Received Date : 09-Jan-2015

Revised Date : 11-Sep-2015

Accepted Date : 17-Sep-2015

Article type : Systematic Review

023-2015.R2

Systematic Review

**Predicting pathological complete response to neoadjuvant
chemoradiotherapy in locally advanced rectal cancer: A systemic
review**

Jennifer E Ryan^{1,2,3}, Satish K Warriar¹, A Craig Lynch¹, Robert G Ramsay^{4,5}, Wayne
A Phillips^{5,6}, Alexander G Heriot¹.

1. Division of Cancer Surgery, Peter MacCallum Cancer Centre, Melbourne, Australia.
2. Epworth Healthcare, Melbourne, Australia.
3. Austin Academic Centre, University of Melbourne, Melbourne, Australia
4. Differentiation and Transcription Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia.
5. Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](https://doi.org/10.1111/codi.13207). Please cite this article as [doi: 10.1111/codi.13207](https://doi.org/10.1111/codi.13207)

This article is protected by copyright. All rights reserved

6. Cancer Biology and Surgical Oncology Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia.

J E Ryan, BSc, MBBS, FRACS

S KWarrier, BSc, MBBS, FRACS

A C Lynch, MBChB MMedSc FCSSANZ FASCRS(int) FRACS

R G Ramsay, BSc(Hons), PhD

W A Phillips, BSc(Hons), PhD

A G Heriot, MA MD MBA FRACS FRCS (Gen.)

Correspondence:

**Dr Jennifer Ryan, Division of Cancer Surgery, Peter MacCallum Cancer Centre,
St Andrews Place, East Melbourne**

Email: Jennifer.Ryan@petermac.org

Word Count: 3452

No authors declare any potential conflicts of interest.

ABSTRACT

Aim: Approximately 20% of patients treated with neoadjuvant chemo-radiotherapy for locally advanced rectal cancer achieve a pathological complete response (pCR) while the remainder derive the benefit of improved local control and down-staging and a small proportion show a minimal response. The ability to predict which patients will benefit would allow for improved patient stratification directing therapy to those who are likely to achieve a good response thereby avoiding ineffective treatment in those unlikely to benefit.

Method: A systematic review of the English language literature was conducted to identify pathological factors, imaging modalities, and molecular factors that predict pCR following chemoradiotherapy. PubMed, MEDLINE and Cochrane Database searches were conducted with the following key words and MeSH search terms: 'rectal neoplasm', 'response', 'neoadjuvant', 'preoperative chemoradiation', 'tumor response'. After review of title and abstracts, 85 articles addressing the prediction of pCR were selected.

Results: Clear methods to predict pCR before chemoradiotherapy have not been defined. Clinical and radiological features of the primary cancer have limited ability to predict response. Molecular profiling holds the greatest potential to predict pCR but adoption of this technology will require greater concordance between cohorts for the biomarkers currently under investigation.

Conclusion: At present no robust markers of prediction of pCR have been identified and the topic remains an area for future research. This review critically evaluates existing literature providing an overview of the methods currently available to predict pCR to neoadjuvant chemoradiotherapy for locally advanced rectal cancer. The review also provides comprehensive comparison of the accuracy of each modality.

INTRODUCTION

The management of rectal cancer has evolved over the last 20 years to become multidisciplinary and, for locally advanced tumours, may include pre-operative neoadjuvant chemoradiotherapy (nCRT), total mesorectal excision (TME) and adjuvant chemotherapy [1-6]. Currently patients are selected for nCRT based on preoperative staging to identify those with a threatened circumferential resection margin (CRM) and node positive disease who have higher rates of recurrence that can be reduced with the combination of nCRT, TME and adjuvant chemotherapy [5, 7, 8]. A pathological complete response (pCR) occurs in approximately 20% of patients, and this appears to confer a survival benefit [9-11]. Potentially this patient group might be spared the morbidity and risks of surgery and there are limited case series reporting this approach [12-14]. The ability reliably to predict which patients will benefit from nCRT would allow improved selection of patients with locally advanced disease and those who are likely to achieve a complete response. For such an approach to be widely adopted, accurate, reliable and reproducible methods to predict response prior to commencement of therapy are needed.

The prediction of response to neoadjuvant chemoradiotherapy has been a topic of discussion and research for many years. Excellent reviews of this topic were published in 2006 [15] and 2011 [16] but there has been increased focus on the subject recently, including a European Consensus meeting in 2014 [17], hence the indication to reassess the current literature on this important topic.

Preoperative prediction of pCR may be possible through a number of tumour-related factors that include clinical, pathological, radiological and molecular markers [18-20]. The present study aims to review the potential of these factors to predict pCR in patients with rectal cancer undergoing chemoradiotherapy.

METHOD

A systematic review of the English language literature was conducted to identify pathological factors, imaging modalities, and molecular factors that predict pCR following neoadjuvant chemoradiotherapy. PubMed and MEDLINE searches were conducted with the following key words and MeSH search terms: 'rectal neoplasm', 'response', 'neoadjuvant', 'preoperative chemoradiation', 'tumor response'. Relevant papers were also identified through manual searching of references in the identified papers.

Of the 694 articles initially identified, 416 were excluded after review of title and abstract. Articles with insufficient information on methodology, radiological parameters, molecular techniques or statistical analysis. , Systematic reviews and meta-analyses were excluded leaving 85 papers with sufficient detail to be included in the final review of the prediction of pCR (Fig 1).

Clinicopathological prediction of pCR

There are few studies in the literature investigating the relationship between pretreatment clinical features and pCR. Three groups have found that pCR was associated with, good tumour differentiation, small tumour diameter, early T and N stage on imaging and low levels of pretreatment CEA[21-23]. In addition, non-circumferential and non-ulcerated tumours have been shown to predict pCR while poor response to treatment is associated with T4 tumours and poor histological differentiation [22, 23]. These studies, however, show poor sensitivity and specificity and have been contradicted in other series[13, 24, 25].

Several studies have investigated the ability of CEA to predict response to nCRT. While most have found low pretreatment CEA to be associated with pCR [26-34], not all have found this to be statistically significant [26, 31, 35]. In contrast, Kalady et al reported that a pretreatment CEA of less than 5mg/mL did not correlate with pCR [24]. These studies are summarised in Table 1.

Radiological Prediction of pCR

Magnetic Resonance Imaging (MRI)

MRI is routinely used for preoperative staging in rectal cancer and may provide features predictive of response such as T-stage and N-stage status and extra mural venous invasion [21, 36]. Volume measurement on standard pretreatment MRI has, however, not been shown accurately to predict response to nCRT [37-39]. The only study identified using standard MRI to predict pCR demonstrated that signal intensity (SI) was higher in those with Tumor Regression Grading (TRG) 1 compared with TRG 2 and 3 but no difference was found between SI in TRG 1 and TRG 4 [40]. Contrast enhanced and diffusion weighted imaging (DWI) show some potential to predict response, as they are markers of perfusion and cellular density that reflect tumour biology and function in addition to morphology. Perfusion index (PI) calculated based on contrast enhanced MRI, may provide an indication of tumour cell activity and has the potential to predict response to nCRT [41]. A high PI has been shown by two groups to predict poor response to nCRT but a low PI has not been reported to predict pCR [41-43].

Diffusion weighted imaging provides an impression of cellular density and architecture through the measurement of the displacement of water molecules, which are more restricted in tissues such as cancer with greater cellular density and can be quantified by the apparent diffusion coefficient (ADC) [44-47]. Diffusion weighted imaging has been investigated by a number of groups, but with conflicting results. Lambrechts et al provided promising data on the prediction of pCR in 22 patients, and found that those with a low pretreatment ADC had a significantly greater rate of pCR after nCRT ($p=0.003$). A pre-treatment mean of ADC of $\leq 1.06 \times 10^3 \text{ mm}^2/\text{sec}$ was 100% sensitive and 86% specific in predicting pCR [38].

