Original article

Histopathological and molecular classification of colorectal cancer and corresponding peritoneal metastases

I. Ubink[1], W. J. van Eden[5], P. Snaebjornsson[6], N. F. M. Kok[5], J. van Kuik[2], W. M. U. van Grevenstein[1], M. M. Laclé[2], J. Sanders[6], R. J. A. Fijneman[6], S. G. Elias[3], I. H. M. Borel Rinkes[1], A. G. J. Aalbers[5] and O. Kranenburg[1,4]

[1]Department of Surgical Oncology, Cancer Centre, [2]Department of Pathology, [3]Julius Centre for Health Sciences and Primary Care, and [4]Division of Biomedical Genetics, University Medical Centre Utrecht, Utrecht, and Departments of [5]Surgical Oncology and [6]Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Correspondence to: Professor O. Kranenburg, Division of Biomedical Genetics and Cancer Centre, University Medical Centre Utrecht, Heidelberglaan 100, 3584CX Utrecht, The Netherlands (e-mail: o.kranenburg@umcutrecht.nl)

Background: Patients with colorectal peritoneal carcinomatosis have a very poor prognosis. The recently developed consensus molecular subtype (CMS) classification of primary colorectal cancer categorizes tumours into four robust subtypes, which could guide subtype-targeted therapy. CMS4, also known as the mesenchymal subtype, has the greatest propensity to form distant metastases. CMS4 status and histopathological features of colorectal peritoneal carcinomatosis were investigated in this study.

Methods: Fresh-frozen tissue samples from primary colorectal cancer and paired peritoneal metastases from patients who underwent cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy were collected. Histopathological features were analysed, and a reverse transcriptase–quantitative PCR test was used to assess CMS4 status of all collected lesions.

Results: Colorectal peritoneal carcinomatosis was associated with adverse histopathological characteristics, including a high percentage of stroma in both primary tumours and metastases, and poor differentiation grade and high-grade tumour budding in primary tumours. Furthermore, CMS4 was significantly enriched in primary tumours with peritoneal metastases, compared with unselected stage I–IV tumours (60 per cent (12 of 20) *versus* 23 per cent; $P = 0.002$). The majority of peritoneal metastases (75 per cent, 21 of 28) were also classified as CMS4. Considerable intrapatient subtype heterogeneity was observed. Notably, 15 of 16 patients with paired tumours had at least one CMS4-positive tumour location.

Conclusion: Significant enrichment for CMS4 was observed in colorectal peritoneal carcinomatosis.

Surgical relevance

Cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC) improves survival of selected patients with colorectal peritoneal carcinomatosis, but recurrence is common. Histopathological and molecular analysis of colorectal peritoneal carcinomatosis could provide clues for development of novel therapies.

In this study, colorectal peritoneal carcinomatosis was found to be enriched for tumours with high stromal content and CMS4-positive status.

To further improve prognosis for patients with colorectal peritoneal carcinomatosis, therapies that target tumour–stroma interaction could be added to CRS-HIPEC.

Paper accepted 16 November 2017
Published online in Wiley Online Library (www.bjs.co.uk). **DOI:** 10.1002/bjs.10788

Introduction

The peritoneum is a common site of metastatic spread in patients with colorectal cancer. Approximately 5 per cent of all patients with colorectal cancer present with colorectal peritoneal carcinomatosis (CRPC) at first diagnosis, and another 5 per cent develop metachronous CRPC[1]. Patients with CRPC have a poor prognosis, with a median survival of only 6 months if left untreated[2]. Currently, cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC) is the only potentially curative treatment option, which may improve median overall survival to more than 30 months in selected patients[3]. Unfortunately, in spite of complete cytoreduction, more

than half of patients experience recurrent disease within 2 years[3].

In addition to classical histopathological features, colorectal cancer can be stratified into four consensus molecular subtypes (CMS1–4), based on gene expression profiling. These subtypes have distinct biological characteristics and prognostic significance. CMS4 has been associated with worse disease-free and overall survival than the other subtypes. CMS4 is characterized by high expression of genes reflecting epithelial-to-mesenchymal transition, transforming growth factor (TGF) β signalling and matrix remodelling[4]. CMS4 tumours often show a profound desmoplastic reaction with high stromal cell content[5].

