Clinical & Experimental Metastasis (2023) 40:431–440
https://doi.org/10.1007/s10585-023-10221-x

RESEARCH PAPER

Association between primary tumor characteristics and histopathological growth pattern of liver metastases in colorectal cancer

Ali Bohlok[1] · Camille Tonneau[1] · Sophie Vankerckhove[1] · Ligia Craciun[2] · Valerio Lucidi[3] · Fikri Bouazza[1] · Alain Hendlisz[4] · Jean Luc Van Laethem[5] · Denis Larsimont[2] · Peter Vermeulen[6] · Vincent Donckier[1] · Pieter Demetter[2]

Received: 19 December 2022 / Accepted: 3 July 2023 / Published online: 15 July 2023

Abstract

Introduction: The microarchitecture of liver metastases (LMs), or histopathological growth pattern (HGP), has been demonstrated to be a significant prognostic factor in patients undergoing resection of colorectal liver metastases (CRLMs). Currently, however, HGP can be only determined on the operative specimen. Therefore, the development of new tools to predict the HGP of CRLMs before surgery and to understand the mechanisms that drive these patterns is important for improving individualization of therapeutic management. In this study, we analyzed data from a retrospective series of patients who underwent surgery for CRLMs to compare primary tumor characteristics, including markers of local aggressiveness and migratory capacity, and HGP of liver metastases. Methods: Data from a retrospective series of 167 patients who underwent curative-intent resection of CRLMs and in whom pathological samples from both primary tumor and liver metastases were available were reviewed. At the primary tumor level, *KRAS* mutational status, grade of differentiation, and tumor budding were assessed. HGP was scored in each resected CRLM, according to consensus guidelines, and classified as desmoplastic (dHGP) or non-desmoplastic (non-dHGP). Associations between primary tumor characteristics and HGP of CRLMs were evaluated using a binary logistic regression model. Overall survival and disease-free survival were evaluated using Kaplan-Meier and multivariable Cox regression analyses. Results: CRLMs were classified as dHGP in 36% of the patients and as non-dHGP in 64%. Higher rates of moderately or poorly differentiated primary tumors were observed in the non-dHGP CRLM group (80%), as compared with the dHGP group (60%) (OR = 3.6; 95%CI: 1.6–7.05; p = 0.001). Higher rates of tumor budding were observed in the non-dHGP CRLM group, with a median tumor budding value of 4 as compared with 2.5 in the dHGP group (p = 0.042). In the entire series, 5-year overall and disease-free survival were 43% and 32.5%, respectively. The non-dHGP CRLM group had worse post-hepatectomy survival, with 5-year overall and disease-free survival of 32.2% and 24.6%, respectively, as compared with 60.8% and 45.9%, respectively, for the dHGP group (p = 0.02). Conclusion: Colorectal tumors with moderate or poor differentiation and those with high tumor budding are more frequently associated with CRLMs with a non-dHGP. This suggests that primary tumor characteristics of local aggressiveness and migratory capacity could preferentially promote the development of CRLMs with an infiltrating pattern and that these parameters should be considered as part of new scores for predicting HGP before surgery. This finding may stimulate new lines of research for more individualized therapeutic decision in patients with CRLM candidate to surgery.

Keywords Colorectal · Primary · Liver metastasis · Histological growth pattern

List of abbreviations

CEA	Carcinoembryonic antigen
CI	Confidence interval
CRLMs	Colorectal liver metastases
CRS	Clinical risk score
DFS	Disease-free survival

Ali Bohlok and Camille Tonneau contributed equally to this manuscript.

