factors ($P < 0.001$) and clinical TNM stage ($P < 0.001$). Most importantly, as could be expected due to the more aggressive nature of the tumours involved, the TSR is also already higher in the LARC patients of the RAPIDO,

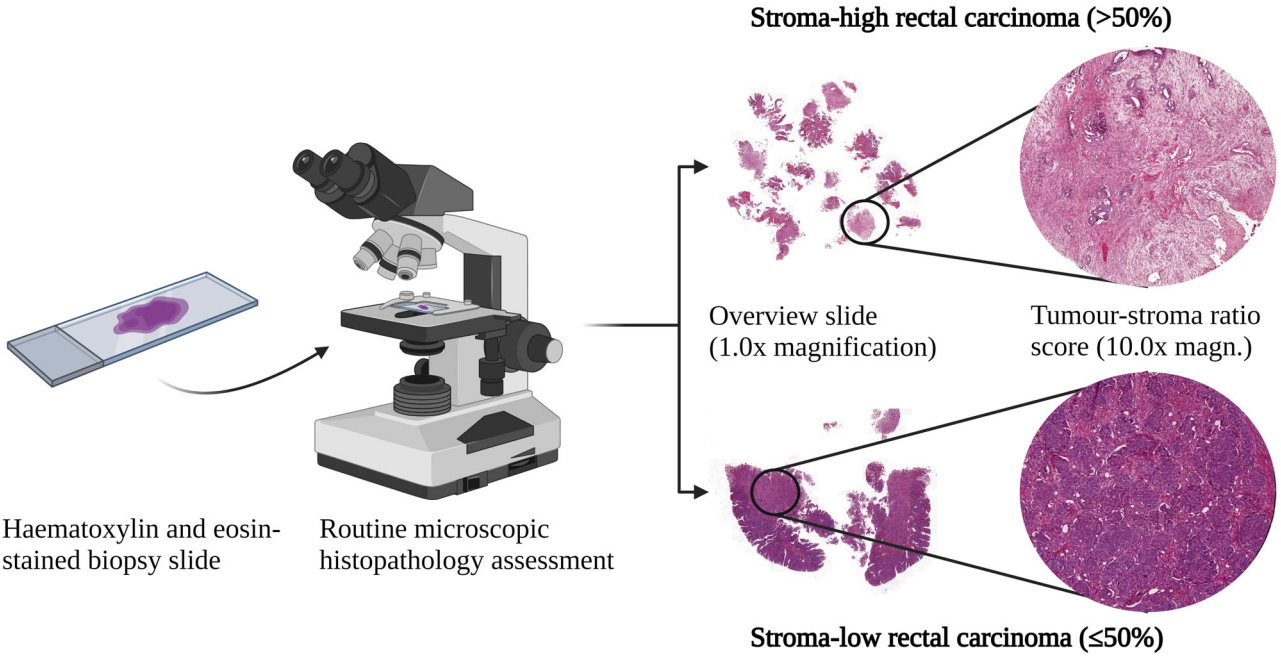

Figure 1. The process of scoring the tumour–stroma ratio (TSR) on haematoxylin and eosin-stained biopsy material using light microscopy. First, using 1.0–2.5× magnification for a general overview of the complete slide, the area with the highest amount of tumour stroma is selected. Subsequently, the TSR is scored on a 10.0× magnifying objective, as per protocol by van Pelt *et al*. Finally, the biopsy is categorised as stroma-high (> 50% intratumoural stroma; example shown above) or stroma-low (≤ 50% intratumoural stroma; example shown below). Created in BioRender.com.

where 57% of patients were stroma-high, compared to approximately one-third in other cohorts and the literature ($P < 0.001$). Hereby, the full spectrum of presentations of RC is covered in our study.

OVERVIEW OF TSR ANALYSES

The TSR scores had high Cohen's interobserver agreement kappas of 0.84 (M.P.-D.H.; RAPIDO biopsies) and 0.77 (M.P.-G.P.; LUMC and PROCTOR-SCRIPT biopsies). Supporting information, Table S2 presents an overview of stroma-low compared to stroma-high clinical variables in the total patient population and per cohort separately. Overall, although more clinical risk factors were found in stroma-high patients as expected ($P = 0.014$), stroma-low patients had more often not undergone neoadjuvant therapy ($P < 0.001$) and were operated on in earlier years (2009 versus 2012 in stroma-high patients; $P < 0.001$).

TSR PREDICTOR OF MAJOR RESPONSE TO NEOADJUVANT THERAPY

Pathological outcomes per TSR category of the total patient population and per therapy type separately are summarised in Supporting information, Table S3. The CRT and RAPIDO regimens are combined in a large neoadjuvant treatment (NAT) group ($N = 182$). Of note, SCRT was not intended to be used to reach pCR, and any significant downsizing can be seen only after more than 7 weeks following neoadjuvant therapy.[7,38] Hence, it almost mimics the situation in treatment-naïve patients: In stroma-high patients, a higher ypT-stages 2–4 ($P = 0.043$) and less response was seen more often ($P = 0.044$). Subsequently, analysing major response rates per treatment type in depth, the biopsy-scored TSR emerged as a valuable predictor, as stroma-high patients reached significantly less major response to NAT than stroma-low patients (HR = 0.63, 95% CI = 0.41–0.99; $P = 0.044$) (Table 2).