Several studies have suggested that the differences in underlying signalling pathways of CMSs account for heterogeneous responses to anticancer therapies. Irinotecan-based therapies appeared effective only in metastatic colorectal cancer with upregulated Wnt pathway signalling (CMS2)[6]. Metastatic colorectal cancer of CMS4 seemed resistant to antiepidermal growth factor receptor therapy independent of *RAS*-mutation status[7,8]. Patients with stage III CMS4 tumours did not benefit from systemic adjuvant oxaliplatin treatment[9], which could be relevant in the context of CRPC as oxaliplatin is a commonly used intraperitoneal chemotherapeutic agent in HIPEC[10].

Patients are currently selected for CRS-HIPEC based on clinical rather than biological features[11]. The aim of this study was to investigate whether CRPC is enriched for stroma-rich and/or CMS4 variants of colorectal cancer. A better understanding of signalling pathways that drive CRPC would allow patient selection and repurposing of (targeted) therapies, which are required to further improve outcome for patients with CRPC. Molecular and histopathological classification of CRPC, as reported here, is a first step towards reaching this goal.

Methods

This study was performed at the Netherlands Cancer Institute (NCI), Amsterdam, and University Medical Centre Utrecht (UMCU), two tertiary oncological referral centres in the Netherlands. Patients who underwent CRS-HIPEC for CRPC, and from whom fresh-frozen tissues from both the primary tumour and one or more peritoneal metastases were sampled, were eligible for the study. Patients were included if at least one of these samples met the quality criteria for reverse transcriptase–quantitative PCR (RT-qPCR) analysis. Clinical data were extracted from prospectively maintained CRS-HIPEC databases at both centres. The extent of CRPC was estimated by the Dutch 7 Region Count[12]. The study protocol was approved by the ethical committees of the Biobanks at NCI and UMCU (project codes CFMPB491 and 17-163 respectively). Collection, storage and use of patient-derived tissue and data were performed either under informed consent, or in compliance with the Code for Proper Secondary Use of Human Tissue in The Netherlands.

Histopathology

All haematoxylin and eosin-stained slides derived from the primary tumours and corresponding peritoneal metastases were reassessed. TNM staging was done according to the UICC fifth edition[13]. Tumour type and differentiation were assessed according to the WHO Classification of Tumours of the Digestive System[14]. Primary tumours were classified as right-sided if they were located in the caecum, ascending or transverse colon; tumours in the descending colon, sigmoid and rectum were considered left-sided. Features evaluated without additional staining of haematoxylin and eosin-stained slides of both the primary tumours and peritoneal metastasis were: venous and lymphatic invasion (using conventional methodology, and including intramural and extramural invasion); amount of mucin (as a percentage of tumour area); number of signet ring cells (as a percentage of tumour cells); tumour border configuration[15]; tumour budding[16]; stroma–carcinoma percentage[17] and inflammatory score[18]. Microsatellite status was determined using immunohistochemistry for mismatch repair proteins. *KRAS* and *BRAF* mutational status was determined with Ion Torrent™ (PGM Cancer Hotspot panel v2Plus; Thermo Fisher Scientific, Waltham, Massachusetts, USA) or MassARRAY® Dx colon panel (Agena Bioscience, San Diego, California, USA).

RNA isolation and analysis

Tumour cell percentage of fresh-frozen tissue was evaluated; only samples that contained at least 10 per cent tumour cells were processed for RNA isolation. Frozen tissue samples were cut in 20–30-μm thick cryosections with a cryostat and immersed in RLT buffer (RNeasy® Mini Kit; Qiagen, Stockholm, Sweden) plus 1 per cent β-mercaptoethanol. RNA isolation, including on-column DNase digestion, was performed according to the manufacturer's instructions. RNA concentration was measured using a NanoDrop™ 2000 instrument (Thermo Fisher Scientific). RNA integrity was assessed using an Agilent RNA 6000 Nano Kit and an Agilent 2100 Bioanalyzer® (Agilent Technologies, Santa Clara, California, USA); only samples with an RNA integrity number (RIN) over 6 were subjected

Table 1 Baseline characteristics

	No. of patients*
Age (years)†	63 (39–72)
Sex ratio (M : F)	12 : 12
Primary tumour location	
Right colon‡	14
Left colon	10
pT category	
pT3	2
pT4a	18
pT4b	4
pN category	
pN0	1
pN1	11
pN2	12
Metastases	
Metachronous	2
Synchronous	22
Primary tumour histological subtype	
Adenocarcinoma	14
Mucinous adenocarcinoma	8
Signet ring cell carcinoma	2
Primary tumour differentiation grade	
Good/moderate	14
Poor	10
Site of metastatic disease	
PC only	20
PC + systemic	4
MMR status	
MMR proficient	21
MMR deficient	3
KRAS mutation status	
Wild-type	14
Mutant	10
BRAF mutation status	
Wild-type	18
Mutant	6

*Unless indicated otherwise; †values are median (range). ‡Includes two poorly differentiated appendiceal carcinomas. PC, peritoneal carcinomatosis; MMR, mismatch repair.