Barbaro et al, however, found that a low pretreatment ADC $< 1 \times 10^3 \text{ mm}^2/\text{sec}$ was associated with a poor response ($p=0.0011$). While the median pretreatment ADC was higher in patients with a pCR compared with a partial or poor response, this was not statistically significant [48]. This conclusion was supported by data from Elmi et al who found that a higher pretreatment ADC was associated with a response to treatment ($p=0.035$), and was able to predict pCR with a sensitivity of 75% and specificity of 48% [49]. Curvo-Semedo et al reported that neither estimates of

pretreatment volume nor ADC were predictive of pCR ($p=0.16$ and $p=0.61$) [39]. These results are summarised in Table 2.

¹⁸F-Fluorodeoxyglucose Positron Emission Tomography–computed tomography (FDG-PET/CT)

Several studies were identified which addressed the ability of FDG PET/CT to assess response to therapy [50, 51], although this technique is rarely used before starting treatment. The few studies that have investigated the pretreatment standardized uptake value (SUV) to predict pCR, have not shown statistical significance [25, 52-56], although Martoni et al uniquely report an association between TRG and pretreatment SUVmax. In this study, a median SUV of <27 was found to predict pCR with a sensitivity of 100% but with a low specificity of only 10.6% [57].

Molecular prediction of pCR

Despite extensive research, the molecular and genetic mechanisms for sensitivity to chemotherapy and radiotherapy are yet to be clearly defined. The varied response to nCRT seen among tumours suggests a complex relationship between tumour biology and response and it is likely that a number of genetic or molecular pathways regulate chemoradiosensitivity [58].

Gene expression profiling

Gene expression microarrays, by assessing large numbers of genes simultaneously, have the potential to identify prognostic markers by comparing gene expression in patients with differing outcomes [20]. For example, microarray techniques have been used to generate predictive models in breast cancer, including Oncotype DX (Genomic Health, USA) and MammaPrint (Agendia Inc. USA) that are used in the clinical setting to individualize patient treatment [18, 20].

To date seven studies have been reported in the literature identifying different signatures for response in rectal cancer [59-65]. While there is little overlap or concordance in the genes identified, common pathways involved in DNA repair, apoptosis and growth signaling pathways provide links between the studies [60, 62, 66]. A summary of these papers is provided in Table 3. Two specifically addressing gene expression and prediction of pCR are elaborated in this review [59, 62].

A Korean group performed microarray analysis on pretreatment biopsies from 31

patients with locally advanced rectal cancer to identify 95 genes predictive of pCR [62]. Pathological complete response was predicted with an accuracy of 84%.

Pretreatment biopsies from a separate cohort of 15 patients were then analyzed to validate the findings. In the second cohort, the 95 genes were able to distinguish between pCR and partial response with an 87% accuracy. The genes identified included those involved in activation of MAPK activity, EGFR signaling pathway, defense response and nucleotide excision repair, but this showed little overlap with the signatures previously reported [62].

Brettingham-Moore et al performed microarray analysis on 51 patients with locally advanced rectal cancer [59]. The authors investigated response according to a number of criteria including TRG, PET metabolic response, TMN down staging and pCR, but were unable to identify any molecular predictors of response. The authors further attempted to validate the gene expressions described Kim et al [62], Ghadimi et al, [60] and Rimkus et al, [66] on their patient cohort but were unable to identify sufficient correlation between response and molecular signature. They thus surmised that differential gene expression across studies may reflect alterations of similar molecular pathways. Indeed, when further analysis was performed, genes from the TNF signaling pathway and the β -estradiol signaling network were found to be commonly expressed between the four signature profiles [59].

RNA expression profiling has been used to assess microRNAs (miRNA) that are small non-coding RNA molecules that can be up- or down-regulated and influence activity of signaling pathways that may be associated with prognosis and response to nCRT. Differential expression of 53 miRNAs was demonstrated between pCR and non-pCR by Della Vittoria Scarpati et al. The greatest differential expression was found in 14 miRNAs. miRNA-622 and miRNA-630 demonstrated an impressive 100% specificity and sensitivity to predict pCR [67]. The authors concluded that miRNA influences genes and signaling pathways involved in cell repair following chemoradiotherapy. In the case of miRNA 630, it has previously been shown to impair a cell's ability to repair DNA damage caused by Cisplatin-based chemotherapy in non-small cell lung cancer. This may explain the benefit seen in this patient cohort receiving Oxaliplatin based nCRT, which may not, however, be transferable to the more standard 5-FU based neoadjuvant treatment. [67] In direct contrast to these findings, a study in rectal cancer cells lines by Ma et al identified miR-622 as a

marker of radioresistance and not of pCR[68].

Kheirelseid et al extracted miRNA from twelve formalin fixed paraffin embedded rectal cancer specimens and found that miRNA-16, miRNA-590-5p and miRNA-153 predicted pCR with 100% accuracy [69]. This is an interesting technique with great potential due to the wide availability of FFPE tissue stored in archives but is yet to be tested on a larger cohort.[69]

Lopes-Ramos et al investigated miRNA expression in 43 patients following LCCRT. Patients were divided into three groups, clinical complete response clinical incomplete response and those with an initial cCR who developed early recurrence. They identified four miRNA with differential expression that predicted cCR miR-21-5p, miR-1246, miR1290-3p and miR-205-5p. The sensitivity and specificity of miR-21-5p to predict complete response was calculated to be 100% and 85%. Importantly those with cCR who developed an early recurrence had levels of expression of miR-21-5p that was similar to those with an incomplete response and statistically significantly lower to those with sustained cCR [70]. While these results are promising individually, the lack of concordance between studies highlights the inconsistencies in the molecular prediction of complete response. (Table 4)

KRAS mutation analysis

KRAS expression and mutation analysis has been extensively reported in the literature. Activating mutations of this oncogene have been identified in a number of colorectal cancers with resultant constitutive activation of the MAPK pathway implicated in carcinogenesis [20], but the influence of KRAS mutations on pCR is inconsistent between studies and likely to be influenced by the use of EGF targeted therapies [71-78]. A summary of KRAS mutation status, chemotherapy regimen and pCR is provided in Table 5.

It has been suggested that one reason for some discrepancy in results may be the influence of KRAS mutations at specific codons[71, 73]. For example, Duldulao et al found that any mutation in KRAS was associated with a lower rate of pCR compared with KRASwt but, more specifically, none of the tumours with KRAS mutation in codon 13 developed a pCR. However, when Gaedcke et al also investigated specific KRAS mutations at codons 12, 13, 61 and 146, they failed to find a correlation with

any of the mutations and complete response [79]. Similarly, Bengala et al found no association between mutations in codon 12 or 13 and response [78].

Protein Expression

Instead of RNA or mutation profiling, Folkvord et al used a multiplex kinase substrate platform to assess tumour kinase activity. Elevated activity of 86 kinases predicted response to nCRT with 95% accuracy in a subset of selected patients with good and poor responses. This was validated on a test set of 47 patients and found to predict response in 85% of samples. A number of these kinases are components of signaling pathways mediated by VEGF, EGFR and phosphoinositide 3- kinase (PI3K)/AKT pathways [80].

The focus of the literature more generally on protein expression, determined by antibody-based immunohistochemical analysis or reverse transcriptase polymerase chain reaction (RT-PCR), has involved the detection of candidate proteins involved in DNA damage repair, proliferation, angiogenesis and apoptosis. Despite there being several studies, no single protein has been consistently identified to be predictive of response to nCRT in rectal cancer. While all studies addressed response to long course chemoradiotherapy, the nCRT regimens were not consistent between studies, nor were the methods used to measure expression, making direct comparison between the studies difficult.

With specific reference to p53, the presence of p53 mutations correlated inversely with pCR in a number of studies but not all authors have found this association to be statistically significant [81-89]. In contrast other authors have failed to identify any association at all between pCR and the presence of p53 mutations [58, 90-92]. These results are summarised in Table 6.

