Systematic review

Meta-analysis of the molecular associations of mucinous colorectal cancer

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Background: **Mucinous differentiation occurs in 5–15 per cent of colorectal adenocarcinomas. This subtype of colorectal cancer responds poorly to chemoradiotherapy and has a worse prognosis. The genetic aetiology underpinning this cancer subtype lacks consensus. The aim of this study was to use meta-analytical techniques to clarify the molecular associations of mucinous colorectal cancer.**

Methods: **This study adhered to MOOSE guidelines. Databases were searched for studies comparing *KRAS*, *BRAF*, microsatellite instability (MSI), CpG island methylator phenotype (CIMP), *p53* and *p27* status between patients with mucinous and non-mucinous colorectal adenocarcinoma. A random-effects model was used for analysis.**

Results: **Data from 46 studies describing 17 746 patients were included. Mucinous colorectal adenocarcinoma was associated positively with *KRAS* (odds ratio (OR) 1·46, 95 per cent c.i. 1·08 to 2·00, $P = 0·014$) and *BRAF* (OR 3·49, 2·50 to 4·87; $P < 0·001$) mutation, MSI (OR 3·98, 3·30 to 4·79; $P < 0·001$) and CIMP (OR 3·56, 2·85 to 4·43; $P < 0·001$), and negatively with altered *p53* expression (OR 0·46, 0·31 to 0·67; $P < 0·001$).**

Conclusion: **The genetic origins of mucinous colorectal adenocarcinoma are predominantly associated with *BRAF*, MSI and CIMP pathways. This pattern of molecular alterations may in part explain the resistance to standard chemotherapy regimens seen in mucinous adenocarcinoma.**

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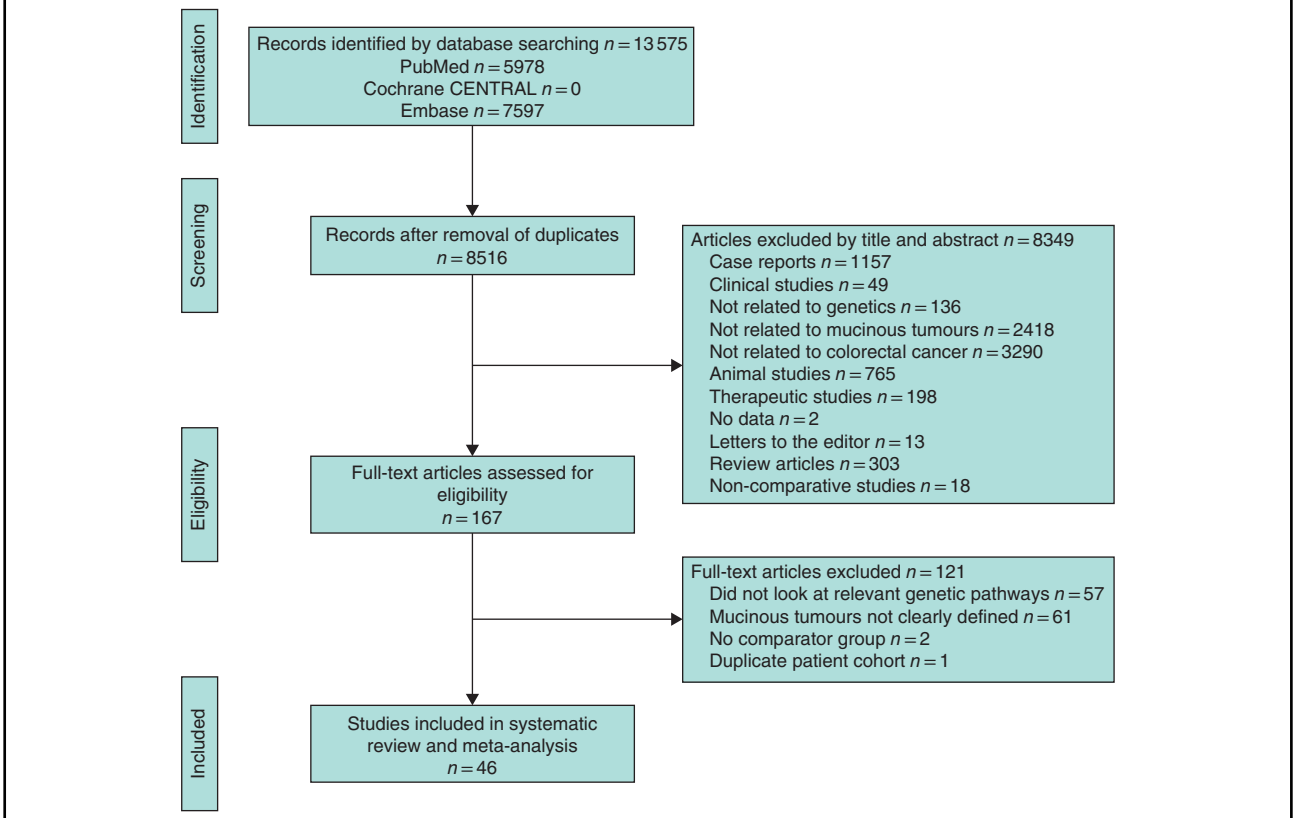
Introduction

Colorectal cancer is a common malignancy[1–3]. Of all colonic and rectal tumours, 5–15 per cent have mucinous differentiation[4]. A mucinous tumour is defined as a tumour in which more than 50 per cent of the lesion is composed of pools of extracellular mucin[5]. Patients with mucinous tumours of the rectum have reduced rates of pathological complete response and tumour downstaging after neoadjuvant chemoradiotherapy compared with patients who have non-mucinous tumours. Mucinous tumours are also associated with worse survival. After rectal excision, higher involved margin rates are seen in patients with mucinous tumours[6]. Mucinous colonic cancers are associated with increased risk of metastasis, poorer survival, and resistance to oxaliplatin- and irinotecan-based chemotherapy[7]. The mechanism of resistance to chemoradiotherapy in mucinous tumours of the rectum is unknown. Resistance may arise from alternative genetic mutations compared with those of non-mucinous adenocarcinoma.