Table 2 Histopathological characteristics

	Primary tumours ($n = 24$)	Peritoneal metastases ($n = 35$)
Venous invasion		
No	14	34
Yes	10	1
Lymphatic invasion		
No	15	32
Yes	9	3
Tumour border configuration		
Pushing	4	17
Infiltrating	20	18
Stroma (% of surface area)		
≤ 50	5	21
> 50	19	14
Tumour budding score		
Bd1	4	18
Bd2	6	5
Bd3	14	12
Mucin (% of surface area)		
0	12	20
1–50	7	6
51–100	5	9
Signet ring cell (% of tumour cells)		
0	19	28
1–50	3	5
51–100	2	2
Inflammatory score		
0–2	18	31
3–6	6	4
7–12	0	0

to further analysis. The CMS4 RT–qPCR test was performed as described previously[19]. The test is based on the expression of four genes (*PDGFRA*, *PDGFRB*, *PDGFC* and *KIT*), and results in a CMS4 probability ranging from 0 to 1, with 0.5 as the cut-off point for CMS4 positivity.

Statistical analysis

Fisher's exact test was used to characterize the relationship between categorical variables. Correlations between continuous variables were tested using linear regression analysis. Disease-free and overall survival rates from the date of CRS-HIPEC were determined by the Kaplan–Meier method. Estimates are reported with 95 per cent confidence intervals. All statistical tests were two-sided with a threshold for statistical significance of 5 per cent. SPSS® for Windows® version 21.0 (IBM, Armonk, New York, USA) was used for statistical analyses. Graphs were created with GraphPad Prism® version 7 (GraphPad Software, San Diego, California, USA).

Results

Twenty-four patients met the inclusion criteria. Baseline characteristics are shown in *Table 1*, and details of the CRS-HIPEC procedure and oncological outcomes in *Table S1* (supporting information). The majority of patients had synchronous CRPC, mostly without evidence of systemic metastases. Primary tumours were distributed equally between the left and right colon. Nearly all patients had regional lymph nodes metastases.

To further characterize the CRPC cohort, histopathological features of both primary tumours and metastases were examined (*Table 2*). Primary tumours frequently showed venous invasion, infiltrating growth at the invasive margin and high-grade tumour budding. None of the included CRPC samples had a high inflammatory score (*Table S2*,