EGFR and VEGF expression has been investigated by several workers using different detection methods, all of which have reported differing results [71, 78, 92-96]. These results are summarised in Table 7. Grimminger et al investigated EGFR and VEGF expression in the context of KRAS wild type tumours. Here, pCR was predicted with a sensitivity of 100% and specificity of 95% in KRASwt tumours with high VEGF expression, but this association was lost when the expression was analyzed independently of KRAS mutation status [71].

Protein expression of thymidylate synthase (TS), p21, Bax, Bcl-2, matrix metalloproteinase-9 and 2 (MMP-9, MMP-2), Ki-67, mismatch repair proteins and thymidine phosphorylase (TP) have been the focus of a number of studies, but the results are again varied with limited concordance between study populations [58, 85, 86, 90, 92, 96-99] (Table 7).

Over-expression of ATP binding cassette subfamily C member 4 (ABCC4), a member of the multidrug transporter and resistance family and its effect on pCR was investigated by Yu et al. High levels of ABCC4 were associated with radio-resistance and, by silencing the gene in an experimental model, the authors demonstrated a significant increase in radiation-induced apoptosis, inhibition of proliferation, and decreased growth [100] (Table. 7).

Hur et al compared a panel of biomarkers identified using IHC and prediction of response to nCRT in 81 patients with locally advanced rectal cancer. The panel consisted of 12 biomarkers including p53mt, p21, Bcl-2, Bax, EGFR, COX-2, MLH-1, MSH-2, Ku-70, VEGF, TS and Ki-67. They found that low levels of p-53mt and high expression of VEGF, p-21 and Ki-67 were correlated with pCR ($p=0.01-0.03$). Patients expressing three or four of these markers were more likely to achieve a pCR ($p=0.001$) [101]. (Table 7)

One group has reported on the presence of phosphorylated extracellular signal related kinase (pERK), PI3K/AKT and pCR. This is potentially instructive as BRAF activation leads to phosphorylation and activation of ERK and ERK is involved in cell proliferation and evasion of apoptosis. PI3K and its downstream target AKT are activated by receptor tyrosine kinases and play a role in the regulation of growth, survival, metastasis and proliferation. Activation of ERK and AKT were significantly associated with response but only AKT was predictive of pCR ($p=0.02$) [76].

Chromosomal aberrations

Gains and losses of chromosomal segments lead to changes in oncogenes and tumour suppressor genes that are important for the development and progression of colorectal cancer. When Chen et al investigated chromosomal copy number alterations (CNA), they found statistically fewer high copy gains ($p=0.01$) and specific loss of chromosomal region 12p13.31 ($p=0.0003$) in those with pCR [102]. Eight genes were

identified in this region associated with tumour response. Further Ingenuity Pathway Analysis (IPA) identified pathways involved in pCR including GABA receptor signaling pathway, the glycolysis/gluconeogenesis pathway and the glutamate receptor signaling pathway. The authors also developed a predictive model of 32 genes that predicted pCR with a sensitivity and specificity of 76% and 97% respectively [102].

Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) in the thymidylate synthase (TS), EGFR Sp1-216 and its ligand encoding gene EGF (A61G) have been investigated by Spindler et al. Using RT-PCR with allele-specific primers on blood samples, they found a small subgroup of patients with TS 2/2 genotype and heterozygous for the EGF A61G gene demonstrated a pCR rate of 100%. The combination of all three polymorphisms predicted pCR with accuracy of 64% [103].

Hu-Lieskovna et al evaluated the relationship between KRAS status and germline SNPs in genes involved in the EGFR pathway (EGF, EGFR, COX 2, KRAS, Cyclin D1), angiogenesis (VEGF and IL-8), antibody-dependent cell-mediated toxicity (FCGR2A/3), DNA repair (XRCC3, Rad 51) and drug metabolism (MTHR, TS). In keeping with the results above, polymorphisms of the EGF (A61G) gene were found in 45% of patients with pCR ($p=0.001$), but, no statistically significant association between pCR or the other polymorphisms studied was found [75]. Hur et al also assessed TS SNPs and tumour response in 44 patients finding it to be associated with tumour downstaging and nodal staging but not significantly associated with pCR [104].

Spontaneous apoptosis

Rates of spontaneous apoptosis have been investigated as a marker of sensitivity to radiation. Most studies on this topic investigate response rather than pCR, but four articles investigate the presence of spontaneous apoptosis in pretreatment biopsies and subsequent complete response to nCRT [105-108]. Three studies demonstrated that increased apoptosis was statistically significantly associated with pCR, but McDowell et al did not find an association between rates of apoptosis and nodal regression. In

contrast, Tannapfel et al did not identify any association between spontaneous apoptosis and pCR or response[108].

Discussion

Prediction of complete response to neoadjuvant chemoradiotherapy is a topical area of investigation. Despite the many studies reporting some promising results no clinical, radiological or molecular features have demonstrated an ability to predict response with adequate sensitivity or specificity to guide management. Preoperative clinicopathological features have limited ability to predict response to nCRT. While it seems logical that increased size and an advanced stage of the tumour should predict response, this has not been borne out by all studies. While MRI and PET/CT are regularly used for pretreatment staging, their ability to predict a complete response has not been demonstrated.

Molecular markers, have been investigated extensively and hold the potential to predict response, but disparate results reported by the studies reviewed suggests that this approach has poor reproducibility and lacks independent validation. Most of the literature investigating individual biomarkers has focused on the detection of candidate proteins involved in DNA damage repair, proliferation, angiogenesis and apoptosis including p53, KRAS, EGFR, VEGF, Bax, and Bcl-2. Despite a number of studies, no single protein has been consistently identified to be predictive of response to nCRT in rectal cancer. An increased rate of spontaneous apoptosis in pretreatment biopsies has been shown in a limited number of studies to be predictive of pCR.

The varied response to nCRT seen between tumours suggests a complex relationship between tumour biology and response and it is likely that several genes or molecular pathways regulate chemoradiosensitivity. Thus gene expression profiles that sequence large number of genes have been used in the hope of identifying a molecular signature for the prediction of response. Despite the theoretical advantage, the two published studies investigating pCR have not identified similar gene profiles and this method would appear to lack sufficient external validity, although the data would suggest that while the molecular patterns identified are different, there are perhaps similar pathways involved that predict response to nCRT. Despite this, the data are not robust enough to modify current treatment paradigms and further research is required.

Overall the key action that may resolve the inconsistencies would be to coordinate multicentre studies to create much larger cohorts and ensure robust methodical platforms that test imaging modalities, IHC, gene expression profiles, combined with agreed standards of pathological review.

REFERENCES

1. Heald RJ, Husband EM, Ryall RD. The mesorectum in rectal cancer surgery--the clue to pelvic recurrence? *Br J Surg* 1982; **69**: 613-6.
2. Kapiteijn E, Marijnen CA, Nagtegaal ID, Putter H, Steup WH, Wiggers T, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. *The New England journal of medicine* 2001; **345**: 638-46.
3. Marijnen CA, Nagtegaal ID, Kapiteijn E, Kranenbarg EK, Noordijk EM, van Krieken JH, et al. Radiotherapy does not compensate for positive resection margins in rectal cancer patients: report of a multicenter randomized trial. *Int J Radiat Oncol Biol Phys* 2003; **55**: 1311-20.
4. Peeters KC, Marijnen CA, Nagtegaal ID, Kranenbarg EK, Putter H, Wiggers T, 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.
5. Ceelen WP, Van Nieuwenhove Y, Fierens K. Preoperative chemoradiation versus radiation alone for stage II and III resectable rectal cancer. *Cochrane Database Syst Rev* 2009: CD006041.
6. Folkesson J, Birgisson H, Pahlman L, Cedermark B, Glimelius B, Gunnarsson U. Swedish Rectal Cancer Trial: long lasting benefits from radiotherapy on survival and local recurrence rate. *J Clin Oncol* 2005; **23**: 5644-50.
7. Janjan NA, Khoo VS, Abbruzzese J, Pazdur R, Dubrow R, Cleary KR, et al. Tumor downstaging and sphincter preservation with preoperative chemoradiation in locally advanced rectal cancer: the M. D. Anderson Cancer Center experience. *Int J Radiat Oncol Biol Phys* 1999; **44**: 1027-38.
8. Ceelen W, Boterberg T, Pattyn P, van Eijkeren M, Gillardin JM, Demetter P, et al. Neoadjuvant chemoradiation versus hyperfractionated accelerated