Several studies[8–10] have tried to determine the genetic aetiology of mucinous adenocarcinoma of the colon and rectum, with heterogeneous outcomes. Mutations in Kirsten rat sarcoma viral oncogene (*KRAS*) and v-Raf murine sarcoma viral oncogene homologue B (*BRAF*), inherited defects or epigenetic silencing of mismatch repair (MMR) proteins resulting in microsatellite instability (MSI), and the presence of the CpG island methylator phenotype (CIMP) are the most commonly studied genetic aberrations[11–14]. The presence or absence of each of these genetic markers may have therapeutic and/or prognostic implications for patients with colorectal cancer. Currently those with *KRAS* wild-type metastatic disease can be offered treatment with an epidermal growth factor receptor (EGFR) inhibitor such as cetuximab. Those with the *BRAF* v600e mutation may respond to treatment with vemurafenib. Nivolumab, an antiprogrammed cell death 1 monoclonal antibody, can be used for the treatment of MSI – high (MSI-H) or MMR-deficient unresectable

Fig. 1 Flow diagram showing selection of articles for review



or metastatic colorectal cancers that have progressed following treatment with a fluoropyrimidine, oxaliplatin and irinotecan[15].

This aim of this meta-analysis was to pool the available data on the molecular characteristics of mucinous adenocarcinoma of the colon and rectum. To address this, a systematic review and meta-analysis of all studies comparing *KRAS*, *BRAF*, MSI, CIMP, *p53* and *p27* status between mucinous and non-mucinous colorectal adenocarcinoma was undertaken.

Table 1 Frequency of molecular alterations

Molecular marker	Frequency (%)	
	Mucinous	Non-mucinous
KRAS mutation	41·6 (26·9–72·7)	33·0 (23·6–57·6)
BRAF mutation	29·1 (7·7–46·2)	9·8 (0–20·0)
MSI – high	33·3 (0–63·6)	10·6 (0–37·5)
CIMP – high	36·4 (33·3–41·4)	13·6 (11·1–17·6)
p27 alteration	43·2 (30·0–58·3)	51·3 (18·8–87·5)
p53 alteration	28·4 (0–75·7)	51·1 (32·2–80·1)

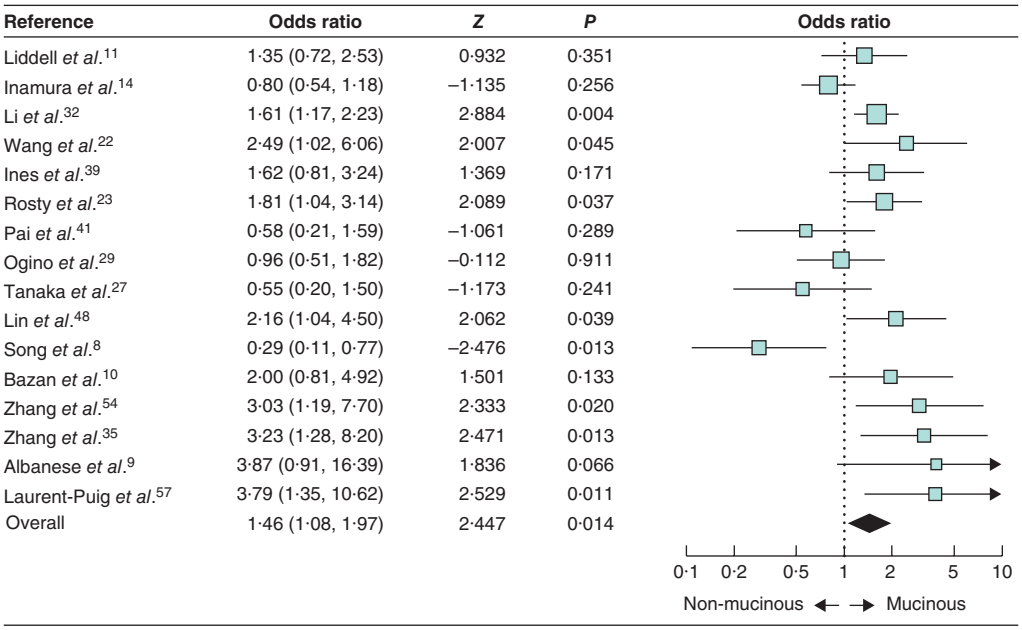
Values are median (range). MSI, microsatellite instability; CIMP, CpG island methylator phenotype.

Methods

Literature search and study selection

This systematic review and meta-analysis adhered to the recommendations of the MOOSE statement[16]. A systematic search of PubMed, Embase and the Cochrane CENTRAL Register of Controlled Trials was performed for all published studies that compared *KRAS*, *BRAF*, MSI, CIMP, *p53* and *p27* status between patients with non-mucinous adenocarcinoma and those with mucinous adenocarcinoma of the colon and rectum. The following search terms were used in the search algorithm: (Mucinous OR Mucin) AND (Colon OR Rectal OR Colorectal). The latest search was performed on 17 July 2017. Two authors examined the title and abstract of each citation independently, and the full texts of potentially eligible

Fig. 2 Forest plot for *KRAS*



Odds ratios are shown with 95 per cent confidence intervals. The analysis included 4975 patients ($P = 0·014$; Cochran Q 41·9, 15 d.f., $P < 0·001$; $I^2 = 64·2$ per cent).

studies were obtained; disagreements were resolved by discussion or if needed, by a third author. The reference lists of all retrieved articles were further screened for additional eligible publications.

Eligibility criteria

Comparative studies of mucinous and non-mucinous adenocarcinoma of the colon and rectum containing data on *KRAS*, *BRAF*, MSI, CIMP, *p53* and *p27* status were eligible for inclusion. Any studies looking at the status of the above markers in mucinous adenocarcinoma only or with no comparative data were excluded, as were studies that did not correctly define mucinous adenocarcinoma according to the WHO definition. Studies in which tumours with mucinous components of less than 50 per cent or where signet ring cell tumours were analysed with the mucinous adenocarcinoma group were also excluded. There were no language restrictions.