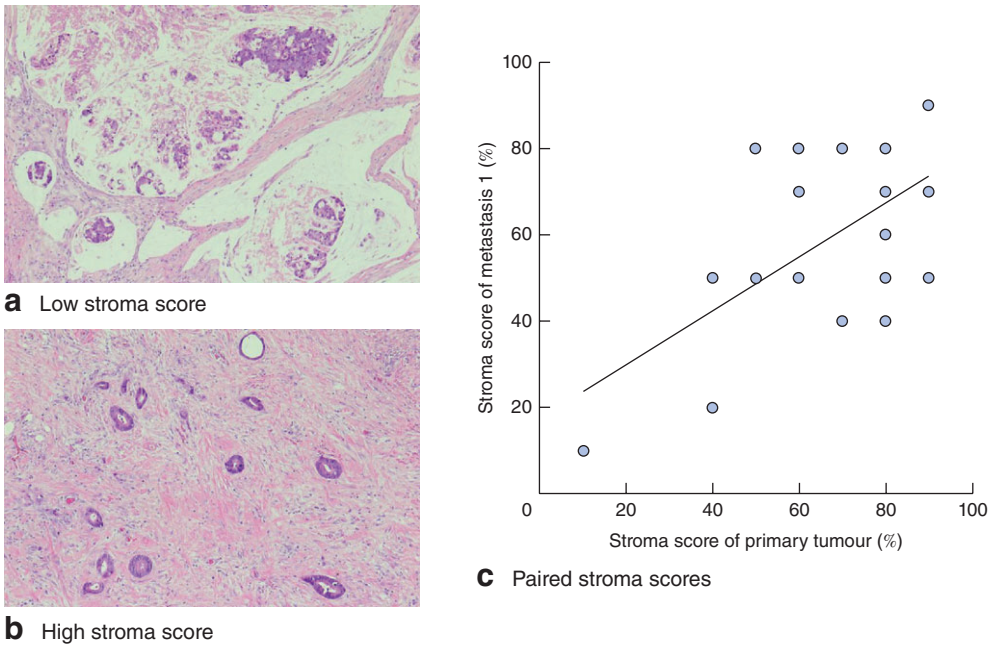

Fig. 1 Stroma score in primary tumours and peritoneal metastases. **a**,**b** Representative micrographs of peritoneal metastases with **a** low stroma score (scored as 10 per cent) and **b** high stroma score (scored as 90 per cent) (haematoxylin and eosin stain, original magnification × 10). **c** Correlation between stroma score of primary tumours and corresponding peritoneal metastases ($R^2 = 0.343$, $P = 0.003$)

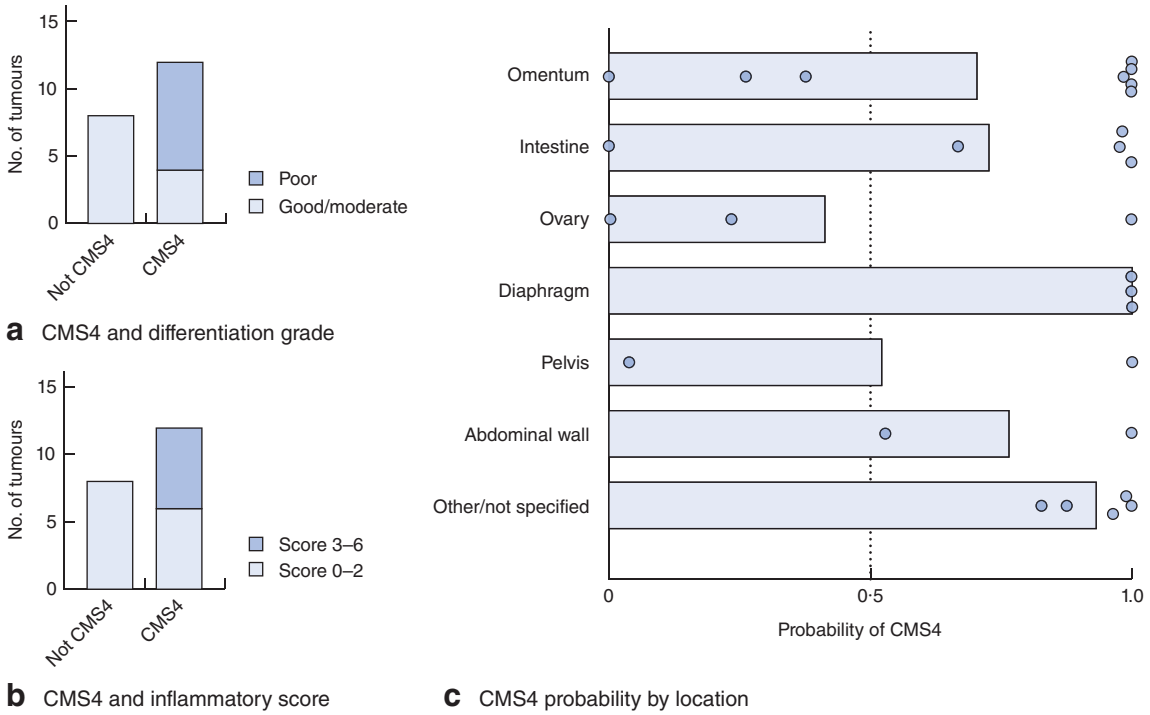

Fig. 2 Consensus molecular subtype (CMS) 4 status in relation to histopathological features: **a** differentiation ($P = 0.005$, Fisher's exact test), **b** inflammation ($P = 0.142$, Fisher's exact test) and **c** site of peritoneal metastases. In **c**, reverse transcriptase–quantitative PCR results for all 28 metastatic lesions are grouped according to site of metastasis; bars represent the mean probability of being CMS4 (0.5 or more classified as CMS4)

supporting information). Both primary tumours and peritoneal metastases often had a high stroma percentage, and there was a significant correlation between stroma percentage in paired primary tumours and metastases (*Fig. 1*).