- radiotherapy in locally advanced rectal cancer. *Ann Surg Oncol* 2007; **14**: 424-31.
9. Brown CL, Terner CA, Thorson AG, Christensen MA, Blatchford GJ, Shashidharan M, et al. Response to preoperative chemoradiation in stage II and III rectal cancer. *Dis Colon Rectum* 2003; **46**: 1189-93.
 10. Martin ST, Heneghan HM, Winter DC. Systematic review and meta-analysis of outcomes following pathological complete response to neoadjuvant chemoradiotherapy for rectal cancer. *Br J Surg* 2012; **99**: 918-28.
 11. de Campos-Lobato LF, Stocchi L, da Luz Moreira A, Geisler D, Dietz DW, Lavery IC, et al. Pathologic complete response after neoadjuvant treatment for rectal cancer decreases distant recurrence and could eradicate local recurrence. *Ann Surg Oncol* 2011; **18**: 1590-8.
 12. Habr-Gama A, Perez R, Proscurshim I, Gama-Rodrigues J. Complete clinical response after neoadjuvant chemoradiation for distal rectal cancer. *Surg Oncol Clin N Am* 2010; **19**: 829-45.
 13. Habr-Gama A, Sabbaga J, Gama-Rodrigues J, Sao Juliao GP, Proscurshim I, Bailao Aguilar P, et al. Watch and wait approach following extended neoadjuvant chemoradiation for distal rectal cancer: are we getting closer to anal cancer management? *Dis Colon Rectum* 2013; **56**: 1109-17.
 14. Maas M, Beets-Tan RG, Lambregts DM, Lammering G, Nelemans PJ, Engelen SM, et al. Wait-and-see policy for clinical complete responders after chemoradiation for rectal cancer. *J Clin Oncol* 2011; **29**: 4633-40.
 15. Smith FM, Reynolds JV, Miller N, Stephens RB, Kennedy MJ. Pathological and molecular predictors of the response of rectal cancer to neoadjuvant radiochemotherapy. *European Journal of Surgical Oncology* 2006; **32**: 55-64.
 16. Perez RO. Predicting response to neoadjuvant treatment for rectal cancer: a step toward individualized medicine. *Dis Colon Rectum* 2011; **54**: 1057-8.
 17. Heald RJ, Beets G, Carvalho C. Report from a consensus meeting: response to chemoradiotherapy in rectal cancer - predictor of cure and a crucial

- new choice for the patient: on behalf of the Champalimaud 2014 Faculty for 'Rectal cancer: when NOT to operate'. *Colorectal Dis* 2014; **16**: 334-7.
18. Akiyoshi T, Kobunai T, Watanabe T. Predicting the response to preoperative radiation or chemoradiation by a microarray analysis of the gene expression profiles in rectal cancer. *Surg Today* 2012; **42**: 713-9.
 19. Barbaro B, Vitale R, Leccisotti L, Vecchio FM, Santoro L, Valentini V, et al. Restaging locally advanced rectal cancer with MR imaging after chemoradiation therapy. *Radiographics* 2010; **30**: 699-716.
 20. Grade M, Wolff HA, Gaedcke J, Ghadimi BM. The molecular basis of chemoradiosensitivity in rectal cancer: implications for personalized therapies. *Langenbecks Arch Surg* 2012; **397**: 543-55.
 21. Garland ML, Vather R, Bunkley N, Pearse M, Bissett IP. Clinical tumour size and nodal status predict pathologic complete response following neoadjuvant chemoradiotherapy for rectal cancer. *Int J Colorectal Dis* 2014; **29**: 301-7.
 22. Qiu HZ, Wu B, Xiao Y, Lin GL. Combination of differentiation and T stage can predict unresponsiveness to neoadjuvant therapy for rectal cancer. *Colorectal Dis* 2011; **13**: 1353-60.
 23. Huh JW, Kim HR, Kim YJ. Clinical prediction of pathological complete response after preoperative chemoradiotherapy for rectal cancer. *Dis Colon Rectum* 2013; **56**: 698-703.
 24. Kalady MF, de Campos-Lobato LF, Stocchi L, Geisler DP, Dietz D, Lavery IC, et al. Predictive factors of pathologic complete response after neoadjuvant chemoradiation for rectal cancer. *Annals of Surgery* 2009; **250**: 582-9.
 25. Hur H, Kim NK, Yun M, Min BS, Lee KY, Keum KC, et al. 18Fluoro-deoxy-glucose positron emission tomography in assessing tumor response to preoperative chemoradiation therapy for locally advanced rectal cancer. *J Surg Oncol* 2011; **103**: 17-24.
 26. Moreno Garcia V, Cejas P, Blanco Codesido M, Feliu Batlle J, de Castro Carpeno J, Belda-Iniesta C, et al. Prognostic value of carcinoembryonic antigen level in rectal cancer treated with neoadjuvant chemoradiotherapy. *Int J Colorectal Dis* 2009; **24**: 741-8.

27. Moureau-Zabotto L, Farnault B, de Chaisemartin C, Esterni B, Lelong B, Viret F, et al. Predictive factors of tumor response after neoadjuvant chemoradiation for locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 2011; **80**: 483-91.
28. Kang JH, Kim YC, Kim H, Kim YW, Hur H, Kim JS, et al. Tumor volume changes assessed by three-dimensional magnetic resonance volumetry in rectal cancer patients after preoperative chemoradiation: the impact of the volume reduction ratio on the prediction of pathologic complete response. *Int J Radiat Oncol Biol Phys* 2010; **76**: 1018-25.
29. Yoon SM, Kim DY, Kim TH, Jung KH, Chang HJ, Koom WS, et al. Clinical parameters predicting pathologic tumor response after preoperative chemoradiotherapy for rectal cancer. *Int J Radiat Oncol Biol Phys* 2007; **69**: 1167-72.
30. Lee SD, Park JW, Park KS, Lim SB, Chang HJ, Kim DY, et al. Influence of anemia on tumor response to preoperative chemoradiotherapy for locally advanced rectal cancer. *Int J Colorectal Dis* 2009; **24**: 1451-8.
31. Yeo SG, Kim DY, Chang HJ, Park JW, Oh JH, Kim BC, et al. Reappraisal of pretreatment carcinoembryonic antigen in patients with rectal cancer receiving preoperative chemoradiotherapy. *Tumori* 2013; **99**: 93-9.
32. Yang KL, Yang SH, Liang WY, Kuo YJ, Lin JK, Lin TC, et al. Carcinoembryonic antigen (CEA) level, CEA ratio, and treatment outcome of rectal cancer patients receiving pre-operative chemoradiation and surgery. *Radiation Oncology* 2013; **8**: 43.
33. Wang L, Zhong XG, Peng YF, Li ZW, Gu J. Prognostic value of pretreatment level of carcinoembryonic antigen on tumour downstaging and early occurring metastasis in locally advanced rectal cancer following neoadjuvant radiotherapy (30 Gy in 10 fractions). *Colorectal Disease* 2014; **16**: 33-9.
34. Restivo A, Zorcolo L, Cocco IM, Manunza R, Margiani C, Marongiu L, et al. Elevated CEA levels and low distance of the tumor from the anal verge are predictors of incomplete response to chemoradiation in patients with rectal cancer. *Annals of Surgical Oncology* 2013; **20**: 864-71.