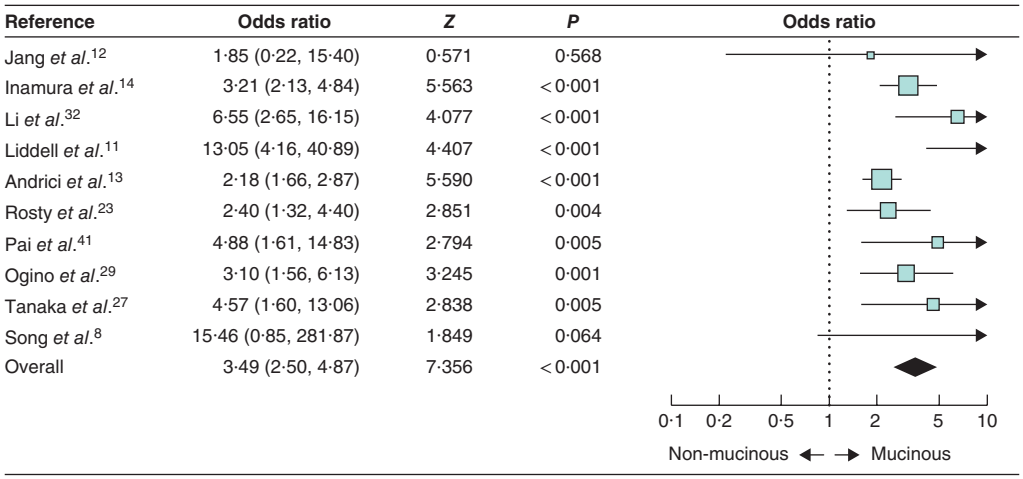
Data extraction and outcomes

The following information regarding each eligible study was recorded: authors, journal, year of publication, country/countries in which the study was undertaken, the method of identification of mucinous adenocarcinoma, numbers of patients with mucinous and non-mucinous adenocarcinoma of the colon or rectum, and *KRAS*, *BRAF*, MSI, CIMP, *p53* and *p27* status.

Statistical analysis

All pooled outcome measures were determined using a random-effects model as described by DerSimonian and Laird[17], and the odds ratio (OR) was estimated with its variance and 95 per cent confidence interval. The random-effects analysis weighted the natural logarithm of each study's OR by the inverse of its variance plus an estimate of the between-study variance in the presence of between-study heterogeneity. Heterogeneity between ORs for the same outcome between different studies was assessed as described previously[18]. This was done by use of the I^2 inconsistency test and χ^2-based Cochran Q statistic test, in which $P < 0·050$ indicates the presence of significant heterogeneity[19]. Ninety-five per cent prediction intervals (PI) were also calculated[20]. Analyses were conducted using Comprehensive Meta-Analysis version 2 (Biostat, Englewood, New Jersey, USA). The quality of included studies was assessed using the Newcastle–Ottawa Scale[21]. The quality of studies was evaluated by examining

Fig. 3 Forest plot for *BRAF*



Odds ratios are shown with 95 per cent confidence intervals. The analysis included 6608 patients ($P < 0·001$; Cochran Q 17·6, 9 d.f., $P = 0·040$; $I^2 = 48·8$ per cent).

three items: patient selection, comparability of the two study groups and assessment of exposure (maximum score 9).

Results

Literature review

The initial search yielded 13 575 papers; this was reduced to 8516 after removal of duplicates. Some 8349 articles were excluded by title and abstract alone, leaving 167 papers for full-text review (*Fig. 1*), of which 121 articles were found ineligible. The remaining 46 articles[8–14,22–60] with information on 17 746 patients were deemed suitable for inclusion in the systematic review and meta-analysis. All included studies had Newcastle–Ottawa scores of between 6 and 9. Details of the papers included in the review are available in *Table S1* (supporting information). The median (range) frequency of molecular alterations for each marker is shown in *Table 1*.

KRAS status

Sixteen studies[8–11,14,22,23,27,29,32,35,39,41,48,54,57] with data on 4975 patients (807 mucinous, 4168 non-mucinous) were eligible for inclusion in the analysis of *KRAS* status. Three of the 16 studies carried out extended *RAS* testing *versus* exon 2 testing alone. Mucinous tumours were weakly associated with *KRAS* mutations (OR 1·46, 95 per cent c.i. 1·08 to 1·97; $P = 0·014$) (95 per cent PI 0·51 to 4·14) (*Fig. 2*). There was significant heterogeneity (Cochran Q, $P < 0·001$; $I^2 = 64·2$ per cent).

BRAF status

Ten studies[8,11–14,23,27,29,32,41] including data on 6608 patients (863 mucinous, 5745 non-mucinous) comparing *BRAF* mutation status were deemed eligible for inclusion in the meta-analysis. Mucinous tumours were associated positively with BRAF mutations (OR 3·49, 95 per cent c.i. 2·50 to 4·87; $P < 0·001$) (95 per cent PI 1·47 to 8·27) (*Fig. 3*). There was significant heterogeneity (Cochran Q, $P = 0·040$; $I^2 = 48·8$ per cent).