CMS4 assessment of primary tumours and peritoneal metastases

Of 59 fresh-frozen samples identified, 48 met the quality requirements for CMS4 RT–qPCR analysis (more than 10 per cent tumour cells and RIN over 6). Twenty primary cancers were analysed with the diagnostic RT–qPCR test for CMS4, of which 12 were classified as CMS4 (60 (95 per cent c.i. 39 to 78) per cent). In the original CMS publication[4], 23 per cent of non-selected primary stage I–IV tumours were classified as CMS4. Thus, CRPC was significantly enriched for CMS4 ($P = 0.002$). Primary tumours in the present CRPC cohort were even more frequently CMS4 than the stage IV tumours in the original CMS publication, although not statistically significantly (60 *versus* 40 per cent; $P = 0.096$). Adenocarcinomas more often appeared CMS4-positive than primary tumours with mucinous histology (8 of 12 *versus* 2 of 6 respectively), but this was not statistically significant in this small cohort ($P = 0.181$). The two primary signet cell carcinomas were both classified as CMS4. CMS4 positivity was associated with poor tumour differentiation and higher inflammatory scores in the primary tumours ($P = 0.005$ and $P = 0.042$ respectively) (*Fig. 2a,b*).

The majority of peritoneal metastases (21 of 28; 75 (95 per cent c.i. 57 to 87) per cent) were also classified as CMS4. This was significantly higher than the incidence of mesenchymal-type liver metastases in two previous studies (34 of 72 (47 per cent)[8], $P = 0.004$; 60 of 129 (46.5 per cent)[20], $P = 0.007$). *Fig. 2c* shows the CMS4 test results for all metastases, grouped by intraperitoneal location. In this small data set, no clear relationship between metastasis location and probability of being CMS4 was observed. For both primary tumours and metastatic lesions, the probability of being CMS4 was not correlated with the stroma percentage (*Fig. S1*, supporting information).

Intrapatient subtype heterogeneity

Good-quality paired samples from primary tumours and corresponding metastases were available from 16 patients. Remarkably, all patients, except patient 21, had at least one CMS4-positive lesion (*Fig. 3*). In eight patients, the CMS4 classification differed between the primary tumour and the metastases, indicating considerable intrapatient heterogeneity with respect to tumour CMS4 status.

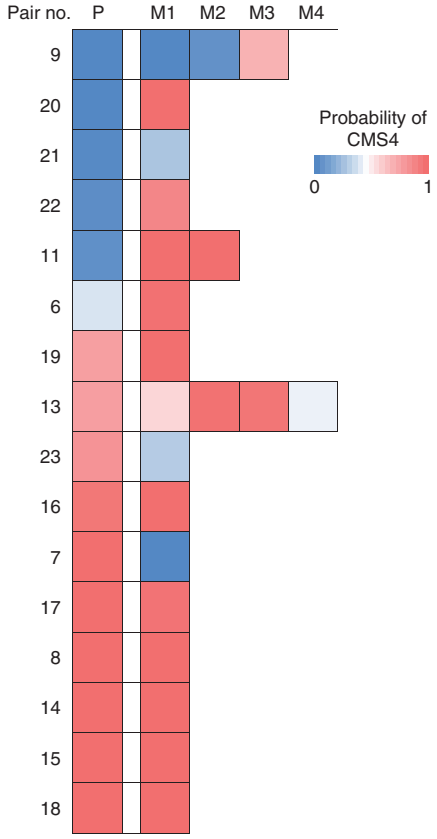

Fig. 3 Intrapatient heterogeneity in consensus molecular subtype (CMS) 4 reverse transcriptase–quantitative PCR test results for paired primary tumours and peritoneal metastases. The test results in a predicted probability of CMS4 ranging from 0 to 1. Samples with a probability below 0.5 are classified as not CMS4 (blue), and those with a probability of 0.5 or greater as CMS4 (red). P, primary; M1–4, metastatic lesions 1–4

Notably, intrapatient heterogeneity was observed in all three patients who had more than one metastasis available for CMS4 testing.