Vincent Donckier and Pieter Demetter co-senior authors

Extended author information available on the last page of the article

dHGP	Desmoplastic histological growth pattern
HE	Hematoxylin and eosin
HGP	Histological growth pattern
HR	Hazard ratio
IQR	Interquartile range
KRAS	Kirsten RAt Sarcoma virus gene
NACT	Neoadjuvant chemotherapy
non-dHGP	Non-desmoplastic histological growth pattern
OS	Overall survival
pHGP	Pushing histological growth pattern
PTB	Primary tumor budding
rHGP	Replacement histological growth pattern
SD	Standard deviation
TLI	Tumor-to-liver interface
VIF	Variance inflation factor
WHO	World Health Organization

Introduction

Surgery remains the only potentially curative treatment in patients with isolated colorectal liver metastases (CRLMs) [1, 2]. Currently, however, in the absence of accurate selection criteria [3], the majority of patients who undergo surgery with curative-intent for CRLMs still recur after surgery, including a significant proportion with rapid and aggressive relapses who carry a very poor prognosis [4–6]. Accordingly, the identification of new (bio)markers of metastatic behavior would represent major progress for improving the oncosurgical management of these patients.

Among various candidate markers, histopathological growth pattern (HGP) has now been demonstrated to be a robust prognostic factor in patients undergoing surgical resection for CRLMs [7–13]. Better postoperative outcomes have been reproducibly reported in patients who underwent surgery for CRLMs with desmoplastic HGP (dHGP), characterized by a peritumor fibrous rim, neoangiogenesis, and dense immune infiltrate at the tumor-to-liver interface (TLI), as compared with those with replacement HGP (rHGP), characterized by a more infiltrating form, without peritumor desmoplastic reaction, and with a direct growth of cancer cells into the liver parenchyma, vessel co-option, or and minimal-to-absent immune infiltrate at the TLI [8, 9, 12, 13].

The reproducibility of the prognostic value of HGP in patients undergoing surgery for CRLM strongly suggests that the tumor microenvironment of the liver metastases may accurately reflect the type of metastatic behavior in individual cases. Currently, however, the HGP status of each patient's CRLM can only be assessed by the complete pathological analysis of the entire TLI of each resected lesion. Targeted biopsies and dedicated imaging techniques are not able to distinguish between liver metastases with dHGP or rHGP before resection. Therefore, this information cannot be included in decision-making therapeutic algorithms for patients who are potentially candidates for surgery. Accordingly, the development of new tools to predict the HGP of CRLMs before surgery, such as dedicated imaging methods [14–16], and a better understanding of the driving mechanisms that lead to the development of liver metastases with distinct patterns could be important for improving individualized therapeutic management of these patients.

From this perspective, it can be hypothesized that primary tumor characteristics may play an important role in influencing the development of liver metastases with different pathological features. To evaluate this, we analyzed data for a retrospective series of patients who underwent surgery for CRLMs and compared primary tumor characteristics, including markers of local aggressiveness and migratory capacity, and HGP of liver metastases.

Patients and methods

Study population

Data from a consecutive series of patients who underwent curative-intent resection of CRLMs between January 2005 and December 2020 at Institut Jules Bordet and Hôpital Erasme (N = 444), Université Libre de Bruxelles, were retrospectively analyzed. Two hundred and fifty-eight patients, in whom both the hematoxylin and eosin (HE)-stained tissue sections of the primary colorectal cancer invasive border and of the entire TLI of the resected CRLMs were available, were included. Among them, 91 were excluded due to lack of data for evaluation of primary tumor budding (PTB), due to mucinous changes (n = 18), due to modifications induced by preoperative chemoradiotherapy (n = 50), or due to the lack of data for scoring HGP in CRLMs in the case of complete pathological response to preoperative chemotherapy (n = 23), leading to a study population of 167 patients (Flow chart, Fig. 1). The study was approved by the Ethical Committees of both institutions (CE2953, P2019/232).

Clinicopathologic and surgical data

Demographic data, primary tumor and CRLM clinicopathologic characteristics, and data on associated treatments were collected (Table 1). In each patient, the Clinical Risk Score (CRS) was calculated according to Fong, ranging from 0 to 5 [17]. Low- and high-risk CRS were defined as CRS scores between 0 and 2, or > 2, respectively [17, 18]. Chemotherapy was defined as neoadjuvant (NACT) or adjuvant when given before or after the surgery for the primary