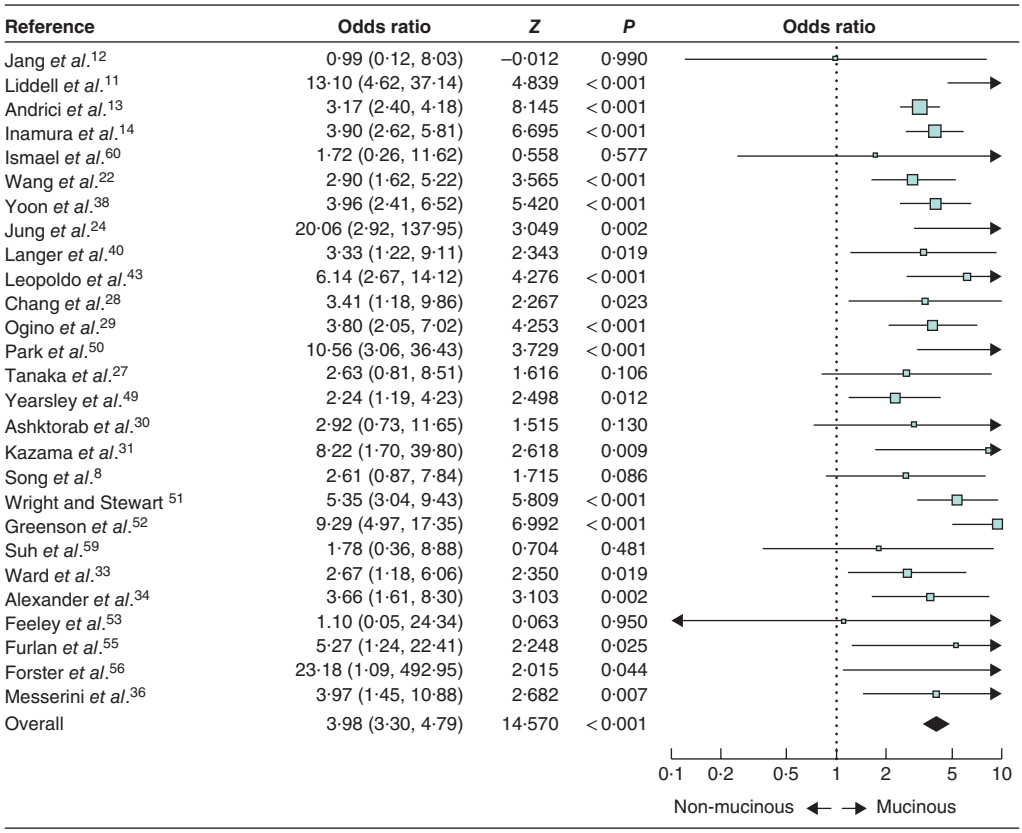
Microsatellite instability status

Twenty-seven studies[8,11–14,22,24,27–31,33,34,36,38,40,43,49–53,55,56,59,60] comparing MSI status, with data on 11 043 patients (1431 mucinous, 9612 non-mucinous), were included in the analysis. Mucinous tumours of the colon and rectum were significantly more likely to be associated with MSI (OR 3·98, 95 per cent c.i. 3·30 to 4·79; $P < 0·001$) (95 per cent PI 2·41 to 6·56) (*Fig. 4*). There was no significant heterogeneity (Cochran Q, $P = 0·121$; $I^2 = 24·8$ per cent).

CpG island methylator phenotype status

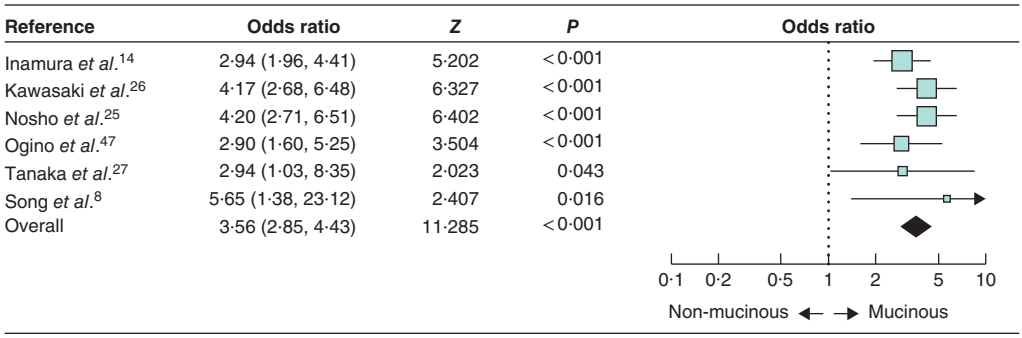
Six studies[8,14,25–27,47] comparing CIMP status, with 3433 patients (474 mucinous, 2959 non-mucinous) were included. Mucinous tumours were more likely to be

Fig. 4 Forest plot for microsatellite instability



Odds ratios are shown with 95 per cent confidence intervals. The analysis included 11 043 patients ($P < 0{\cdot}001$; Cochran Q 34·6, 26 d.f., $P = 0{\cdot}121$; $I^2 = 24{\cdot}8$ per cent).

Fig. 5 Forest plot for CpG island methylator phenotype

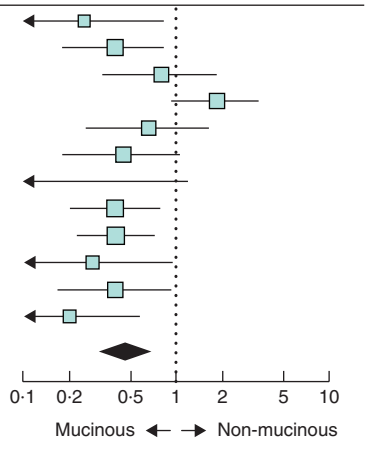


Odds ratios are shown with 95 per cent confidence intervals. The analysis included 3433 patients ($P < 0{\cdot}001$; Cochran Q 2·9, 5 d.f., $P = 0{\cdot}717$; $I^2 = 0$ per cent).

Fig. 6 Forest plots for *p53* and *p27*

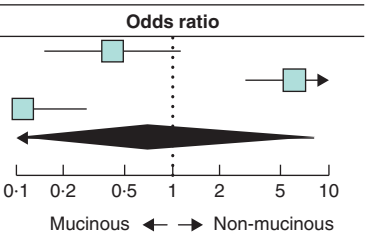
a *p53*

Reference	Odds ratio	*Z*	*P*
Wang *et al.*[22]	0·24 (0·07, 0·83)	–2·262	0·024
Ines *et al.*[39]	0·39 (0·18, 0·83)	–2·441	0·015
Lam *et al.*[42]	0·77 (0·33, 1·81)	–0·598	0·550
Leopoldo *et al.*[43]	1·77 (0·92, 3·41)	1·715	0·086
Lan *et al.*[46]	0·64 (0·26, 1·61)	–0·941	0·347
Tozawa *et al.*[44]	0·43 (0·18, 1·04)	–1·867	0·062
Georgescu *et al.*[58]	0·06 (0·00, 1·19)	–1·847	0·065
Ogino *et al.*[29]	0·40 (0·20, 0·78)	–2·660	0·008
Park *et al.*[50]	0·40 (0·22, 0·72)	–3·046	0·002
Song *et al.*[8]	0·28 (0·08, 0·94)	–2·068	0·039
Zhang *et al.*[54]	0·40 (0·17, 0·93)	–2·123	0·034
Hanski *et al.*[37]	0·20 (0·07, 0·58)	–2·974	0·003
Overall	0·46 (0·31, 0·67)	–3·984	< 0·001



b *p27*

Reference	Odds ratio	*Z*	*P*
Wang *et al.*[22]	0·41 (0·15, 1·11)	–1·750	0·080
Leopoldo *et al.*[43]	6·07 (2·94, 12·53)	4·872	0·000
Sarli *et al.*[45]	0·11 (0·04, 0·28)	–4·575	0·000
Overall	0·66 (0·05, 8·02)	–0·331	0·741



Odds ratios are shown with 95 per cent confidence intervals. **a** The analysis of *p53* included 2234 patients ($P < 0·001$; Cochran Q 24·5, 11 d.f., $P = 0·011$; $I^2 = 55·1$ per cent). **b** The analysis of *p27* included 442 patients ($P = 0·741$; Cochran Q 54·2, 2 d.f., $P < 0·001$; $I^2 = 95·8$ per cent).

associated with CIMP – high status (OR 3·56, 95 per cent c.i. 2·85 to 4·43; $P < 0·001$) (95 per cent PI 2·60 to 4·86) (*Fig. 5*). There was no significant heterogeneity (Cochran Q, $P = 0·717$, $I^2 = 0$ per cent).

p53 and *p27* status

Twelve studies[8,22,29,37,39,42–44,46,50,54,58] with 2234 patients (449 mucinous, 1785 non-mucinous) were included in the analysis of *p53* status. Mucinous tumours were less likely to be associated with altered *p53* expression (OR 0·46, 95 per cent c.i. 0·31 to 0·67; $P < 0·001$) (95 per cent PI 0·14 to 1·47) (*Fig. 6a*). There was significant heterogeneity (Cochran Q, $P = 0·011$; $I^2 = 55·1$ per cent).