SURVIVAL ANALYSES

To assess the predictive value of the TSR on DFS and OS, Kaplan–Meier analysis with log-rank tests were first performed for the complete cohort and separate therapy groups (Supporting information, Table S4 and Figures S1 and S2). No significant influence of the TSR was observed here. Subsequently, we used

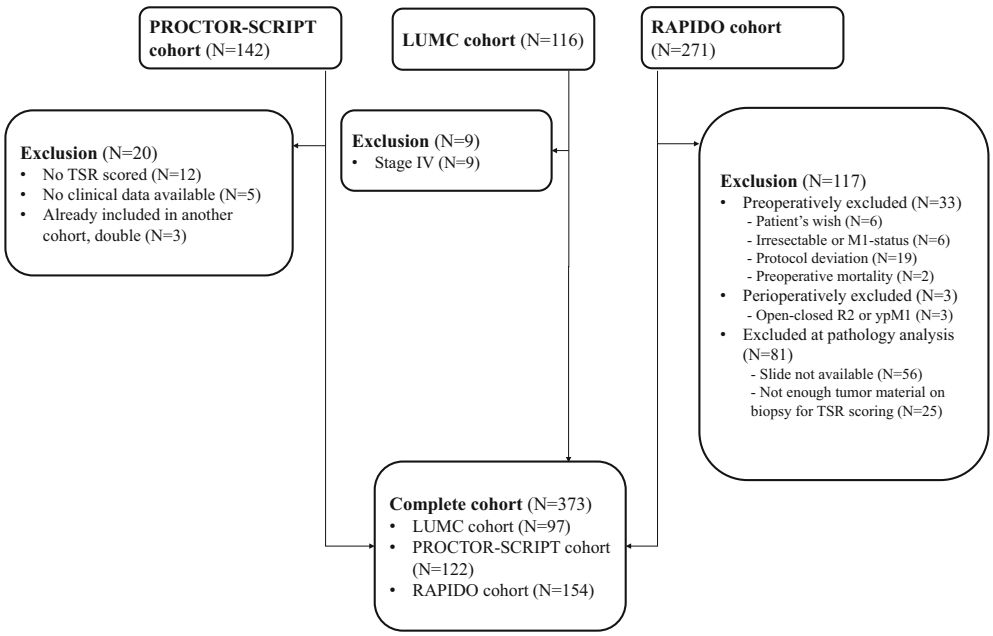

Figure 2. Flowchart showing the patient population with initial inclusion rates per separate cohort [PROCTOR-SCRIPT, Leiden University Medical Center (LUMC) or RAPIDO] and exclusion numbers and reasons, leading to the ultimately included final patient cohort (N = 373). TSR, tumour–stroma ratio.

Table 1. Baseline characteristics of the eligible patients in the complete cohort and separate cohorts

Baseline characteristics	Complete cohort (N = 373)	LUMC (N = 97)	PROCTOR-SCRIPT (N = 122)	RAPIDO (N = 154)	P-value
Participating centres					
Total no. participating centres	28	1	22	13	NA
Sex					
Female	117 (31)	26 (27)	41 (34)	50 (33)	0.520‡
Male	256 (69)	71 (73)	81 (66)	104 (68)	
Age (years)					
Median age	62 (55–69)	65 (57–73)	60 (55–68)	64 (54–69)	**0.017**†
Aged > 70	75 (20)	29 (30)	19 (16)	27 (18)	**0.018**‡
Treatment type					
Neoadjuvant treatment and surgery	289 (78)	74 (76)	64 (52)	152 (99)	< **0.001**‡
Neoadjuvant treatment, surgery and adjuvant treatment	64 (17)	3 (3)	58 (48)	2 (1)	
Surgery and adjuvant treatment	4 (1)	4 (4)	0 (0)	0 (0)	
Surgery alone	16 (4)	16 (17)	0 (0)	0 (0)	

Continued

Table 1. (*Continued*)