Discussion

In this study, histopathological features and CMS4 status were assessed in a cohort of patients who underwent CRS-HIPEC for CRPC. CMS4 positivity was observed in 60 per cent of primary tumours in the cohort (12 of 20), which is significantly higher than the reported incidence of CMS4 in unselected stage I–IV colorectal cancer[4]. CMS4 colorectal cancer has a higher propensity for relapse and distant metastasis, and worse overall survival[4]. Furthermore, the majority of primary tumours in this CRPC cohort (79 per cent, 19 of 24) were found to have a high stroma percentage. The carcinoma–stroma ratio has been

identified as an individual predictor of survival in colorectal cancer, with a high stroma score (over 50 per cent) related to poor prognosis[17]. In an analysis of 701 stage II–III cancers, 71 per cent of tumours were scored as stroma-low (50 per cent or less stroma)[17], which contrasts strongly with this CRPC cohort of predominantly stroma-high tumours.

Although CMS4 is characterized by high stromal content[4,5], stroma score and CMS4 status were not correlated in the CRPC cohort studied here. There are several possible explanations. Although stroma percentage provides an estimate of the quantity of stroma within a tumour, it does not provide information on the abundance and types of cells within the stroma. Functional differences in cancer stroma[21] may be more important determinants of aggressive tumour behaviour than simply the percentage of tumour stroma. The mesenchymal subtype is not solely determined by the stromal component, but tumour cell-intrinsic gene expression also contributes to the mesenchymal phenotype of the poor-prognosis colorectal cancer subtype[22,23]. Extensive infiltration with cancer-associated fibroblasts and tumour cell-intrinsic mesenchymal gene expression may be two distinct means of acquiring aggressive cancer behaviour.

The primary tumours in this CRPC cohort frequently had histological features that have previously been associated with poor prognosis, including poor differentiation grade, mucinous histology, venous invasion, high-grade tumour budding, infiltrating tumour border configuration, and low inflammatory score[15,16,18,24]. Furthermore, one-quarter of the tumours had a *BRAF* mutation, which is higher than the reported incidence of *BRAF* mutations in metastatic colorectal cancer (approximately 10 per cent[25]). Indeed, *BRAF* mutations have been associated with higher rates of peritoneal dissemination[25] and with a poor prognosis[26] in colorectal cancer.

Three-quarters of the peritoneal metastases analysed in this study were also classified as CMS4. CMS4 is characterized by TGF-β signalling[4,5], and TGF-β can stimulate transdifferentiation of peritoneal fibroblasts and mesothelial cells into activated myofibroblasts[27]. The interaction between tumour cells and fibroblasts is thought to be important in the establishment of peritoneal metastases[28–30]. The dependence of peritoneal metastases on TGF-β signalling could explain the enrichment of CMS4 in this cohort. As no association was observed between the stroma percentage and probability of CMS4 in the peritoneal metastases, further research into the type of reactive stroma in CMS4 and non-CMS4 metastases in relation to TGF-β pathway activation is needed.

Heterogeneity in CMS4 status between primary tumours and metastatic lesions was frequently observed. Considerable intratumoral heterogeneity (with respect to CMS4 status when analysing multiple regions within a primary tumour) was recently reported by this group[19]. Subtype heterogeneity between primary tumours and peritoneal metastases could thus be a consequence of intratumour heterogeneity. Alternatively, molecular classification of metastases could be influenced by the specific intra-abdominal location of the metastases, as gene expression in tumour cells is influenced by the tumour microenvironment[22]. Although the small cohort studied here provides insufficient data to draw firm conclusions, both CMS4-positive and -negative lesions were found at most metastatic sites, which does not support this hypothesis.

Currently, patients with CRPC are treated based on clinicopathological features, regardless of genetic alterations or molecular subtyping. CMS4 has been associated with a poorer response to anticancer drugs[4,7,9], although these findings need prospective validation. The observation that nearly all patients had at least one CMS4-positive tumour lesion could thus have clinical implications. Mitomycin C and oxaliplatin are the most frequently used chemotherapeutic agents in HIPEC. Retrospective comparisons between these two drugs are contradictory; one study[10] favoured mitomycin C, whereas another[31] showed a clear benefit of oxaliplatin. When given as adjuvant therapy in stage III colorectal cancer, oxaliplatin did not benefit patients with CMS4 cancers[9]. Given the enrichment of CMS4 in CRPC, and its potential resistance to oxaliplatin, prospective studies are required to study the benefit of oxaliplatin in the HIPEC procedure in relation to CMS4 status.