Three studies[22,43,45], which included 442 patients (124 mucinous, 318 non-mucinous), were included in the analysis of *p27* status. Mucinous tumours were not associated with altered *p27* expression (OR 0·66, 95 per cent c.i. 0·05 to 8·02; $P = 0·741$) (*Fig. 6b*). There was significant heterogeneity (Cochran Q, $P < 0·001$, $I^2 = 95·8$ per cent).

Discussion

The present study found that mucinous colorectal adenocarcinoma was associated positively with *BRAF* mutation, MSI and CIMP, and negatively associated with altered *p53* expression. It was also weakly associated with *KRAS* mutation although there was significant statistical heterogeneity associated with this result.

Sporadic colorectal cancer is thought to develop through one of two distinct mechanisms. The first, chromosomal instability, results from loss of heterozygosity at multiple tumour suppressor loci. This accounts for 80–85 per cent of colorectal cancers. *p53* mutations are frequently found in tumours with chromosomal instability[61]. The second mechanism is MSI. These tumours have MMR deficiency,

resulting in an inability to repair single-nucleotide DNA mismatches. *MLH1* silencing is characteristic of sporadic MSI tumours[62]. This group of tumours frequently has associated *BRAF* mutations. Mucinous adenocarcinoma tends to demonstrate the characteristics of MSI tumours. Recently a third mechanism has been described, known as epigenetic instability. This pathway results in aberrant methylation and silencing of tumour suppressor genes[63]. The findings from the present meta-analysis show that mucinous tumours are more likely to display MMR deficiency, *BRAF* mutations and epigenetic instability as demonstrated by the association with the CIMP. This suggests that mucinous tumours develop and progress through different molecular pathways compared with sporadic colorectal cancers. Mucinous tumours appear to be right-sided more frequently, and are associated with higher tumour stage and histological grade[64–66], a feature shared with *BRAF*-associated colorectal cancer[67].

Guinney and colleagues[2] have described the consensus molecular subtypes (CMSs) of colorectal cancer, comprising four different types, each with distinguishing features. Based on the present findings, it appears that mucinous colorectal tumours would be classified as CMS1. This subtype is hypermutated, with MSI and has strong immune activation; there is frequent occurrence of *BRAF* mutations in the CMS1 group. Classifying tumours in this manner provides insight into prognosis, and it is known that CMS1 tumours tend to present with a higher histopathological grade and are associated with poor survival after relapse[2].

The mechanism underlying the poor response of mucinous colorectal cancers to traditional chemotherapy regimens is poorly understood, but the demonstrated association with MSI may in part provide an explanation. Retrospective studies have shown that the clinical behaviour of MSI-H tumours is different. Studies examining adjuvant chemotherapy for patients with stage III MSI-H tumours have shown that they do not benefit from regimens containing fluorouracil, unlike patients whose tumours demonstrate chromosomal instability[68]. In mucinous rectal cancer, the lack of response to chemoradiotherapy may be attributed to decreased sensitization of tumour cells to radiotherapy owing to the reduced efficacy of 5-fluorouracil in mucinous tumours that are MSI-H. The use of chemotherapy regimens containing irinotecan has shown promise in MSI-H tumours[62], and novel immunotherapy agents such as ipilimumab have already been shown to be beneficial[69]. The increased rate of *KRAS* mutations in patients with mucinous adenocarcinoma means that this group is less likely to benefit from EGFR inhibitors if they develop metastatic disease.

It is important to recognize the limitations of this meta-analysis, including the significant statistical heterogeneity found in the *KRAS*, *BRAF* and *p53* analyses. This heterogeneity may reflect methodological differences between studies included in the analysis, unknown study characteristics and publication bias. Also of note were the different methods used across studies to detect altered expression of *p53* and *p27*. These differences reflect real-life clinical practice, with different laboratories using different assays. With regard to the heterogeneity in the *BRAF* and *p53* analyses, it is noteworthy that the rate of *BRAF* mutations was higher in the mucinous groups in all included studies, and the rate of *p53* alterations was lower in the mucinous groups in all but one study. Heterogeneity may have been underestimated in the CIMP analysis, given the difficulties reported in determining and assigning CIMP status owing to the variety of markers currently used[70,71]. The majority of studies included in the analysis were retrospective, which may increase the risk of sampling bias and data collection bias. The results are reported as ORs; however, it is important to recognize that ORs can often overestimate the effect compared with relative risks[72].

The progression of mucinous colorectal cancer along alternative genetic pathways may account partly for the resistance to treatment and worse prognosis of mucinous adenocarcinomas. In-depth research into the genetics of mucinous adenocarcinoma may identify potential therapeutic targets.

Acknowledgements

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Disclosure: The authors declare no conflict of interest.

References

1 American Cancer Society. *Key Statistics for Colorectal Cancer American Cancer Society Website*; 2017. https://www.cancer.org/cancer/colon-rectal-cancer/about/key-statistics.html [accessed 13 May 2018].
2 Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C *et al.* The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015; **21**: 1350–1356.
3 Cancer Research UK. *Bowel Cancer Statistics*; 2018. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer#heading-Zero [accessed 13 May 2018].
4 Kang H, O'Connell JB, Maggard MA, Sack J, Ko CY. A 10-year outcomes evaluation of mucinous and signet-ring cell carcinoma of the colon and rectum. *Dis Colon Rectum* 2005; **48**: 1161–1168.