Baseline characteristics	Complete cohort (*N* = 373)	LUMC (*N* = 97)	PROCTOR-SCRIPT (*N* = 122)	RAPIDO (*N* = 154)	*P*-value
Clinical TNM stage					
II/III	4 (1)	4 (4)	NA	0 (0)	< **0.001**‡
II	42 (11)	28 (29)	NA	14 (9)	
III	202 (54)	62 (64)	NA	140 (91)	
Unknown	125 (34)	3 (3)	122 (100)	0 (0)	
Clinical locally advanced rectal carcinoma (LARC)					
No, no clinical LARC	23 (6)	23 (24)	NA	0 (0)	< **0.001**‡
Yes, clinical LARC	221 (59)	67 (69)	NA	154 (0)	
Unknown	129 (35)	7 (7)	122 (100)	0 (0)	
Tumour location					
Low rectum (< 5 cm anal verge)	133 (36)	42 (43)	40 (34)	51 (33)	0.463‡
Mid-rectum (5–10 cm anal verge)	108 (29)	27 (28)	34 (28)	46 (31)	
High rectum (> 10 cm anal verge)	126 (34)	27 (28)	44 (36)	55 (36)	
Unknown	6 (2)	1 (1)	4 (3)	2 (1)	
Clinical risk factors					
No, no additional risk factors	24 (10)	24 (26)	NA	0 (0)	
Yes, 1 clinical risk factor present	48 (20	32 (35)	NA	16 (10)	
Yes, 2 clinical risk factors present	85 (35)	19 (21)	NA	66 (43)	
Yes, 3 or more clinical risk factors present	88 (36)	16 (18)	NA	72 (47)	
Not (enough) determined, unknown	128 (34)	6 (6)	122 (100)	0 (0)	
Clinical risk factors					
Extramural venous invasion	19 (5)	1 (1)	NA	18 (12)	< **0.001**‡
Mesorectal fascia involvement	95 (26)	16 (17)	NA	79 (51)	
Clinical lateral lymph nodes	42 (11)	17 (18)	NA	25 (16)	
Clinical T4 stage	38 (10)	7 (7)	NA	31 (20)	
Clinical N + stage	202 (54)	62 (64)	NA	140 (91)	
Clinical N2 stage	122 (33)	22 (23)	NA	100 (65)	
Tumour–stroma ratio biopsy					
Stroma-low (≤ 50%)	215 (58)	69 (71)	80 (66)	66 (43)	< **0.001**‡
Stroma-high (> 50%)	158 (42)	28 (29)	42 (34)	88 (57)	

All variables are given as absolute numbers with associated percentages or medians with interquartile ranges. Sum of percentages can be less or more than 100 due to rounding.
NA, not applicable; TNM, tumour–node–metastasis; LARC, locally advanced rectal carcinoma.
†Calculated with χ^2 test.
‡Calculated with one-way analysis of variance analysis.

Table 2. Univariate Cox regression analysis on the hazard of major response of TSR in total cohort and per treatment type

Therapy type	Variable	Number of major responders (%)	Major response—univariate analysis[†]		
			Hazard ratio	95% confidence interval	*P*-value
Complete cohort (*N* = 337)[‡]	Stroma-low (*N* = 188)	39 (21)	1		0.071
	Stroma-high (*N* = 149)	41 (28)	0.666	0.428–1.035	
NAT (CRT + RAPIDO; *N* = 182)	Stroma-low (*N* = 81)	38 (47)	1		**0.044**
	Stroma-high (*N* = 101)	40 (40)	0.632	0.405–0.989	
CRT (*N* = 107)	Stroma-low (*N* = 56)	26 (46)	1		0.308
	Stroma-high (*N* = 51)	17 (33)	0.726	0.393–1.343	
RAPIDO (*N* = 75)	Stroma-low (*N* = 25)	12 (48)	1		0.839
	Stroma-high (*N* = 50)	23 (42)	0.930	0.461–1.877	

CRT, chemoradiation (25 × 1.8–2 Gray and capecitabine monotherapy); NAT, neoadjuvant treatment; RAPIDO (5 × 5 Gray followed by six cycles capecitabine and oxaliplatin).

[†]The period between surgery (for pathology) and diagnosis (LUMC), first radiotherapy dose (PROCTOR-SCRIPT) or randomisation (RAPIDO). The event is defined as major response (TRG1 + 2/pathological complete response).

[‡]Complete cohort here pertains to those patients who had undergone neoadjuvant therapy and with a known and determined response.

Cox hazard regression in the complete cohort. In univariate analysis, higher (y)pT and/or (y)pN stages, as well as residual tumour status or not reaching a major response, were of such significant influence it potentially introduced bias, and the TSR reached no significance (DFS, $P = 0.800$; OS, $P = 0.856$) (Table 3). Aiming to analyse the effect of the TSR relative to the other, well-known risk factors we added the TSR as a variable in the multivariate analysis. However, even correcting for these variables, the TSR did not add significant information (DFS, $P = 0.438$; OS, $P = 0.934$).

Discussion

The present study set out to determine the predictive effect of biopsy-scored TSR on response to neoadjuvant therapy. In a large and varied multicentre patient population, we first observed that LARC, characterised through imaging as a more aggressive and invasive tumour, was significantly more often categorised as stroma-high. We subsequently validated the TSR's predictive value on response. In RC patients undergoing a NAT approach, significantly fewer major response rates were achieved in those with a stroma-high biopsy. Identifying potential major therapy responders can ultimately aid upfront selection of patients for treatment strategies, e.g. watch-and-wait.

