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

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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 though 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 $\leq 1.06 \times 10^3$ mm^2/sec was 100% sensitive and 86% specific in predicting pCR [38].
Barbaro et al, however, found that a low pretreatment ADC $<1 \times 10^3$ mm^2/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.

^{18}F-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.

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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%	
				ADC_{Median}	pCR			0.052
Elmi[49]	2013	49	DWI	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

[a]na, Not available; [b]Sens, Sensitivity; [c]Spec, 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
Kheirelseid[69]	2013	12	3 miRNA

Lopes-Ramos[70]	2014	43	4 miRNA

miRNA, MicroRNA.

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)	3144.3)	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

[a]NS, not significant; [b]NR, 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]
Kudrimoti[90]	2007	17	80	75	NS
Brophy[91]	2009	69	50	49	NR[b]
Kelley[88]	2005	50	47	71	NR
Charara[86]	2005	47	31	50	NR
Diez[89]	2003	73	67	76	0.4
Luna-Perez[74]	1998	26	0	36	NR
Spitz[81]	1997	42	23	69	0.005
Suzuki[113]	2013	101	13.9	12.3	1.0
Bertolini[92]	2007	91	NR	NR	NS
Hur[101]	2014	81	55.6	31.5	0.03
RT-PCR[d]					
Kandioler[83]	2002	64	0	45	NS
Chen[84]	2011	96	27	64	0.19
Rebischung	2002	86	20	55	0.01
Huh[85]	2014	123	45	40	0.695

[a]NS, not significant; [b]NR, not reported; [c] p53 mutation detected by immunohistochemistry; [d]p53 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

[a]NS, not significant; [b]protein detection by immunohistochemistry; [c]protein detection by reverse transcriptase polymerase chain reaction; [d]fluorescence *in situ* hybridization; [e]low expression; [f]NR, not reported.

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