5 Hamilton SR, Aaltonen LA. In *World Health Organization Classification of Tumours*, Kleihues P, Sobin LH (eds). IARC Press: Lyon, 2000.
6 McCawley N, Clancy C, O'Neill BD, Deasy J, McNamara DA, Burke JP. Mucinous rectal adenocarcinoma is associated with a poor response to neoadjuvant chemoradiotherapy: a systematic review and meta-analysis. *Dis Colon Rectum* 2016; **59**: 1200–1208.
7 Debunne H, Ceelen W. Mucinous differentiation in colorectal cancer: molecular, histological and clinical aspects. *Acta Chir Belg* 2013; **113**: 385–390.
8 Song GA, Deng G, Bell I, Kakar S, Sleisenger MH, Kim YS. Mucinous carcinomas of the colorectum have distinct molecular genetic characteristics. *Int J Oncol* 2005; **26**: 745–750.
9 Albanese I, Rinaudo C, Alberti M, Bazan V, Russo A, Migliavacca M *et al. ras* and *myc* analysis in primary and metastatic colorectal carcinomas. *Int J Oncol* 1997; **10**: 293–300.
10 Bazan V, Migliavacca M, Zanna I, Tubiolo C, Grassi N, Latteri MA *et al.* Specific codon 13 *K-ras* mutations are predictive of clinical outcome in colorectal cancer patients, whereas codon 12 *K-ras* mutations are associated with mucinous histotype. *Ann Oncol* 2002; **13**: 1438–1446.
11 Liddell C, Droy-Dupré L, Métairie S, Airaud F, Volteau C, Bezieau S *et al.* Mapping clinicopathological entities within colorectal mucinous adenocarcinomas: a hierarchical clustering approach. *Mod Pathol* 2017; **30**: 1177–1189.
12 Jang MH, Kim S, Hwang DY, Kim WY, Lim SD, Kim WS *et al. BRAF*-mutated colorectal cancer exhibits distinct clinicopathological features from wild-type *BRAF*-expressing cancer independent of the microsatellite instability status. *J Korean Med Sci* 2017; **32**: 38–46.
13 Andrici J, Farzin M, Sioson L, Clarkson A, Watson N, Toon CW *et al.* Mismatch repair deficiency as a prognostic factor in mucinous colorectal cancer. *Mod Pathol* 2016; **29**: 266–274.
14 Inamura K, Yamauchi M, Nishihara R, Kim SA, Mima K, Sukawa Y *et al.* Prognostic significance and molecular features of signet-ring cell and mucinous components in colorectal carcinoma. *Ann Surg Oncol* 2015; **22**: 1226–1235.
15 Yan L, Zhang W. Precision medicine becomes reality – tumor type–agnostic therapy. *Cancer Commun (Lond)* 2018; **38**: 6.
16 Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D *et al.* Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008–2012.
17 DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177–188.
18 Clancy C, Burke JP, Coffey JC. *KRAS* mutation does not predict the efficacy of neo-adjuvant chemoradiotherapy in rectal cancer: a systematic review and meta-analysis. *Surg Oncol* 2013; **22**: 105–111.
19 Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. *Ann Intern Med* 1997; **127**: 820–826.
20 Borenstein M, Higgins JP, Hedges LV, Rothstein HR. Basics of meta-analysis: I^2 is not an absolute measure of heterogeneity. *Res Synth Methods* 2017; **8**: 5–18.
21 Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M *et al. The Newcastle–Ottawa Scale (NOS) for Assessing the Quality of Non-Randomized Studies in Meta-Analyses*. http//www.ohri.ca/programs/clinical epidemiology/oxford.htm [accessed 15 May 2018].
22 Wang MJ, Ping J, Li Y, Holmqvist A, Adell G, Arbman G *et al.* Prognostic significance and molecular features of colorectal mucinous adenocarcinomas: a Strobe-compliant study. *Medicine (Baltimore)* 2015; **94**: e2350.
23 Rosty C, Young JP, Walsh MD, Clendenning M, Walters RJ, Pearson S *et al.* Colorectal carcinomas with *KRAS* mutation are associated with distinctive morphological and molecular features. *Mod Pathol* 2013; **26**: 825–834.
24 Jung SB, Lee HI, Oh HK, Shin IH, Jeon CH. Clinico-pathologic parameters for prediction of microsatellite instability in colorectal cancer. *Cancer Res Treat* 2012; **44**: 179–186.
25 Nosho K, Irahara N, Shima K, Kure S, Kirkner GJ, Schernhammer ES *et al.* Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. *PLoS One* 2008; **3**: e3698.
26 Kawasaki T, Ohnishi M, Suemoto Y, Kirkner GJ, Liu Z, Yamamoto H *et al.* WRN promoter methylation possibly connects mucinous differentiation, microsatellite instability and CpG island methylator phenotype in colorectal cancer. *Mod Pathol* 2008; **21**: 150–158.
27 Tanaka H, Deng G, Matsuzaki K, Kakar S, Kim GE, Miura S *et al. BRAF* mutation, CpG Island methylator phenotype and microsatellite instability occur more frequently and concordantly in mucinous than non-mucinous colorectal cancer. *Int J Cancer* 2006; **118**: 2765–2771.
28 Chang SC, Lin JK, Yang SH, Wang HS, Li AF, Chi CW. Relationship between genetic alterations and prognosis in sporadic colorectal cancer. *Int J Cancer* 2006; **118**: 1721–1727.
29 Ogino S, Brahmandam M, Cantor M, Namgyal C, Kawasaki T, Kirkner G *et al.* Distinct molecular features of colorectal carcinoma with signet ring cell component and colorectal carcinoma with mucinous component. *Mod Pathol* 2006; **19**: 59–68.
30 Ashktorab H, Smoot DT, Farzanmehr H, Fidelia-Lambert M, Momen B, Hylind L *et al.* Clinicopathological features and microsatellite instability (MSI) in colorectal cancers from African Americans. *Int J Cancer* 2005; **116**: 914–919.
31 Kazama Y, Watanabe T, Kanazawa T, Tada T, Tanaka J, Nagawa H. Mucinous carcinomas of the colon and rectum show higher rates of microsatellite instability and lower rates of chromosomal instability: a study matched for T classification and tumor location. *Cancer* 2005; **103**: 2023–2029.