As a secondary endpoint, the prognostic effect on DFS and OS of the TSR in RC patients was assessed. Despite correction for the significant influence of well-known risk factors such as (y)pTNM substages and residual tumour status, the TSR did not significantly show a predictive influence on DFS or OS in our cohort. Response, however, remained an important prognosticator, to which the TSR is a major contributor. This is due most probably to the presence of large multifactorial causal relationships of various risk factors on survival, such as increasing sequential treatment options, underlining the need for a multidisciplinary and patient-tailored approach.[4] Moreover, underlying biological processes influencing response and tumour behaviour, such as mutational status, are determined only in a minority of included patients but are increasingly analysed in current practice.[39]

In the past decades, the traditional neoplastic cell-centred view has incrementally been expanded to include the surrounding tumour microenvironment.[40,41] The complex interaction between these entities has hence been subject to increasing research.[20,42] In 2007, our research group first explored the absolute ratio of the tumour epithelium compartment compared to the stromal compartment: the TSR.[23] Since then, the field of the TSR has exponentially gained interest worldwide.[22,24,25,28,30,43] Recently, the detrimental influence of tumour stromal abundance on

Table 3. Univariate and multivariate analysis of disease-free survival and overall survival with Cox regression analysis in the complete cohort

	Disease-free survival						Overall survival					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
Variable (unit)	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Sex												
Male	1		0.781	NA			1		0.611	NA		
Female	0.951	0.667–1.356					0.897	0.590–1.363				
Age at surgery—older category												
≤ 70 years of age	1		**0.022**	1		0.136	1		**0.001**	1		**< 0.001**
> 70 years of age	1.534	1.063–2.238		1.624	0.858–3.074		2.004	1.323–3.306		2.310	1.431–3.731	
Tumour location												
High rectum (> 10 cm anal verge)	1		0.170	NA			1		0.051	1		0.425
Mid-rectum (5–10 cm anal verge)	1.257	0.831–1.902	0.278				1.651	1.000–2.726	**0.050**	1.137	0.589–2.193	0.702
Low rectum (< 5 cm anal verge)	1.500	0.983–2.289	0.060				1.849	1.109–3.083	**0.018**	1.430	0.803–2.545	0.224
Clinical risk factors												
No, no additional risk factors	1		0.763	NA			1		0.385	NA		
Yes, 1 or more clinical risk factors present	1.119	0.540–2.319					1.451	0.627–3.359				
Neoadjuvant therapy received												
Yes, received	1		0.731	NA			1		0.444	NA		
No, not received	0.875	0.409–1.871					0.703	0.286–1.730				
Received therapy, grouped												
NAT	1		0.373	NA			1		0.520	NA		
SCRT	0.995	0.455–2.173	0.990				0.778	0.308–1.962	0.595			
No neoadjuvant therapy	1.269	0.901–1.786	0.173				1.194	0.799–1.782	0.387			
Adjuvant treatment												
Yes, adjuvant treatment received	1		0.384	NA			1		0.704	NA		
No, no adjuvant treatment received	0.830	0.546–1.263					0.904	0.538–1.521				

Continued

Table 3. (*Continued*)

	Disease-free survival						Overall survival					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
Variable (unit)	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Surgery type, grouped												
Low anterior resection (LAR)	1		0.511	NA			1		**0.012**	1		0.114
Abdominoperineal resection (APR)	1.219	0.870–1.709	0.250				1.753	1.181–2.602	**0.005**	1.641	0.966–2.789	0.067
Hartmann	1.157	0.503–2.660	0.731				2.090	0.892–4.896	0.090	1.924	0.651–5.690	0.237
Residual tumour (Wittekind 2009)												
R0 resection	1		< **0.001**	1		0.493	1		< **0.001**	1		< **0.001**
R+ resection	3.082	1.874–5.069		1.354	0.569–3.220		3.367	1.907–5.946		3.401	1.782–6.490	
Major response												
TRG1 + 2	1		< **0.001**	1		0.275	1		**0.017**	1		0.701
TRG3-5	2.449	1.466–4.092		3.407	0.377–30.83		1.968	1.129–3.432		1.183	0.501–2.793	
y(p)T-category												
y(p)T-stage 0 + 1	1		< **0.001**	1		0.973	1		**0.023**	1		0.398
y(p)T-stage 2–4	2.748	1.520–4.966		NA	NA		2.065	1.105–3.860		1.521	0.575–4.020	
Lymph nodes (LN)												
LN examined ≥ 12	1		0.716	NA			1		0.995	NA		
LN examined < 12	0.941	0.676–1.308					1.001	0.682–1.471				
y(p)N-category												
y(p)N-stage 0	1		< **0.001**	1		0.210	1		< **0.001**	1		**0.004**
y(p)N-stage +	2.694	1.900–3.819		1.678	0.748–3.764		2.152	1.452–3.187		2.117	1.274–3.517	
Differentiation grade tumour												
Low grade (well–moderate)	1		0.691	NA			1		0.152	NA		
High grade (poor–undifferentiated)	1.124	0.631–2.002					1.564	0.849–2.882				

Continued

Table 3. (*Continued*)

Variable (unit)	Disease-free survival						Overall survival					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Pathology risk factors												
No risk factors present	1		**0.005**	1		0.056	1		0.118	NA		
Yes, 1 or more risk factors present	2.301	1.290–4.107		1.889	0.983–3.623		1.746	0.868–3.513				
TSR biopsy												
Stroma-low	1		0.800	1		0.438	1		0.856	1		0.934
Stroma-high	1.044	0.750–1.453		0.798	0.451–1.412		1.036	0.705–1.523		0.982	0.637–1.512	

Disease-free survival and overall survival are censored at 10 years. Variables with a significant ($P \leq 0.050$) influence on outcome in univariate analysis are included in multivariate analysis.