35. Wallin U, Rothenberger D, Lowry A, Luepker R, Mellgren A. CEA - a predictor for pathologic complete response after neoadjuvant therapy for rectal cancer. *Diseases of the Colon & Rectum* 2013; **56**: 859-68.
36. Yu SK, Tait D, Chau I, Brown G. MRI predictive factors for tumor response in rectal cancer following neoadjuvant chemoradiation therapy-- implications for induction chemotherapy? *Int J Radiat Oncol Biol Phys* 2013; **87**: 505-11.
37. Hotker AM, Garcia-Aguilar J, Gollub MJ. Multiparametric MRI of rectal cancer in the assessment of response to therapy: a systematic review. *Diseases of the Colon & Rectum* 2014; **57**: 790-9.
38. Lambrecht M, Deroose C, Roels S, Vandecaveye V, Penninckx F, Sagaert X, et al. The use of FDG-PET/CT and diffusion-weighted magnetic resonance imaging for response prediction before, during and after preoperative chemoradiotherapy for rectal cancer. *Acta Oncol* 2010; **49**: 956-63.
39. Curvo-Semedo L, Lambregts DM, Maas M, Thywissen T, Mehsen RT, Lammering G, et al. Rectal cancer: assessment of complete response to preoperative combined radiation therapy with chemotherapy-- conventional MR volumetry versus diffusion-weighted MR imaging. *Radiology* 2011; **260**: 734-43.
40. Kluza E, Rozeboom ED, Maas M, Martens M, Lambregts DM, Slenter J, et al. T2 weighted signal intensity evolution may predict pathological complete response after treatment for rectal cancer. *European Radiology* 2013; **23**: 253-61.
41. Devries AF, Griebel J, Kremser C, Judmaier W, Gneiting T, Kreczy A, et al. Tumor microcirculation evaluated by dynamic magnetic resonance imaging predicts therapy outcome for primary rectal carcinoma. *Cancer research* 2001; **61**: 2513-6.
42. DeVries AF, Kremser C, Hein PA, Griebel J, Kreczy A, Ofner D, et al. Tumor microcirculation and diffusion predict therapy outcome for primary rectal carcinoma. *Int J Radiat Oncol Biol Phys* 2003; **56**: 958-65.
43. Kremser C, Trieb T, Rudisch A, Judmaier W, de Vries A. Dynamic T(1) mapping predicts outcome of chemoradiation therapy in primary rectal

- carcinoma: sequence implementation and data analysis. *J Magn Reson Imaging* 2007; **26**: 662-71.
44. Costa-Silva L, Brown G. Magnetic resonance imaging of rectal cancer. *Magnetic resonance imaging clinics of North America* 2013; **21**: 385-408.
 45. Koh DM, Collins DJ. Diffusion-weighted MRI in the body: applications and challenges in oncology. *AJR Am J Roentgenol* 2007; **188**: 1622-35.
 46. Padhani AR, Liu G, Koh DM, Chenevert TL, Thoeny HC, Takahara T, et al. Diffusion-weighted magnetic resonance imaging as a cancer biomarker: consensus and recommendations. *Neoplasia* 2009; **11**: 102-25.
 47. Dzik-Jurasz A, Domenig C, George M, Wolber J, Padhani A, Brown G, et al. Diffusion MRI for prediction of response of rectal cancer to chemoradiation. *Lancet* 2002; **360**: 307-8.
 48. Barbaro B, Vitale R, Valentini V, Illuminati S, Vecchio FM, Rizzo G, et al. Diffusion-weighted magnetic resonance imaging in monitoring rectal cancer response to neoadjuvant chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2012; **83**: 594-9.
 49. Elmi A, Hedgire SS, Covarrubias D, Abtahi SM, Hahn PF, Harisinghani M. Apparent diffusion coefficient as a non-invasive predictor of treatment response and recurrence in locally advanced rectal cancer. *Clinical Radiology* 2013; **68**: e524-31.
 50. Guillem JG, Moore HG, Akhurst T, Klimstra DS, Ruo L, Mazumdar M, et al. Sequential preoperative fluorodeoxyglucose-positron emission tomography assessment of response to preoperative chemoradiation: a means for determining longterm outcomes of rectal cancer. *J Am Coll Surg* 2004; **199**: 1-7.
 51. Akhurst T, Fong Y. Positron emission tomography in surgical oncology. *Advances in surgery* 2002; **36**: 309-31.
 52. Goldberg N, Kundel Y, Purim O, Bernstine H, Gordon N, Morgenstern S, et al. Early prediction of histopathological response of rectal tumors after one week of preoperative radiochemotherapy using 18 F-FDG PET-CT imaging. A prospective clinical study. *Radiat Oncol* 2012; **7**: 124.
 53. Kim JW, Kim HC, Park JW, Park SC, Sohn DK, Choi HS, et al. Predictive value of (18)FDG PET-CT for tumour response in patients with locally

- advanced rectal cancer treated by preoperative chemoradiotherapy. *Int J Colorectal Dis* 2013
54. Konski A, Hoffman J, Sigurdson E, Haluszka O, Engstrom P, Cheng JD, et al. Can molecular imaging predict response to preoperative chemoradiation in patients with rectal cancer? A Fox Chase Cancer Center prospective experience. *Semin Oncol* 2005; **32**: S63-7.
 55. Konski A, Li T, Sigurdson E, Cohen SJ, Small W, Jr., Spies S, et al. Use of molecular imaging to predict clinical outcome in patients with rectal cancer after preoperative chemotherapy and radiation. *Int J Radiat Oncol Biol Phys* 2009; **74**: 55-9.
 56. Lee SJ, Kim JG, Lee SW, Chae YS, Kang BW, Lee YJ, et al. Clinical implications of initial FDG-PET/CT in locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy. *Cancer Chemother Pharmacol* 2013; **71**: 1201-7.
 57. Martoni AA, Di Fabio F, Pinto C, Castellucci P, Pini S, Ceccarelli C, et al. Prospective study on the FDG-PET/CT predictive and prognostic values in patients treated with neoadjuvant chemoradiation therapy and radical surgery for locally advanced rectal cancer. *Ann Oncol* 2011; **22**: 650-6.
 58. Chang HJ, Jung KH, Kim DY, Jeong SY, Choi HS, Kim YH, et al. Bax, a predictive marker for therapeutic response to preoperative chemoradiotherapy in patients with rectal carcinoma. *Hum Pathol* 2005; **36**: 364-71.
 59. Brettingham-Moore KH, Duong CP, Greenawalt DM, Heriot AG, Ellul J, Dow CA, et al. Pretreatment transcriptional profiling for predicting response to neoadjuvant chemoradiotherapy in rectal adenocarcinoma. *Clin Cancer Res* 2011; **17**: 3039-47.
 60. Ghadimi BM, Grade M, Difilippantonio MJ, Varma S, Simon R, Montagna C, et al. Effectiveness of gene expression profiling for response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy. *J Clin Oncol* 2005; **23**: 1826-38.
 61. Rimkus C, Friederichs J, Boulesteix AL, Theisen J, Mages J, Becker K, et al. Microarray-based prediction of tumor response to neoadjuvant