32 Li W, Qiu T, Zhi W, Shi S, Zou S, Ling Y *et al.* Colorectal carcinomas with *KRAS* codon 12 mutation are associated with more advanced tumor stages. *BMC Cancer* 2015; **15**: 340.
33 Ward R, Meagher A, Tomlinson I, O'Connor T, Norrie M, Wu R *et al.* Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. *Gut* 2001; **48**: 821–829.
34 Alexander J, Watanabe T, Wu TT, Rashid A, Li S, Hamilton SR. Histopathological identification of colon cancer with microsatellite instability. *Am J Pathol* 2001; **158**: 527–535.
35 Zhang H, Nordenskjöld B, Dufmats M, Söderkvist P, Sun XF. *K-ras* mutations in colorectal adenocarcinomas and neighbouring transitional mucosa. *Eur J Cancer* 1998; **34**: 2053–2057.
36 Messerini L, Vitelli F, De Vitis LR, Mori S, Calzolari A, Palmirotta R *et al.* Microsatellite instability in sporadic mucinous colorectal carcinomas: relationship to clinico-pathological variables. *J Pathol* 1997; **182**: 380–384.
37 Hanski C, Bornhoeft G, Shimoda T, Hanski ML, Lane DP, Stein H *et al.* Expression of p53 protein in invasive colorectal carcinomas of different histologic types. *Cancer* 1992; **70**: 2772–2777.
38 Yoon YS, Kim J, Hong SM, Lee JL, Kim CW, Park IJ *et al.* Clinical implications of mucinous components correlated with microsatellite instability in patients with colorectal cancer. *Colorectal Dis* 2015; **17**: O161–O167.
39 Ines C, Donia O, Rahma B, Ben Ammar A, Sameh A, Khalfallah T *et al.* Implication of *K-ras* and *p53* in colorectal cancer carcinogenesis in Tunisian population cohort. *Tumour Biol* 2014; **35**: 7163–7175.
40 Langner C, Harbaum L, Pollheimer MJ, Kornprat P, Lindtner RA, Schlemmer A *et al.* Mucinous differentiation in colorectal cancer – indicator of poor prognosis? *Histopathology* 2012; **60**: 1060–1072.
41 Pai RK, Jayachandran P, Koong AC, Chang DT, Kwok S, Ma L *et al. BRAF*-mutated, microsatellite-stable adenocarcinoma of the proximal colon: an aggressive adenocarcinoma with poor survival, mucinous differentiation, and adverse morphologic features. *Am J Surg Pathol* 2012; **36**: 744–752.
42 Lam AK, Ong K, Ho YH. hTERT expression in colorectal adenocarcinoma: correlations with p21, p53 expressions and clinicopathological features. *Int J Colorectal Dis* 2008; **23**: 587–594.
43 Leopoldo S, Lorena B, Cinzia A, Gabriella DC, Angela Luciana B, Renato C *et al.* Two subtypes of mucinous adenocarcinoma of the colorectum: clinicopathological and genetic features. *Ann Surg Oncol* 2008; **15**: 1429–1439.
44 Tozawa E, Ajioka Y, Watanabe H, Nishikura K, Mukai G, Suda T *et al.* Mucin expression, p53 overexpression, and peritumoral lymphocytic infiltration of advanced colorectal carcinoma with mucus component: is mucinous carcinoma a distinct histological entity? *Pathol Res Pract* 2007; **203**: 567–574.
45 Sarli L, Bottarelli L, Azzoni C, Campanini N, Di Cola G, Barilli AL *et al.* Loss of *p27* expression and microsatellite instability in sporadic colorectal cancer. *Surg Oncol* 2006; **15**: 97–106.
46 Lan YT, Chang SC, Li AF, Lin TC, Chen WS, Jiang JK *et al.* p53 protein accumulation as a prognostic marker in sporadic colorectal cancer. *Int J Colorectal Dis* 2007; **22**: 499–506.
47 Ogino S, Odze RD, Kawasaki T, Brahmandam M, Kirkner GJ, Laird PW *et al.* Correlation of pathologic features with CpG island methylator phenotype (CIMP) by quantitative DNA methylation analysis in colorectal carcinoma. *Am J Surg Pathol* 2006; **30**: 1175–1183.
48 Lin JK, Chang SC, Wang HS, Yang SH, Jiang JK, Chen WC *et al.* Distinctive clinicopathological features of *Ki-ras* mutated colorectal cancers. *J Surg Oncol* 2006; **94**: 234–241.
49 Yearsley M, Hampel H, Lehman A, Nakagawa H, de la Chapelle A, Frankel WL. Histologic features distinguish microsatellite-high from microsatellite-low and microsatellite-stable colorectal carcinomas, but do not differentiate germline mutations from methylation of the *MLH1* promoter. *Hum Pathol* 2006; **37**: 831–838.
50 Park SY, Lee HS, Choe G, Chung JH, Kim WH. Clinicopathological characteristics, microsatellite instability, and expression of mucin core proteins and p53 in colorectal mucinous adenocarcinomas in relation to location. *Virchows Arch* 2006; **449**: 40–47.
51 Wright CL, Stewart ID. Histopathology and mismatch repair status of 458 consecutive colorectal carcinomas. *Am J Surg Pathol* 2003; **27**: 1393–1406.
52 Greenson JK, Bonner JD, Ben-Yzhak O, Cohen HI, Miselevich I, Resnick MB *et al.* Phenotype of microsatellite unstable colorectal carcinomas: well-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability. *Am J Surg Pathol* 2003; **27**: 563–570.
53 Feeley KM, Fullard JF, Heneghan MA, Smith T, Maher M, Murphy RP *et al.* Microsatellite instability in sporadic colorectal carcinoma is not an indicator of prognosis. *J Pathol* 1999; **188**: 14–17.
54 Zhang H, Evertsson S, Sun X. Clinicopathological and genetic characteristics of mucinous carcinomas in the colorectum. *Int J Oncol* 1999; **14**: 1057–1061.
55 Furlan D, Tibiletti MG, Taborelli M, Albarello L, Cornaggia M, Capella C. The value of microsatellite instability in the detection of HNPCC families and of sporadic colorectal cancers with special biological features: an investigation on a series of 100 consecutive cases. *Ann Oncol* 1998; **9**: 901–906.
56 Forster S, Sattler HP, Hack M, Romanakis K, Rohde V, Seitz G *et al.* Microsatellite instability in sporadic carcinomas of the proximal colon: association with diploid DNA content, negative protein expression of p53, and distinct histomorphologic features. *Surgery* 1998; **123**: 13–18.
57 Laurent-Puig P, Olschwang S, Delattre O, Validire P, Melot T, Mosseri V *et al.* Association of *Ki-ras* mutation with