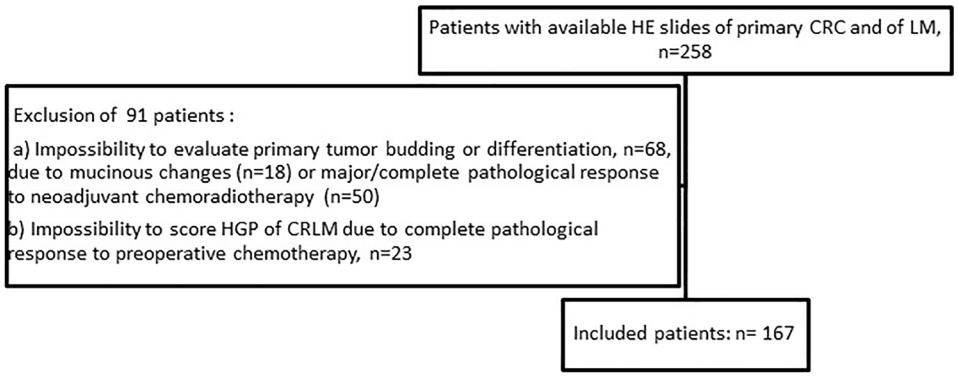

Fig. 1 Flowchart of the included patients

Table 1 Clinicopathological characteristics of the patient population and comparisons between patients with desmoplastic and non-desmoplastic histological growth patterns

	Overall population (n = 167)	dHGP (n = 60)	Non-dHGP (n = 107)	p	Multivariate rergression analysis	
					OR	P
Age, years (mean ± sd)	63.3 ± 10.1	62.2 ± 9	64 ± 10.7	0.122		
Female sex	101 (60.5%)	38 (63.3%)	63 (58.9%)	0.623		
Colonic localization						
Right colonic	23 (13.8%)	8 (13.3%)	15 (14%)	1		
Transverse colon	12 (7.2%)	4 (6.7%)	8 (7.5%)	1		
Left colon	86 (51.5%)	34 (56.7%)	52 (48.6%)	0.337		
Rectal	46 (27.5%)	14 (23.3%)	32 (29.9%)	0.470		
KRAS mutation (missing: 23)	54 (38%)	19 (37.3%)	35 (38.3%)	1		
T3-4	134 (80.2%)	51 (85%)	83 (77.5%)	0.827		
LN+ (missing = 9)	108 (68.4%)	41 (69.5%)	67 (67.7%)	0.861		
Synchronous	122 (73%)	45 (75%)	77 (72%)	0.719		
CEA > 200 (missing = 5)	15 (9.3%)	5 (8.6%)	10 (9.7%)	1		
Multiple LMs	116 (69.5%)	44 (73.3%)	72 (67.3%)	0.485		
Diam > 50 mm (missing = 1)	40 (24.1%)	12 (20.3%)	28 (26.2%)	0.452		
High risk CRS (3–5)	82 (49.1%)	32 (53.3%)	50 (46.7%)	0.621		
Differentiation (Well vs. others)				0.001	2.7 (1.29–5.62)	0.008
Well	47 (28.1%)	26 (43.3%)	21 (19.6%)			
Moderate	110 (65.9%)	27 (45%)	83 (77.6%)			
Poor	10 (6%)	7 (11.7%)	3 (2.8%)			
Tumor Budding cont (med [IQR])	4 [6]	2.5 [4]	4 [6]	0.042	0.94 (0.88–1.02)	0.122
TB categories				0.282		
Grade 1 (0–4)	99 (59.3%)	39 (65%)	60 (56.1%)			
Grade 2 (5–9)	45 (26.9%)	16 (26.7%)	29 (27.1%)			
Grade 3 (≥ 10)	23 (13.8%)	5 (8.3%)	18 (16.8%)			
Absent budding	31 (19.1%)	11 (19%)	20 (19.2%)	1		
Preoperative chemotherapy	102 (61.8%)	45 (77.6%)	57 (53.3%)	0.001	2.59 (1.23–5.47)	0.013

Abbreviations: CEA = carcinoembryonic antigen; CRS = clinical risk score; dHGP = desmoplastic histological growth pattern; non-dHGP = non-desmoplastic histological growth pattern; KRAS = Kirsten rat sarcoma viral oncogene homolog; LMs, liver metastases; LN + = lymph node positive; TB = tumor budding; IQR = interquartile range

tumor, respectively, and preoperative or postoperative when given within the 6 months before or after liver surgery, respectively.