This study was limited by the small sample size, and the findings deserve validation in a larger cohort. Because fresh-frozen samples were not collected routinely, no consecutive series of CRPC was available. The interest in paired samples of both primary tumours and peritoneal metastases resulted in predominant inclusion of patients with synchronous CRPC, as primary cancer surgery in patients with metachronous CRPC was usually performed at another hospital. These factors may have resulted in considerable selection bias. This small series is insufficient to define the relationship between molecular subtype and outcome following HIPEC. Despite these limitations, the findings provide an incentive to explore the molecular classification of CRPC further. Combined with clinical and histological parameters, molecular classification could advance the personalized treatment of peritoneal metastases.

Acknowledgements

The authors acknowledge the NKI-AVL Core Facility Molecular Pathology and Biobanking for supplying NKI-AVL Biobank material. I.U. is supported by a grant from the Dutch Cancer Society (UU2014-6617).
Disclosure: The authors declare no conflict of interest.

References

1 Segelman J, Granath F, Holm T, Machado M, Mahteme H, Martling A. Incidence, prevalence and risk factors for peritoneal carcinomatosis from colorectal cancer. *Br J Surg* 2012; **99**: 699–705.
2 Razenberg LG, Lemmens VE, Verwaal VJ, Punt CJ, Tanis PJ, Creemers GJ *et al.* Challenging the dogma of colorectal peritoneal metastases as an untreatable condition: results of a population-based study. *Eur J Cancer* 2016; **65**: 113–120.
3 Kuijpers AM, Mirck B, Aalbers AG, Nienhuijs SW, de Hingh IH, Wiezer MJ *et al.* Cytoreduction and HIPEC in the Netherlands: nationwide long-term outcome following the Dutch protocol. *Ann Surg Oncol* 2013; **20**: 4224–4230.
4 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.
5 Calon A, Lonardo E, Berenguer-Llergo A, Espinet E, Hernando-Momblona X, Iglesias M *et al.* Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. *Nat Genet* 2015; **47**: 320–329.
6 Del Rio M, Mollevi C, Bibeau F, Vie N, Selves J, Emile JF *et al.* Molecular subtypes of metastatic colorectal cancer are associated with patient response to irinotecan-based therapies. *Eur J Cancer* 2017; **76**: 68–75.
7 Trinh A, Trumpi K, De Sousa EMF, Wang X, de Jong JH, Fessler E *et al.* Practical and robust identification of molecular subtypes in colorectal cancer by immunohistochemistry. *Clin Cancer Res* 2017; **23**: 387–398.
8 De Sousa EMF, Wang X, Jansen M, Fessler E, Trinh A, de Rooij LP *et al.* Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat Med* 2013; **19**: 614–618.
9 Song N, Pogue-Geile KL, Gavin PG, Yothers G, Kim SR, Johnson NL *et al.* Clinical outcome from oxaliplatin treatment in stage II/III colon cancer according to intrinsic subtypes: secondary analysis of NSABP C-07/NRG oncology randomized clinical trial. *JAMA Oncol* 2016; **2**: 1162–1169.
10 Prada-Villaverde A, Esquivel J, Lowy AM, Markman M, Chua T, Pelz J *et al.* The American Society of Peritoneal Surface Malignancies evaluation of HIPEC with mitomycin C *versus* oxaliplatin in 539 patients with colon cancer undergoing a complete cytoreductive surgery. *J Surg Oncol* 2014; **110**: 779–785.
11 Simkens GA, Rovers KP, Nienhuijs SW, de Hingh IH. Patient selection for cytoreductive surgery and HIPEC for the treatment of peritoneal metastases from colorectal cancer. *Cancer Manag Res* 2017; **9**: 259–266.
12 Verwaal VJ, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H *et al.* Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy *versus* systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. *J Clin Oncol* 2003; **21**: 3737–3743.
13 Fleming ID, Cooper JS, Henson DE, Hutter RVF, Kennedy BJ, Murphy GF (eds). *AJCC Cancer Staging Manual* (5th edn). J. B. Lippincott: Philadelphia, 1997.
14 Bosman FT, Carneiro F, Hruban RH, Theise ND (eds). *WHO Classification of Tumours of the Digestive System*. International Agency for Research on Cancer (IARC) WHO Classification of Tumours. IARC: Lyon, 2010.
15 Compton C, Fenoglio-Preiser CM, Pettigrew N, Fielding LP. American Joint Committee on Cancer Prognostic Factors Consensus Conference: Colorectal Working Group. *Cancer* 2000; **88**: 1739–1757.
16 Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H *et al.* Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. *Mod Pathol* 2017; **30**: 1299–1311.
17 Huijbers A, Tollenaar RA, van Pelt GW, Zeestraten EC, Dutton S, McConkey CC *et al.* The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. *Ann Oncol* 2013; **24**: 179–185.
18 Ogino S, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K *et al.* Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. *Clin Cancer Res* 2009; **15**: 6412–6420.
19 Ubink I, Elias SG, Moelans CB, Laclé MM, van Grevenstein WMU, van Diest PJ *et al.* A novel diagnostic tool for selecting patients with mesenchymal-type colon cancer reveals intratumor subtype heterogeneity. *J Natl Cancer Inst* 2017; **109**.
20 Trumpi K, Ubink I, Trinh A, Djafarihamedani M, Jongen JM, Govaert KM *et al.* Neoadjuvant chemotherapy affects molecular classification of colorectal tumors. *Oncogenesis* 2017; **6**: e357.
21 Berdiel-Acer M, Sanz-Pamplona R, Calon A, Cuadras D, Berenguer A, Sanjuan X *et al.* Differences between CAFs and their paired NCF from adjacent colonic mucosa reveal functional heterogeneity of CAFs, providing prognostic information. *Mol Oncol* 2014; **8**: 1290–1305.
22 Vellinga TT, den Uil S, Rinkes IH, Marvin D, Ponsioen B, Alvarez-Varela A *et al.* Collagen-rich stroma in aggressive colon tumors induces mesenchymal gene expression and tumor cell invasion. *Oncogene* 2016; **35**: 5263–5271.
23 Isella C, Brundu F, Bellomo SE, Galimi F, Zanella E, Porporato R *et al.* Selective analysis of cancer-cell intrinsic transcriptional traits defines novel clinically relevant subtypes of colorectal cancer. *Nat Commun* 2017; **8**: 15107.