- radiochemotherapy of patients with locally advanced rectal cancer.
Clinical Gastroenterology & Hepatology 2008; **6**: 53-61.
62. Kim IJ, Lim SB, Kang HC, Chang HJ, Ahn SA, Park HW, et al. Microarray gene expression profiling for predicting complete response to preoperative chemoradiotherapy in patients with advanced rectal cancer. *Dis Colon Rectum* 2007; **50**: 1342-53.
63. Watanabe T, Komuro Y, Kiyomatsu T, Kanazawa T, Kazama Y, Tanaka J, et al. Prediction of sensitivity of rectal cancer cells in response to preoperative radiotherapy by DNA microarray analysis of gene expression profiles. *Cancer research* 2006; **66**: 3370-4.
64. Watanabe T, Kobunai T, Akiyoshi T, Matsuda K, Ishihara S, Nozawa K. Prediction of response to preoperative chemoradiotherapy in rectal cancer by using reverse transcriptase polymerase chain reaction analysis of four genes. *Diseases of the Colon & Rectum* 2014; **57**: 23-31.
65. Nishioka M, Shimada M, Kurita N, Iwata T, Morimoto S, Yoshikawa K, et al. Gene expression profile can predict pathological response to preoperative chemoradiotherapy in rectal cancer. *Cancer genomics & proteomics* 2011; **8**: 87-92.
66. Rimkus C, Friederichs J, Boulesteix AL, Theisen J, Mages J, Becker K, et al. Microarray-based prediction of tumor response to neoadjuvant radiochemotherapy of patients with locally advanced rectal cancer. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2008; **6**: 53-61.
67. Della Vittoria Scarpati G, Falcetta F, Carlomagno C, Ubezio P, Marchini S, De Stefano A, et al. A specific miRNA signature correlates with complete pathological response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 2012; **83**: 1113-9.
68. Ma W, Yu J, Qi X, Liang L, Zhang Y, Ding Y, et al. Radiation-induced microRNA-622 causes radioresistance in colorectal cancer cells by down-regulating Rb. *Oncotarget* 2015
69. Kheirleiseid EA, Miller N, Chang KH, Curran C, Hennessey E, Sheehan M, et al. miRNA expressions in rectal cancer as predictors of response to

- neoadjuvant chemoradiation therapy. *Int J Colorectal Dis* 2013; **28**: 247-60.
70. Lopes-Ramos CM, Habr-Gama A, Quevedo Bde S, Felicio NM, Bettoni F, Koyama FC, et al. Overexpression of miR-21-5p as a predictive marker for complete tumor regression to neoadjuvant chemoradiotherapy in rectal cancer patients. *BMC medical genomics* 2014; **7**: 68.
71. Grimminger PP, Danenberg P, Dellas K, Arnold D, Rodel C, Machiels JP, et al. Biomarkers for cetuximab-based neoadjuvant radiochemotherapy in locally advanced rectal cancer. *Clin Cancer Res* 2011; **17**: 3469-77.
72. Garcia-Aguilar J, Chen Z, Smith DD, Li W, Madoff RD, Cataldo P, et al. Identification of a biomarker profile associated with resistance to neoadjuvant chemoradiation therapy in rectal cancer. *Ann Surg* 2011; **254**: 486-92; discussion 92-3.
73. Duldulao MP, Lee W, Nelson RA, Li W, Chen Z, Kim J, et al. Mutations in Specific Codons of the KRAS Oncogene are Associated with Variable Resistance to Neoadjuvant Chemoradiation Therapy in Patients with Rectal Adenocarcinoma. *Ann Surg Oncol* 2013
74. Luna-Perez P, Segura J, Alvarado I, Labastida S, Santiago-Payan H, Quintero A. Specific c-K-ras gene mutations as a tumor-response marker in locally advanced rectal cancer treated with preoperative chemoradiotherapy. *Ann Surg Oncol* 2000; **7**: 727-31.
75. Hu-Lieskovan S, Vallbohmer D, Zhang W, Yang D, Pohl A, Labonte MJ, et al. EGF61 polymorphism predicts complete pathologic response to cetuximab-based chemoradiation independent of KRAS status in locally advanced rectal cancer patients. *Clin Cancer Res* 2011; **17**: 5161-9.
76. Davies JM, Trembath D, Deal AM, Funkhouser WK, Calvo BF, Finnegan T, et al. Phospho-ERK and AKT status, but not KRAS mutation status, are associated with outcomes in rectal cancer treated with chemoradiotherapy. *Radiat Oncol* 2011; **6**: 114.
77. Zauber NP, Marotta SP, Berman E, Grann A, Rao M, Komati N, et al. Molecular genetic changes associated with colorectal carcinogenesis are not prognostic for tumor regression following preoperative

- chemoradiation of rectal carcinoma. *Int J Radiat Oncol Biol Phys* 2009; **74**: 472-6.
78. Bengala C, Bettelli S, Bertolini F, Sartori G, Fontana A, Malavasi N, et al. Prognostic role of EGFR gene copy number and KRAS mutation in patients with locally advanced rectal cancer treated with preoperative chemoradiotherapy. *Br J Cancer* 2010; **103**: 1019-24.
79. Gaedcke J, Grade M, Jung K, Schirmer M, Jo P, Obermeyer C, et al. KRAS and BRAF mutations in patients with rectal cancer treated with preoperative chemoradiotherapy. *Radiother Oncol* 2010; **94**: 76-81.
80. Folkvord S, Flatmark K, Dueland S, de Wijn R, Groholt KK, Hole KH, et al. Prediction of response to preoperative chemoradiotherapy in rectal cancer by multiplex kinase activity profiling. *Int J Radiat Oncol Biol Phys* 2010; **78**: 555-62.
81. Spitz FR, Giacco GG, Hess K, Larry L, Rich TA, Janjan N, et al. p53 immunohistochemical staining predicts residual disease after chemoradiation in patients with high-risk rectal cancer. *Clin Cancer Res* 1997; **3**: 1685-90.
82. Rebischung C, Gerard JP, Gayet J, Thomas G, Hamelin R, Laurent-Puig P. Prognostic value of P53 mutations in rectal carcinoma. *Int J Cancer* 2002; **100**: 131-5.
83. Kandoler D, Zwrtek R, Ludwig C, Janschek E, Ploner M, Hofbauer F, et al. TP53 genotype but not p53 immunohistochemical result predicts response to preoperative short-term radiotherapy in rectal cancer. *Ann Surg* 2002; **235**: 493-8.
84. Chen Z, Duldulao MP, Li W, Lee W, Kim J, Garcia-Aguilar J. Molecular diagnosis of response to neoadjuvant chemoradiation therapy in patients with locally advanced rectal cancer. *J Am Coll Surg* 2011; **212**: 1008-17 e1.
85. Huh JW, Lee JH, Kim HR. Pretreatment expression of 13 molecular markers as a predictor of tumor responses after neoadjuvant chemoradiation in rectal cancer. *Annals of Surgery* 2014; **259**: 508-15.
86. Charara M, Edmonston TB, Burkholder S, Walters R, Anne P, Mitchell E, et al. Microsatellite status and cell cycle associated markers in rectal cancer

- patients undergoing a combined regimen of 5-FU and CPT-11 chemotherapy and radiotherapy. *Anticancer Res* 2004; **24**: 3161-7.
87. Luna-Perez P, Arriola EL, Cuadra Y, Alvarado I, Quintero A. p53 protein overexpression and response to induction chemoradiation therapy in patients with locally advanced rectal adenocarcinoma. *Ann Surg Oncol* 1998; **5**: 203-8.
 88. Kelley ST, Coppola D, Yeatman T, Marcet J. Tumor response to neoadjuvant chemoradiation therapy for rectal adenocarcinoma is mediated by p53-dependent and caspase 8-dependent apoptotic pathways. *Clin Colorectal Cancer* 2005; **5**: 114-8.
 89. Diez M, Ramos P, Medrano MJ, Muguerza JM, Villeta R, Lozano O, et al. Preoperatively irradiated rectal carcinoma: analysis of the histopathologic response and predictive value of proliferating cell nuclear antigen immunostaining. *Oncology* 2003; **64**: 213-9.
 90. Kudrimoti M, Lee EY, Kang Y, Ahmed M, Mohiuddin M. Genetic markers predictive of response to induction chemoradiotherapy for locally advanced rectal cancers. *The Journal of the Kentucky Medical Association* 2007; **105**: 18-22.
 91. Brophy S, Sheehan KM, McNamara DA, Deasy J, Bouchier-Hayes DJ, Kay EW. GLUT-1 expression and response to chemoradiotherapy in rectal cancer. *Int J Cancer* 2009; **125**: 2778-82.
 92. Bertolini F, Bengala C, Losi L, Pagano M, Iachetta F, Dealis C, et al. Prognostic and predictive value of baseline and posttreatment molecular marker expression in locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2007; **68**: 1455-61.
 93. Motlagh A, Azadeh P, Fazlalizadeh A, Shahrada B, Shafaghi B, Fudazi M, et al. Expression of epidermal growth factor receptor as a predictive factor for rectal cancer. *Archives of Iranian medicine* 2007; **10**: 301-8.
 94. Kim JS, Kim JM, Li S, Yoon WH, Song KS, Kim KH, et al. Epidermal growth factor receptor as a predictor of tumor downstaging in locally advanced rectal cancer patients treated with preoperative chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2006; **66**: 195-200.