Primary tumor pathological analyses

The analyses of the primary tumors were carried out by an expert pathologist in the field (PD), assisted by a PhD candidate (AB) and a surgical fellow student (CT), blinded to patient and HGP data. For each colorectal tumor, all of the HE slides at the invasive border were assessed and the most representative field was selected at 40X magnification. At this magnification, the grade of tumor differentiation (well, moderately, or poorly differentiated) was scored according to WHO criteria [19]. PTB was evaluated according to international guidelines [20]. The presence of buds, defined by the identification of a single cell or a group of a maximum of 4 cells detaching from the primary tumor at the level of the cell tumor invasion front, was evaluated at 200X magnification after selection of the most representative field at 40X magnification. In each tumor, PTB was categorized, according to current recommendations, into 4 grades, grade 0: no buds, grade 1: 1 to 4 buds, grade 2: 5 to 9 buds, and grade 3: >9 buds [20] (Fig. 2a and b).

Histopathological growth pattern of liver metastases

HGPs were evaluated according to international consensus guidelines [8]. In each patient, all available HE sections of the TLI of all available metastases were evaluated using light microscopy. All tissue sections were examined during a consensus HGP scoring session with three researchers, including 2 experienced pathologists (PD and PV). As different HGPs can be observed in the same metastasis, any HGP component of the interface with the adjacent liver was considered, even when only present in a limited fraction. According to the guidelines, in case of unique metastasis or when multiple slides were available, the HGP was determined as the mean percentage of each HGP per slide. In case of multiple metastases, the HGP was determined as the mean between the different metastases [8].

The dHGP was defined as the presence of a characteristic fibrotic rim surrounding the tumor with no direct liver cell-to cancer-cell interaction. In this pattern, the blood supply relies on angiogenesis, mirrored by endothelial proliferation and regions of high vessel density, called “hot spots”. The rHGP was defined as the absence of a peri-tumor fibrous rim. In these cases, cancer cells replace the hepatocytes, mimic the architecture of the surrounding liver parenchyma, and co-opt the liver sinusoidal vasculature for blood supply. In this form, there is often only a minimal or absent inflammatory infiltrate (Fig. 3a and b). A third pattern, defined as the pushing HGP (pHGP), was observed in rare cases. In these cases, the liver metastasis pushes away and compresses the surrounding liver tissue without fibrotic reaction. Similar to dHGP, the metastatic architecture is distinct from the surrounding hepatic parenchyma [8]. As it has been shown that the most discriminant prognostic categorization is obtained when comparing patients with a complete dHGP of all CRLMs with those in whom any of the metastases contain any proportion of rHGP or pHGP [8], we identified patients as dHGP or non-dHGP. Due to a minimal representation of patients classified as 100% desmoplastic in our retrospective cohort, we categorized patients into dHGP when this pattern represented ≥ 95% of the TLI of all resected CRLMs, and as non-dHGP when rHGP or pHGP was present in > 5% of the TLI.