24 Massalou D, Benizri E, Chevallier A, Duranton-Tanneur V, Pedeutour F, Benchimol D *et al.* Peritoneal carcinomatosis of colorectal cancer: novel clinical and molecular outcomes. *Am J Surg* 2017; **213**: 377–387.
25 Franko J, Shi Q, Meyers JP, Maughan TS, Adams RA, Seymour MT *et al.*; Analysis and Research in Cancers of the Digestive System (ARCAD) Group. Prognosis of patients with peritoneal metastatic colorectal cancer given systemic therapy: an analysis of individual patient data from prospective randomised trials from the Analysis and Research in Cancers of the Digestive System (ARCAD) database. *Lancet Oncol* 2016; **17**: 1709–1719.
26 Richman SD, Seymour MT, Chambers P, Elliott F, Daly CL, Meade AM *et al.* KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol* 2009; **27**: 5931–5937.
27 Yao Q, Qu X, Yang Q, Wei M, Kong B. CLIC4 mediates TGF-beta1-induced fibroblast-to-myofibroblast transdifferentiation in ovarian cancer. *Oncol Rep* 2009; **22**: 541–548.
28 Kojima M, Higuchi Y, Yokota M, Ishii G, Saito N, Aoyagi K *et al.* Human subperitoneal fibroblast and cancer cell interaction creates microenvironment that enhances tumor progression and metastasis. *PLoS One* 2014; **9**: e88018.
29 Cai J, Tang H, Xu L, Wang X, Yang C, Ruan S *et al.* Fibroblasts in omentum activated by tumor cells promote ovarian cancer growth, adhesion and invasiveness. *Carcinogenesis* 2012; **33**: 20–29.
30 Kenny HA, Krausz T, Yamada SD, Lengyel E. Use of a novel 3D culture model to elucidate the role of mesothelial cells, fibroblasts and extra-cellular matrices on adhesion and invasion of ovarian cancer cells to the omentum. *Int J Cancer* 2007; **121**: 1463–1472.
31 Leung V, Huo YR, Liauw W, Morris DL. Oxaliplatin *versus* mitomycin C for HIPEC in colorectal cancer peritoneal carcinomatosis. *Eur J Surg Oncol* 2017; **43**: 144–149.

Supporting information

Additional supporting information can be found online in the supporting information tab for this article.