95. Bengala C, Bettelli S, Bertolini F, Sartori G, Fontana A, Malavasi N, et al. Prognostic role of EGFR gene copy number and KRAS mutation in patients with locally advanced rectal cancer treated with preoperative chemoradiotherapy. *British Journal of Cancer* 2010; **103**: 1019-24.
96. Carlomagno C, Pepe S, D'Armiento FP, D'Armiento M, Cannella L, De Stefano A, et al. Predictive factors of complete response to neoadjuvant chemoradiotherapy in patients with rectal cancer. *Oncology* 2010; **78**: 369-75.
97. Suzuki T, Sadahiro S, Tanaka A, Okada K, Kamata H, Kamijo A, et al. Biopsy specimens obtained 7 days after starting chemoradiotherapy (CRT) provide reliable predictors of response to CRT for rectal cancer. *Int J Radiat Oncol Biol Phys* 2013; **85**: 1232-8.
98. Chiorean EG, Sanghani S, Schiel MA, Yu M, Burns M, Tong Y, et al. Phase II and gene expression analysis trial of neoadjuvant capecitabine plus irinotecan followed by capecitabine-based chemoradiotherapy for locally advanced rectal cancer: Hoosier Oncology Group GI03-53. *Cancer Chemother Pharmacol* 2012; **70**: 25-32.
99. Unsal Kilic D, Uner A, Akyurek N, Erpolat P, Dursun A, Pak Y. Matrix metalloproteinase-9 expression correlated with tumor response in patients with locally advanced rectal cancer undergoing preoperative chemoradiotherapy. *Int J Radiat Oncol Biol Phys* 2007; **67**: 196-203.
100. Yu ZQ, Zhang C, Wang H, Lao XY, Chai R, Gao XH, et al. Downregulation of ATP-binding cassette subfamily C member 4 increases sensitivity to neoadjuvant radiotherapy for locally advanced rectal carcinoma. *Diseases of the Colon & Rectum* 2013; **56**: 600-8.
101. Hur H, Kim NK, Min BS, Baik SH, Lee KY, Koom WS, et al. Can a biomarker-based scoring system predict pathologic complete response after preoperative chemoradiotherapy for rectal cancer? *Diseases of the Colon & Rectum* 2014; **57**: 592-601.
102. Chen Z, Liu Z, Li W, Qu K, Deng X, Varma MG, et al. Chromosomal copy number alterations are associated with tumor response to chemoradiation in locally advanced rectal cancer. *Genes, chromosomes & cancer* 2011; **50**: 689-99.

103. Spindler KL, Nielsen JN, Lindebjerg J, Jakobsen A. Germline polymorphisms may act as predictors of response to preoperative chemoradiation in locally advanced T3 rectal tumors. *Dis Colon Rectum* 2007; **50**: 1363-9.
104. Hur H, Kang J, Kim NK, Min BS, Lee KY, Shin SJ, et al. Thymidylate synthase gene polymorphism affects the response to preoperative 5-fluorouracil chemoradiation therapy in patients with rectal cancer. *Int J Radiat Oncol Biol Phys* 2011; **81**: 669-76.
105. Scott N, Hale A, Deakin M, Hand P, Adab FA, Hall C, et al. A histopathological assessment of the response of rectal adenocarcinoma to combination chemo-radiotherapy: relationship to apoptotic activity, p53 and bcl-2 expression. *Eur J Surg Oncol* 1998; **24**: 169-73.
106. Rodel C, Grabenbauer GG, Papadopoulos T, Bigalke M, Gunther K, Schick C, et al. Apoptosis as a cellular predictor for histopathologic response to neoadjuvant radiochemotherapy in patients with rectal cancer. *Int J Radiat Oncol Biol Phys* 2002; **52**: 294-303.
107. McDowell DT, Smith FM, Reynolds JV, Maher SG, Adida C, Crotty P, et al. Increased spontaneous apoptosis, but not survivin expression, is associated with histomorphologic response to neoadjuvant chemoradiation in rectal cancer. *Int J Colorectal Dis* 2009; **24**: 1261-9.
108. Tannapfel A, Nusslein S, Fietkau R, Katalinic A, Kockerling F, Wittekind C. Apoptosis, proliferation, bax, bcl-2 and p53 status prior to and after preoperative radiochemotherapy for locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 1998; **41**: 585-91.
109. Kalady MF, de Campos-Lobato LF, Stocchi L, Geisler DP, Dietz D, Lavery IC, et al. Predictive factors of pathologic complete response after neoadjuvant chemoradiation for rectal cancer. *Ann Surg* 2009; **250**: 582-9.
110. Lambrecht M, Vandecaveye V, De Keyser F, Roels S, Penninckx F, Van Cutsem E, et al. Value of diffusion-weighted magnetic resonance imaging for prediction and early assessment of response to neoadjuvant radiochemotherapy in rectal cancer: preliminary results. *Int J Radiat Oncol Biol Phys* 2012; **82**: 863-70.

111. Sun YS, Zhang XP, Tang L, Ji JF, Gu J, Cai Y, et al. Locally advanced rectal carcinoma treated with preoperative chemotherapy and radiation therapy: preliminary analysis of diffusion-weighted MR imaging for early detection of tumor histopathologic downstaging. *Radiology* 2010; **254**: 170-8.
112. Erben P, Strobel P, Horisberger K, Popa J, Bohn B, Hanfstein B, et al. KRAS and BRAF mutations and PTEN expression do not predict efficacy of cetuximab-based chemoradiotherapy in locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 2011; **81**: 1032-8.
113. Suzuki T, Sadahiro S, Tanaka A, Okada K, Kamata H, Kamijo A, et al. Biopsy specimens obtained 7 days after starting chemoradiotherapy (CRT) provide reliable predictors of response to CRT for rectal cancer. *International Journal of Radiation Oncology, Biology, Physics* 2013; **85**: 1232-8.

Table 1: Carcino-embryonic antigen (CEA) and the prediction of pathological complete response (pCR)

First Author	Year	n	CEA	p value
Wang[33]	2014	240	≤5	not significant
Huh[23]	2013	391	≤5	0.002
Yeo[31]	2013	609	≤5	na
Yang[32]	2013	138	≤6	0.152
Wallin[35]	2013	267	≤3.4	0.008
Restivo[34]	2013	260	≤5	0.001
Hur[25]	2011	37	≤3	0.54
Moureau-Zabbotto[27]	2011	168	≤5	0.019
Kang[28]	2010	84	≤3	0.01

Kalady [109]	2009	242	≤ 2.5	0.19
Lee[30]	2009	490	≤ 5	0.004
Moreno Garcia[26]	2009	148	≤ 2.5	0.05
Yoon[29]	2007	351	≤ 5	0.004

CEA, Carcinoembryonic antigen

Table 2: Prediction of **pathological complete response** (pCR) with magnetic resonance imaging (MRI)

First Author	Year	n	MRI	MRI parameter	Predictive of:	Sens	Spec	p
Kluza[40]	2012	39	Standard	SI	pCR			NS
Devries[41]	2001	17	Contrast	PI <12.6	Response			0.001
Devries[42]	2003	34	Contrast	PI _{mean} 7.5	Response			0.001
Barbaro[48]	2012	62	DWI	ADC low	Poor response			0.001
					pCR			0.052
				ADC <1	Poor response	84%	78%	
Elmi[49]	2013	49	DWI	ADC _{Median}	pCR			0.052
				ADC high	Response			0.035
				ADC <0.88	Response	75%	48%	
Curvo- semedo[39]	2011	50	Standard	Volume	pCR			0.46
			DWI	Volume	pCR			0.16
				Mean ADC	pCR			0.61
Lambrect[110]	2012	22	DWI	ADC1	pCR			0.003
				ADC<1.06		100%	86%	

SI, Signal Intensity; PI, perfusion index; NS, not statistically significant; Sens, Sensitivity; Spec, Specificity

Table 3: Microarray analysis of gene signature and **pathological complete response** (pCR).