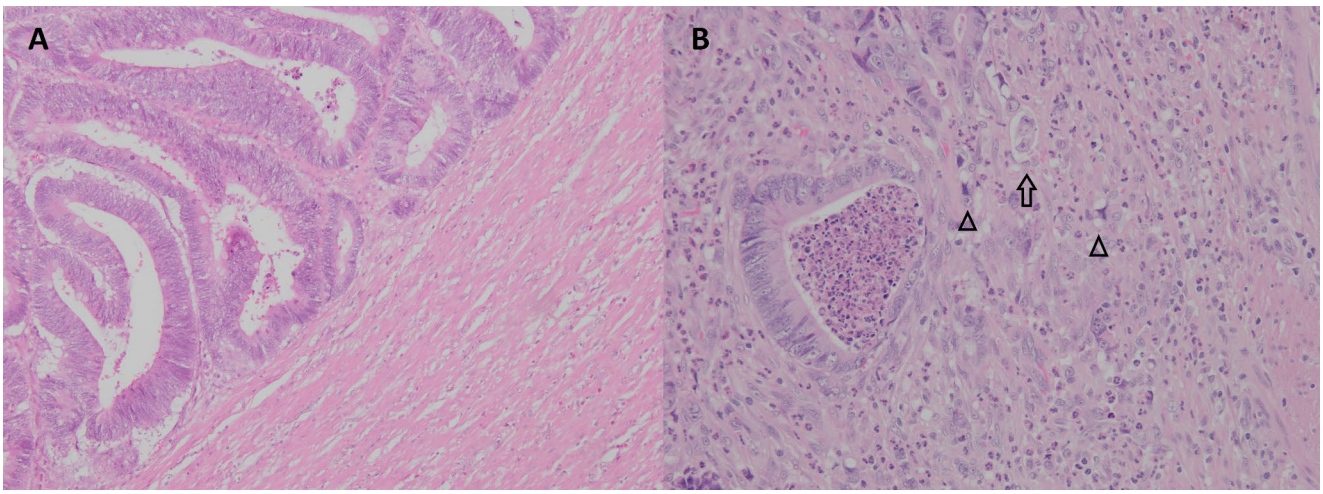

Fig. 2 H&E-stained slides of primary colorectal adenocarcinoma. **a:** well differentiated colonic adenocarcinoma with low-grade tumor budding indicated by black arrow (Magnification 200X); **b:** moderately differentiated colonic adenocarcinoma with high-grade tumor budding indicated by black arrows (Magnification 200X). H&E, hematoxylin and eosin

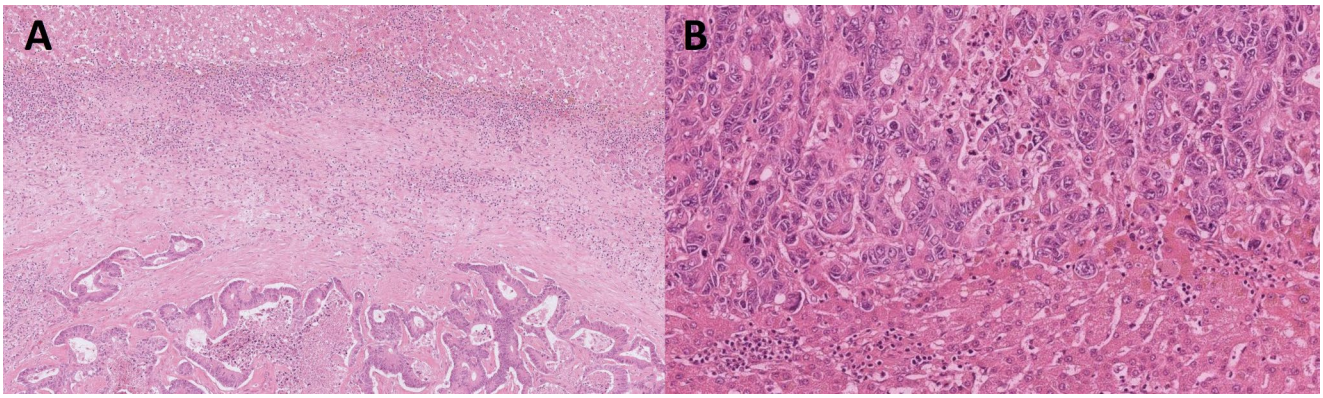

Fig. 3 H&E-stained slides of colorectal liver metastases (CRLMs). **a:** CRLM with a dHGP showing the desmoplastic rim that separates the tumor tissue from the liver parenchyma; **b:** CRLM with a rHGP showing regions where cancer cells grow into the liver cell plates and replace the hepatocytes. Cancer cells are in contact with the hepatocytes. dHGP, desmoplastic histological growth pattern; H&E, hematoxylin and eosin; rHGP, replacement histological growth pattern