NA, not applicable; NAT, neoadjuvant treatment; SCRT, short course radiotherapy; TNM, tumour–node–metastasis classification; TRG, tumour regression grade; TSR, tumour–stroma ratio; HR, hazard ratio; CI, confidence interval.

patient-related outcomes was proved in our UNITED study, where stroma-high colon carcinomas indeed led to worse DFS.[26,27] This parameter has therefore been proposed[27] as a novel factor in international guidelines; for instance, in the TNM classification.

Although the TSR had already previously proved to be of predictive value in biopsies and RC,[32,33,44] large, novel and multicentre studies were lacking.[34,45] As upfront selection of RC patients for personalised therapeutic strategies is gaining importance, with the shift towards a watch-and-wait strategy,[2,4,46] implementing biopsy-based biomarkers to aid this selection is pivotal. Potential implications of our results, proving that biopsy-scored stroma-high RC patients are less prone to reach major response in a NAT approach, could be far-reaching. Including the TSR in the panel with all other important clinicopathological variables currently used by multidisciplinary meetings could lead to an improved tailored approach where, in a shared-decision making setting, patients could even be advised to be spared the potentially less efficient although burdensome treatment, and instead be recommended for immediate surgery, especially in frail elderly people with comorbidities.

The main strength of this study—though also a limitation—was the choice of a large and varied patient population; the differences between the cohorts prohibited large pooling, as this could lead to skewed results. Of interest, it was observed, for example, that the RAPIDO trail, including only LARC patients, had significantly more stroma-high patients than the other cohorts with less advanced tumours. While this also emphasises the importance of the stromal compartment and influence of its abundance on tumour invasiveness, it also gave rise to potential selection bias. Therefore, we aimed to answer our research questions in specific, although smaller, subgroups. Another limitation pertains to the retrospective aspect of this study. Moreover, despite the use of large clinical trials, these are still somewhat dated. With the rise of various neoadjuvant approaches,[47] scoring more recent trials, or even aiming to include the TSR scored on biopsies as a parameter in prospective or randomised controlled trials in the future, would therefore be imperative.

The current European Society for Medical Oncology (ESMO) guidelines in management of RC state that there are several regression grade classifications, including the Mandard[36] and the Dworak[48] classifications, without indicating an optimal method for scoring tumour regression. Despite the fact that the

Mandard classification may be not the most widely used for determining tumour regression, and TRG has been criticised as potentially underestimating tumour shrinkage compared to, for example, a fragmentation pattern,[49] many studies, including ours, use the Mandard score,[32] which enhances potential comparison. In any case, a reliable score should include a 'complete–partial–no response' category.[4] Although the International Collaboration on Cancer Reporting (ICCR) colorectal pathology guidelines[50] mention another classification, the Ryan four-tier system,[51] ultimately all classifications for scoring result in similar endpoints.

Future studies including more parameters of the tumour microenvironment could enhance our understanding of the dynamic interplay between the various components of this entity. The immune cell component,[52–54] tumour budding[55] or biomarkers such as circulating tumour DNA (ctDNA) and other liquid biopsy methods, show promise in colon[56,57] as well as RC,[58] and could provide valuable insights. TSR is, in contrast to most biomarkers, uncomplicated and cost-effective,[35] taught by e-learning,[59] and hence standardised with high interobserver agreement.[60] Furthermore, in this study, we collected scanned H&E-stained biopsy slides. Artificial intelligence is exponentially being researched in pathology, currently successfully completing high-performance tasks such as classification.[61] Future studies could use this collection for developing algorithms, for example, supervised deep-learning models trained by pathologists' annotations to characterise tissue types,[62] resulting in automatised TSR quantification in biopsies, thus supporting the pathologists' workload. Currently, some studies show promise in various TSR scoring methods, mainly performed on primary tumours in the colon.[63–65] However, studies are still scarce for RC,[66] even fewer aiming to predict a response from TSR in biopsies.[67] Moreover, unsupervised learning could potentially discover novel histological patterns on unannotated slides, improving personalised predictions on therapy response and survival.[68]

We have hereby presented a large multicentre study validating the predictive significance of biopsy-scored TSR on neoadjuvant therapy response in RC. Using well-established clinical trials, we conclude that patients with stroma-high RC biopsies will reach a major response using a NAT approach. The TSR should thus be implemented in routine pathology diagnostics and the current clinicopathological panel of parameters, which multidisciplinary meetings consider for RC patients in personalised selection of treatment strategies.

Acknowledgements

This work was supported by the Bollenstreekfonds, Hillegom, the Netherlands (no grant number). This funder had no role in study design, data collection and analysis, nor in the decision to publish or in the preparation of the manuscript. The original PROCTOR-SCRIPT trial was supported by the Dutch Cancer Society (KWF 1999-03 and KWF 2003-16), the Dutch Colorectal Cancer Group, and the Swedish Cancer Society. Furthermore, Roche has provided an unrestricted educational grant. The original RAPIDO trial was funded by the Dutch Cancer Foundation, Swedish Cancer Society, Spanish Ministry of Economy and Competitiveness, and Spanish Clinical Research Network. We thank the PROCTOR-SCRIPT and RAPIDO steering committees for use of their data and support. We also thank pathologist Arantza Farina Sarasqueta for aiding in the initial scoring procedures. We thank Sonay Kuş Öztürk (Radboudumc) for scanning of the RAPIDO slides, and Ronald van Vlierberghe and Geeske Dekker-Ensink (both LUMC) for scanning of the PROCTOR-SCRIPT and LUMC slides, as well as laboratory support.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Clinical trial registration

PROCTOR-SCRIPT: Dutch Colorectal Cancer group, CKTO 2003-16, ISRCTN36266738. RAPIDO: EudraCT 2010-023957-12, and ClinicalTrials.gov NCT01558921.