differentiation and tumor-formation pathways in colorectal carcinoma. *Int J Cancer* 1991; **49**: 220–223.
58 Georgescu CV, Săftoiu A, Georgescu CC, Ciurea R, Ciurea T. Correlations of proliferation markers, p53 expression and histological findings in colorectal carcinoma. *J Gastrointestin Liver Dis* 2007; **16**: 133–139.
59 Suh JH, Lim SD, Kim JC, Hong SH, Kang GH. Comparison of clinicopathologic characteristics and genetic alterations between microsatellite instability-positive and microsatellite instability-negative sporadic colorectal carcinomas in patients younger than 40 years old. *Dis Colon Rectum* 2002; **45**: 219–228.
60 Ismael NE, El Sheikh SA, Talaat SM, Salem EM. Mismatch repair proteins and microsatellite instability in colorectal carcinoma (MLH1, MSH2, MSH6 and PMS2): histopathological and immunohistochemical study. *Open Access Maced J Med Sci* 2017; **5**: 9–13.
61 Fearon ER. Molecular genetics of colorectal cancer. *Annu Rev Pathol* 2011; **6**: 479–507.
62 Bertagnolli MM, Niedzwiecki D, Compton CC, Hahn HP, Hall M, Damas B *et al.* Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. *J Clin Oncol* 2009; **27**: 1814–1821.
63 Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterology* 2008; **135**: 1079–1099.
64 Nitsche U, Zimmermann A, Späth C, Müller T, Maak M, Schuster T *et al.* Mucinous and signet-ring cell colorectal cancers differ from classical adenocarcinomas in tumor biology and prognosis. *Ann Surg* 2013; **258**: 775–783.
65 Hugen N, Verhoeven RH, Radema SA, de Hingh IH, Pruijt JF, Nagtegaal ID *et al.* Prognosis and value of adjuvant chemotherapy in stage III mucinous colorectal carcinoma. *Ann Oncol* 2013; **24**: 2819–2824.
66 Lee DW, Han SW, Lee HJ, Rhee YY, Bae JM, Cho NY *et al.* Prognostic implication of mucinous histology in colorectal cancer patients treated with adjuvant FOLFOX chemotherapy. *Br J Cancer* 2013; **108**: 1978–1984.
67 Clancy C, Burke JP, Kalady MF, Coffey JC. *BRAF* mutation is associated with distinct clinicopathological characteristics in colorectal cancer: a systematic review and meta-analysis. *Colorectal Dis* 2013; **15**: e711–e718.
68 Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM *et al.* Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003; **349**: 247–257.
69 Overman MJ, Lonardi S, Wong KYM, Lenz HJ, Gelsomino F, Aglietta M *et al.* Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J Clin Oncol* 2018; **36**: 773–779.
70 Williamson JS, Harris DA, Beynon J, Jenkins GJ. Review of the development of DNA methylation as a marker of response to neoadjuvant therapy and outcomes in rectal cancer. *Clin Epigenetics* 2015; **7**: 70.
71 Kokelaar RF, Jones H, Beynon J, Evans ME, Harris DA. Meta-analysis of the prognostic value of CpG island methylator phenotype in rectal cancer. *Int J Colorectal Dis* 2018; **33**: 995–1000.
72 Davies HT, Crombie IK, Tavakoli M. When can odds ratios mislead? *BMJ* 1998; **316**: 989–991.

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the article.

European Colorectal Congress

28 November – 1 December 2022, St.Gallen, Switzerland

Monday, 28 November 2022

09.50
Opening and welcome
Jochen Lange, St.Gallen, CH

10.00
It is leaking! Approaches to salvaging an anastomosis
Willem Bemelman, Amsterdam, NL

10.30
Predictive and diagnostic markers of anastomotic leak
Andre D'Hoore, Leuven, BE

11.00
SATELLITE SYMPOSIUM
ETHICON

11.45
Of microbes and men – the unspoken story of anastomotic leakage
James Kinross, London, UK

12.15
LUNCH

13.45
Operative techniques to reduce anastomotic recurrence in Crohn's disease
Laura Hancock, Manchester, UK

14.15
Innovative approaches in the treatment of complex Crohn Diseases perianal fistula
Christianne Buskens, Amsterdam, NL

14.45
To divert or not to divert in Crohn surgery – technical aspects and patient factors
Pär Myrelid, Linköping, SE

15.15
COFFEE BREAK

15.45
Appendiceal neoplasia – when to opt for a minimal approach, when and how to go for a maximal treatment
Tom Cecil, Basingstoke, Hampshire, UK

16.15
SATELLITE SYMPOSIUM
Medtronic
Further,Together

17.00
Outcomes of modern induction therapies and Wait and Watch strategies, Hope or Hype
Antonino Spinelli, Milano, IT

17.30
EAES Presidential Lecture - Use of ICG in colorectal surgery: beyond bowel perfusion
Salvador Morales-Conde, Sevilla, ES

18.00
Get-Together with your colleagues
Industrial Exhibition

Tuesday, 29 November 2022

9.00
CONSULTANT'S CORNER
Michel Adamina, Winterthur, CH

10.30
COFFEE BREAK

11.00
SATELLITE SYMPOSIUM
INTUITIVE

11.45
Trends in colorectal oncology and clinical insights for the near future
Rob Glynne-Jones, London, UK

12.15
LUNCH

13.45
VIDEO SESSION

14.15
SATELLITE SYMPOSIUM
BD

15.00
COFFEE BREAK

15.30
The unsolved issue of TME: open, robotic, transanal, or laparoscopic – shining light on evidence and practice
Des Winter, Dublin, IE
Jim Khan, London, UK
Brendan Moran, Basingstoke, UK

16.30
SATELLITE SYMPOSIUM
Takeda

17.15
Lars Pahlman lecture
Søren Laurberg, Aarhus, DK

Thursday, 1 December 2022
Masterclass in Colorectal Surgery
Proctology Day

Wednesday, 30 November 2022

9.00
Advanced risk stratification in colorectal cancer – choosing wisely surgery and adjuvant therapy
Philip Quirke, Leeds, UK

09.30
Predictors for Postoperative Complications and Mortality
Ronan O'Connell, Dublin, IE

10.00
Segmental colectomy versus extended colectomy for complex cancer
Quentin Denost, Bordeaux, FR

10.30
COFFEE BREAK

11.00
Incidental cancer in polyp - completion surgery or endoscopy treatment alone?
Laura Beyer-Berjot, Marseille, FR

11.30
SATELLITE SYMPOSIUM

12.00
Less is more – pushing the boundaries of full-thickness rectal resection
Xavier Serra-Aracil, Barcelona, ES

12.30
LUNCH

14.00
Management of intestinal neuroendocrine neoplasia
Frédéric Ris, Geneva, CH

14.30
Poster Presentation & Best Poster Award
Michel Adamina, Winterthur, CH

15.00
SATELLITE SYMPOSIUM
OLYMPUS

15.45
COFFEE BREAK

16.15
Reoperative pelvic floor surgery – dealing with perineal hernia, reoperations, and complex reconstructions
Guillaume Meurette, Nantes, FR

16.45
Salvage strategies for rectal neoplasia
Roel Hompes, Amsterdam, NL

17.15
Beyond TME – technique and results of pelvic exenteration and sacrectomy
Paris Tekkis, London, UK

19.30
FESTIVE EVENING

Information & Registration **www.colorectalsurgery.eu**