First Author	Year	n	Gene signature	Accuracy	Sens ^b	Spec ^c	Outcome measure
Ghahimi[60]	2005	30	54 genes		78%	83%	Response
Rimkus[66]	2008	43	42 genes		71%	86%	Response
Kim[62]	2007	31	95 genes	84%			pCR
		15	95 genes	87%			pCR
Brettingham-Moore[59]	2011	51	No genes identified	na ^a	na	na	pCR
Watanabe[63]	2006	35	33 genes	88.6%	71.4%	92.9%	Response
Nishioka[65]	2011	20	17 genes	na	na	na	Response
Watanabe[64]	2014	46	4 genes	89.1%	87.5%	90.9%	Response
		16	4 genes	81.3%	100%	62.5%	Response

^ana, Not available; ^bSens, Sensitivity; ^cSpec, Specificity

Table 4: Micro RNA expression and prediction of **pathological complete response** (pCR).

First Author	Year	n	miRNA Signature
Della Vittoria Scarpati[67]	2012	38	13 miRNA
Kheirleaid[69]	2013	12	3 miRNA

Lopes-Ramos[70]	2014	43	4 miRNA
------------------------	------	----	---------

miRNA, MicroRNA.

Author Manuscript

Table 5: KRAS mutation status and prediction of **pathological complete response** (pCR).

First author	Year	n	Prevalence of KRAS mt in study n (%)	Prevalence of KRAS mt in pCR n(%)	Prevalence of KRAS mt in Non-pCR n(%)	p value	nCRT regimen
Duldulao[73]	2013	148	60(40.5)	8(21.6)	52(46.8)	0.006	5-FU/ FOLFOX
Sun[111]	2012	63	19(30.1)	2(37.5)	17(30.9)	1.0	5-FU/cetuximab
Garcia-Aguilar[72]	2011	132	57(43.2)	8(24.2)	49(49.5)	0.0145	5-FU/ FOLFOX
Grimminger [71]	2011	101	42(41.6)	NR	NR	NR	5-FU/ cetuximab
Chen[84]	2011	96	36(37.5)	5(19.2)	31(44.3)	NR	5-FU/FOLFOX
Hu-Lieskovan	2011	101	42(46.1)	5(14.7)	37(55.2)	0.214	5-FU/cetuximab
Erben[112]	2011	57	18(35.6)	2(3.33)	16(31.4)	NS	Folfiri/cetuximab
Davies[76]	2011	67	24(35.8)	3(27.3)	21(37.5)	0.7	
Gaedcke[79]	2010	94	45(47.9)	1	48	0.22	5-FU/FOLFOX
Bengala[78]	2010	146	28(19.2)	5(20.8)	23(19.8)	0.18	
Luna Perez[74]	2000	37	12 (32.4)	2(100)	10(28.6)	NR	5-FU

^aNS, not significant; ^bNR, not reported; 5-FU, 5 Fluorouracil; FOLFOX, 5 Fluorouracil, Oxaliplatin; Folfiri, 5 Fluorouracil, Irinotecan.

Table 6: p53 mutation status and prediction of **pathological complete response** (pCR)

First Author	Year	n	Prevalence of mt p53 in pCR (%)	Prevalence of mt p53 in Non- pCR (%)	p value	
IHC^c						
Chang[58]	2005	130	79	63	NS ^a	^a NS,
Kudrimoti[90]	2007	17	80	75	NS	not
Brophy[91]	2009	69	50	49	NR ^b	signif
Kelley[88]	2005	50	47	71	NR	icant;
Charara[86]	2005	47	31	50	NR	^b NR,
Diez[89]	2003	73	67	76	0.4	not
Luna-Perez[74]	1998	26	0	36	NR	repor
Spitz[81]	1997	42	23	69	0.005	ted; ^c
Suzuki[113]	2013	101	13.9	12.3	1.0	p53
Bertolini[92]	2007	91	NR	NR	NS	mutat
Hur[101]	2014	81	55.6	31.5	0.03	ion
RT-PCR^d						
Kandioler[83]	2002	64	0	45	NS	detec
Chen[84]	2011	96	27	64	0.19	ted
Rebischung	2002	86	20	55	0.01	by
Huh[85]	2014	123	45	40	0.695	immu
						nohis
						toche

mistry; ^dp53 mutation detected by reverse transcriptase polymerase chain reaction.

Table 7: Protein expression and prediction of **pathological complete response** (pCR)

First Author	Year	Prevalence of protein in pCR (%)	Prevalence of protein in Non- pCR(%)	p value
EGFR^e				
Motlagh[93] ^b	2007	87.5	33.3	NR
Carlomagno[96] ^b	2010	88.9	38.2	0.007
Grimminger[71] ^c	2011	NR	NR	0.122
Kim[94] ^b	2006	67	33	0.57
Bertolini[92] ^c	2007	NR	NR	NR
Bengala[78] ^d	2010	71.4	80	NR
VEGF				
Carlomagno[96] ^b	2010	22.2	55.9	0.07
Grimminger[71] ^c	2011	NR	NR	0.122
Hur[101] ^b	2014	77.8	53.7	0.03
TS				
Carlomagno[96] ^b	2010	88.9	32.4	0.002
Grimminger[71] ^c	2011	NR ^a	NR	NS ^f
Huh[85] ^c	2014	40	50.9	0.428
p21				
Chang[58] ^b	2005	43	37	NS
Kudrimoti[90] ^b	2007	60	67	NS
Hur[101] ^b	2014	66.7	38.9	0.01
Suzuki[113] ^b	2013	19.7	NR	0.03
Bcl-2				
Kudrimoti[90] ^b	2007	60	16	NS
Chang[58] ^b	2005	36	47	NS
Charara[86] ^b	2004	NR	NR	0.12
Bax				

Chang[58]^b	2005	54	29	0.017
Huh[85]^c	2014	40	46.7	0.745
Ki67				
Chang[58]^b	2005	53	54	NS
Carlomagno[96]^b	2010	77.8	41.2	0.05
Kudrimoti[90]^b	2007	100	100	NS
Hur[101]^b	2014	70	42.6	0.001
Suzuki[113]^b	2013	9.7	NR	0.37
MMP 2				
Unsal Kilic[99]^b	2006	NR	NR	NS
Huh[85]^c	2014	60	49.1	0.428
MMP 9				
Unsal Kilic[99]^b	2006	25	52	0.001
Huh[85]^c	2014	53.3	50.0	0.809
MLH1				
Bertolini[92]^b	2007	24.3	9.3	0.055
ABCC4				
Yu[100]^b	2013	57.6	71	0.001
TP				
Huh[85]^c	2014	56.3	45.4	0.562
Chiorean[98]^c	2012	NR	NR	0.03

^aNS, not significant; ^bprotein detection by immunohistochemistry; ^cprotein detection by reverse transcriptase polymerase chain reaction; ^dfluorescence *in situ* hybridization; ^elow expression; ^fNR, not reported.

Figure 1: Flow diagram showing selection of articles for review.

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Ryan, JE;Warrier, SK;Lynch, AC;Ramsay, RG;Phillips, WA;Heriot, AG

Title:

Predicting pathological complete response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer: a systematic review

Date:

2016-03-01

Citation:

Ryan, J. E., Warrier, S. K., Lynch, A. C., Ramsay, R. G., Phillips, W. A. & Heriot, A. G. (2016). Predicting pathological complete response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer: a systematic review. COLORECTAL DISEASE, 18 (3), pp.234-246. <https://doi.org/10.1111/codi.13207>.

Persistent Link:

<http://hdl.handle.net/11343/290999>