Data availability statement

Data generated in this study are only available after approval of both the PROCTOR-SCRIPT and RAPIDO trial steering committees, to which a reasonable request can be submitted.

References

1. Iv AA, Koprowski MA, Nabavizadeh N, Tsikitis VL. The evolution of rectal cancer treatment: the journey to total neoadjuvant therapy and organ preservation. *Ann. Gastroenterol.* 2022; **35**: 226–233.
2. Beets GL, Figueiredo NL, Habr-Gama A, van de Velde CJH. A new paradigm for rectal cancer: organ preservation:

introducing the International Watch & Wait Database (IWWD). *Eur. J. Surg. Oncol.* 2015; **41**; 1562–1564.
3. Weiser MR. AJCC 8th edition: colorectal cancer. *Ann. Surg. Oncol.* 2018; **25**; 1454–1455.
4. Glynne-Jones R, Wyrwicz L, Tiret E *et al*. Rectal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 2018; **29**; iv263.
5. Beets-Tan RGH, Lambregts DMJ, Maas M *et al*. Magnetic resonance imaging for clinical management of rectal cancer: updated recommendations from the 2016 European Society of Gastrointestinal and Abdominal Radiology (ESGAR) consensus meeting. *Eur. Radiol.* 2018; **28**; 1465–1475.
6. Kapiteijn E, Putter H, van de Velde CJ. Impact of the introduction and training of total mesorectal excision on recurrence and survival in rectal cancer in The Netherlands. *Br. J. Surg.* 2002; **89**; 1142–1149.
7. Peeters KC, Marijnen CA, Nagtegaal ID *et al*. The TME trial after a median follow-up of 6 years: increased local control but no survival benefit in irradiated patients with resectable rectal carcinoma. *Ann. Surg.* 2007; **246**; 693–701.
8. 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.
9. 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.
10. 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.
11. Garcia-Aguilar J, Patil S, Gollub MJ *et al*. Organ preservation in patients with rectal adenocarcinoma treated with total neoadjuvant therapy. *J. Clin. Oncol.* 2022; **40**; 2546–2556.
12. Conroy T, Bosset JF, Etienne PL *et al*. Neoadjuvant chemotherapy with folfirinox and preoperative chemoradiotherapy for patients with locally advanced rectal cancer (UNICANCER-PRODIGE 23): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol.* 2021; **22**; 702–715.
13. van der Valk MJ, 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.
14. Greenbaum A, Martin DR, Bocklage T, Lee J-H, Ness SA, Rajput A. Tumor heterogeneity as a predictor of response to neoadjuvant chemotherapy in locally advanced rectal cancer. *Clin. Colorectal Cancer* 2019; **18**; 102–109.
15. Lambregts DMJ, Boellaard TN, Beets-Tan RGH. Response evaluation after neoadjuvant treatment for rectal cancer using modern MR imaging: a pictorial review. *Insights Imaging* 2019; **10**; 15.
16. Temmink SJ, Martling A, Angenete E, Nilsson PJ. Complete response rates in rectal cancer: temporal changes over a decade in a population-based nationwide cohort. *Eur. J. Surg. Oncol.* 2023; **49**; 106991.
17. Nahas SC, Nahas CSR, Cama GM *et al*. Diagnostic performance of magnetic resonance to assess treatment response after neoadjuvant therapy in patients with locally advanced rectal cancer. *Abdom. Radiol. (NY)* 2019; **44**; 3632–3640.
18. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J. Clin.* 2023; **73**; 233–254.
19. Custers PA, van der Sande ME, Grotenhuis BA *et al*. Long-term quality of life and functional outcome of patients with rectal cancer following a Watch-and-Wait Approach. *JAMA Surg.* 2023; **158**; e230146.
20. Werb Z, Lu P. The role of stroma in tumor development. *Cancer J.* 2015; **21**; 250–253.
21. Bremnes RM, Dønnem T, Al-Saad S *et al*. The role of tumor stroma in cancer progression and prognosis: emphasis on carcinoma-associated fibroblasts and non-small cell lung cancer. *J. Thorac. Oncol.* 2011; **6**; 209–217.
22. van Pelt GW, Sandberg TP, Morreau H *et al*. The tumour-stroma ratio in colon cancer: the biological role and its prognostic impact. *Histopathology* 2018; **73**; 197–206.
23. Mesker WE, Junggeburt JM, Szuhai K *et al*. The carcinoma-stromal ratio of colon carcinoma is an independent factor for survival compared to lymph node status and tumor stage. *Cell. Oncol.* 2007; **29**; 387–398.
24. Huijbers A, Tollenaar RA, v Pelt GW *et al*. The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the victor trial. *Ann. Oncol.* 2013; **24**; 179–185.
25. Park JH, Richards CH, McMillan DC, Horgan PG, Roxburgh CSD. The relationship between tumour stroma percentage, the tumour microenvironment and survival in patients with primary operable colorectal cancer. *Ann. Oncol.* 2014; **25**; 644–651.
26. Smit M, van Pelt G, Roodvoets A *et al*. Uniform noting for international application of the tumor-stroma ratio as an easy diagnostic tool: protocol for a multicenter prospective cohort study. *JMIR Res. Protoc.* 2019; **8**; e13464.
27. Polack M, Smit MA, van Pelt GW *et al*. Results from the united study: a multicenter study validating the prognostic effect of the tumor–stroma ratio in colon cancer. *ESMO Open* 2024; **9**; 102988.
28. Zunder SM, van Pelt GW, Gelderblom HJ *et al*. Predictive potential of tumour-stroma ratio on benefit from adjuvant bevacizumab in high-risk stage II and stage III colon cancer. *Br. J. Cancer* 2018; **119**; 164–169.
29. van Pelt GW, Krol JA, Lips IM *et al*. The value of tumor-stroma ratio as predictor of pathologic response after neoadjuvant chemoradiotherapy in esophageal cancer. *Clin. Transl. Radiat. Oncol.* 2020; **20**; 39–44.
30. Hansen TF, Kjær-Frifeldt S, Lindebjerg J *et al*. Tumor-stroma ratio predicts recurrence in patients with colon cancer treated with neoadjuvant chemotherapy. *Acta Oncol.* 2018; **57**; 528–533.
31. Strous MTA, Faes TKE, Gubbels A *et al*. A high tumour-stroma ratio (TSR) in colon tumours and its metastatic lymph nodes predicts poor cancer-free survival and chemo resistance. *Clin. Transl. Oncol.* 2022; **24**; 1047–1058.
32. Strous MTA, Faes TKE, Heemskerk J *et al*. Tumour-stroma ratio to predict pathological response to neo-adjuvant treatment in rectal cancer. *Surg. Oncol.* 2022; **45**; 101862.
33. Park JH, van Wyk H, McMillan DC *et al*. Preoperative, biopsy-based assessment of the tumour microenvironment in patients with primary operable colorectal cancer. *J. Pathol. Clin. Res.* 2020; **6**; 30–39.
34. Scheer R, Baidoshvili A, Zoidze S *et al*. Tumor-stroma ratio as prognostic factor for survival in rectal adenocarcinoma: a retrospective cohort study. *World J. Gastrointest. Oncol.* 2017; **9**; 466–474.

35. van Pelt GW, Kjaer-Frifeldt S, van Krieken J *et al*. Scoring the tumor-stroma ratio in colon cancer: procedure and recommendations. *Virchows Arch.* 2018; **473**; 405–412.
36. Mandard AM, Dalibard F, Mandard JC *et al*. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. *Cancer* 1994; **73**; 2680–2686.
37. Nagtegaal ID, Glynne-Jones R. How to measure tumour response in rectal cancer? An explanation of discrepancies and suggestions for improvement. *Cancer Treat. Rev.* 2020; **84**; 101964.
38. 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.
39. Glynne-Jones R, Wyrwicz L, Tiret E *et al*. Rectal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 2017; **28**; iv22–iv40.
40. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. *J. Cell Sci.* 2012; **125**; 5591–5596.
41. Ueno H, Shinto E, Shimazaki H *et al*. Histologic categorization of desmoplastic reaction: its relevance to the colorectal cancer microenvironment and prognosis. *Ann. Surg. Oncol.* 2015; **22**; 1504–1512.
42. Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. *Nat. Rev. Clin. Oncol.* 2018; **15**; 366–381.
43. Strous MT, van der Linden RL, Gubbels AL *et al*. Node-negative colon cancer: histological, molecular, and stromal features predicting disease recurrence. *Mol. Med.* 2023; **29**; 77.
44. Zhu Y, Jin Z, Qian Y, Shen Y, Wang Z. Prognostic value of tumor-stroma ratio in rectal cancer: a systematic review and meta-analysis. *Front. Oncol.* 2021; **11**; 685570.
45. Tian W, Yang Y, Qin Q *et al*. Vimentin and tumor-stroma ratio for neoadjuvant chemoradiotherapy response prediction in locally advanced rectal cancer. *Cancer Sci.* 2023; **114**; 619–629.
46. Temmink SJD, Peeters K, Bahadoer RR *et al*. Watch and wait after neoadjuvant treatment in rectal cancer: comparison of outcomes in patients with and without a complete response at first reassessment in the International Watch & Wait Database (IWWD). *Br. J. Surg.* 2023; **110**; 676–684.
47. Kagawa Y, Smith JJ, Fokas E *et al*. Future direction of total neoadjuvant therapy for locally advanced rectal cancer. *Nat. Rev. Gastroenterol. Hepatol.* 2024; **21**; 444–455.
48. Dworak O, Keilholz L, Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. *Int. J. Color. Dis.* 1997; **12**; 19–23.
49. Kus Ozturk S, Graham Martinez C, Sheahan K *et al*. Relevance of shrinkage versus fragmented response patterns in rectal cancer. *Histopathology* 2023; **83**; 870–879.
50. Loughrey MB, Webster F, Arends MJ *et al*. Dataset for pathology reporting of colorectal cancer: Recommendations from the International Collaboration On Cancer Reporting (ICCR). *Ann. Surg.* 2022; **275**; e549–e561.
51. Ryan R, Gibbons D, Hyland J *et al*. Pathological response following long-course neoadjuvant chemoradiotherapy for locally advanced rectal cancer. *Histopathology* 2005; **47**; 141–146.
52. Ravensbergen CJ, Polack M, Roelands J *et al*. Combined assessment of the tumor-stroma ratio and tumor immune cell infiltrate for immune checkpoint inhibitor therapy response prediction in colon cancer. *Cells* 2021; **10**(11); 2935.
53. Bagaev A, Kotlov N, Nomie K *et al*. Conserved pan-cancer microenvironment subtypes predict response to immunotherapy. *Cancer Cell* 2021; **39**; e847.
54. 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.
55. Aherne S, Donnelly M, Ryan ÉJ *et al*. Tumour budding as a prognostic biomarker in biopsies and resections of neoadjuvant-treated rectal adenocarcinoma. *Histopathology* 2024; **85**; 224–243.
56. Kramer A, Greuter MJE, Schraa SJ *et al*. Early evaluation of the effectiveness and cost-effectiveness of CTDNA-guided selection for adjuvant chemotherapy in stage II colon cancer. *Ther. Adv. Med. Oncol.* 2024; **16**; 17588359241266164.
57. Ravensbergen CJ, Kuruc M, Polack M *et al*. The stroma liquid biopsy panel contains a stromal-epithelial gene signature ratio that is associated with the histologic tumor-stroma ratio and predicts survival in colon cancer. *Cancers (Basel)* 2021; **14**(1); 163.
58. Sahin IH, Yanes R, Saridogan T, Holder-Murray J, Dasari AN. The role of circulating tumor DNA for management of patients with rectal cancer: challenges and opportunities. *Cancer J.* 2024; **30**; 290–296.
59. Smit MA, van Pelt GW, Dequeker EM *et al*. E-learning for instruction and to improve reproducibility of scoring tumor-stroma ratio in colon carcinoma: performance and reproducibility assessment in the united study. *JMIR Form. Res.* 2021; **5**; e19408.
60. Smit MA, van Pelt GW, Terpstra V *et al*. Tumour-stroma ratio outperforms tumour budding as biomarker in colon cancer: a cohort study. *Int. J. Color. Dis.* 2021; **36**; 2729–2737.
61. Chen RJ, Ding T, Lu MY *et al*. Towards a general-purpose foundation model for computational pathology. *Nat. Med.* 2024; **30**; 850–862.
62. Coudray N, Ocampo PS, Sakellaropoulos T *et al*. Classification and mutation prediction from non-small cell lung cancer histopathology images using deep learning. *Nat. Med.* 2018; **24**; 1559–1567.
63. Smit MA, Ciompi F, Bokhorst JM *et al*. Deep learning based tumor-stroma ratio scoring in colon cancer correlates with microscopic assessment. *J. Pathol. Inform.* 2023; **14**; 100191.
64. Firmbach D, Benz M, Kuritcyn P *et al*. Tumor-stroma ratio in colorectal cancer-comparison between human estimation and automated assessment. *Cancers (Basel)* 2023; **15**(10); 2675.
65. Sinicrope FA, Nelson GD, Saberzadeh-Ardestani B *et al*. Use of deep learning to evaluate tumor microenvironmental features for prediction of colon cancer recurrence. *Cancer Res. Commun.* 2024; **4**; 1344–1350.
66. Geessink OGF, Baidoshvili A, Klaase JM *et al*. Computer aided quantification of intratumoral stroma yields an independent prognosticator in rectal cancer. *Cell. Oncol. (Dordr)* 2019; **42**; 331–341.
67. Liang Y, Zhu Y, Lin H *et al*. The value of the tumour-stroma ratio for predicting neoadjuvant chemoradiotherapy response in locally advanced rectal cancer: a case control study. *BMC Cancer* 2021; **21**; 729.
68. Liu B, Polack M, Coudray N *et al*. Self-supervised learning reveals clinically relevant histomorphological patterns for therapeutic strategies in colon cancer. 2024 *bioRxiv*. 2002.2026.582106.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Detailed overview of the treatment types within the three separate cohorts.

Table S2. Characteristics of the biopsy-scored stroma-low compared to stroma-high patients in total cohort and per cohort.

Table S3. Overview of the pathology outcomes between biopsy-scored stroma-low compared to stroma-high patients total cohort and per treatment type.

Table S4. Overview of the clinical outcomes between biopsy-scored stroma-low compared to stroma-high patients total cohort and per treatment type.

Figure S1. Kaplan–Meier analyses of the TSR on DFS per treatment type.

Figure S2. Kaplan–Meier analyses of the TSR on OS per treatment type.