Statistical analysis

The data were analyzed with the statistical software SPSS version 28. Values are expressed as medians (interquartile range [IQR]), means (standard deviations [SD]), or the number of patients with percentages. The median follow-up for survivors was calculated using the reverse Kaplan–Meier Method. Overall survival (OS) was defined as the time from resection of liver metastases to death or loss to follow-up. Disease-free survival (DFS) was defined as the time from resection of liver metastasis to recurrence or death. Survival estimates were obtained using the Kaplan–Meier method, and compared with the log-rank test. The factors affecting survival were evaluated using a univariate and multivariate cox regression analysis. Potential multi-collinearity in our Cox regression model was evaluated using the variance inflation factor (VIF). The VIFs for all variables in the multivariable Cox regression were determined. A VIF value below 3 indicates that there is no evidence of a multi-collinearity affecting the model, but should ideally be close to 1 [21, 22]. Proportional hazard regression results are reported using hazard ratio (HR) and corresponding 95% confidence interval (95%CI). Factors with a p- value of < 0.1 in univariate analysis were entered to a multivariate cox regression model. As a result, *KRAS* mutation status, lymph node status, and HGP were considered for multivariate analysis for OS and DFS. In addition, multiple LMs was considered for only DFS. A p-value $p < 0.05$ was considered statistically significant. For the comparison of the two groups (dHGP vs. non-dHGP), medians and means were compared using the Mann-Whitney U test and Student's t test, respectively. Differences in proportions were evaluated using the chi-square test. Factors with a p-value < 0.05 were entered into a multivariate logistic regression model. A p-value < 0.05 was considered statistically significant.

Results

Patient demographics and clinicopathological characteristics of primary tumors and liver metastases

The characteristics of the 167 patients are detailed in Table 1. Briefly, mean age was 63.3 ± 10.1 years and females represented 60.5% of the population. Primary tumors, including rectal tumors in 27.5%, were classified as T3 or T4 in 80.2% and associated with nodal metastases in 68.4%. Data for ***KRAS*** mutation evaluation were missing in 23 patients. Among the remaining cases ***KRAS*** mutation was present in 54 patients (38%). Primary tumors were well, moderately, and poorly differentiated in 28%, 66% and 6% of the cases, respectively. The median PTB was 4 [IQR = 6]. Grade I PTB (0 to 4) was present in 99 patients (59.3%), of whom 31 patients (19.1% of the whole population) had no PTB, Grade II was present in 45 patients (26.9%), and Grade III was present in 23 patients (13.8%).

For the CRLM, the majority of the patients had synchronous (73%) and multiple (69.5%) metastases, leading to a high-risk CRS in almost half of the cases (49.1%). Most of the patients (61.8%) received preoperative chemotherapy before surgery for CRLMs.

The mean number of slides analyzed per patient was 5 ± 3 slides. In the entire series, the median percentage of dHGP, rHGP, and pHGP was 68 [IQR = 82], 32 [IQR = 81], and 0[IQR = 0], respectively. Overall, 60 patients (36%) were scored with dHGP and 107 patients (64%) with non-dHGP (Table 1).

Associations between patient and primary tumor characteristics and histopathological growth pattern of the liver metastases

Overall, there was no difference between the groups of patients with dHGP or non-dHGP in terms of demographics, primary tumor localization and stage, *KRAS* mutated status, and extent of liver metastases (Table 1). Among the primary tumor histological characteristics, an association was observed between primary tumor differentiation and PTB and the HGP of the liver metastases (Table 1). In the non-dHGP group, 80% of the patients had moderately or poorly differentiated primary tumors as compared with 60% in the dHGP group (p = 0.001) (Table 1). In multivariable analysis, moderately and poorly differentiated primary tumors remained independently associated with non-dHGP (OR = 3.36; 95%CI: 1.60–7.05; p = 0.001) (Table 1). Similarly, the presence of PTB appeared to be preferentially associated with the development of CRLMs with a non-dHGP (Table 1). The median PTB was 2.5 [IQR = 4] in the dHGP group as compared with a median 4 [IQR = 6] in the non-dHGP group (OR = 0.93; 95%CI: 0.86–0.99; p = 0.042). This association did not reach statistical significance in multivariable analysis (Table 1).

Finally, the development of CRLMs with dHGP appeared to be significantly associated with the use of preoperative chemotherapy. 75% of the patients in the dHGP group received chemotherapy before liver surgery as compared to 53% in the non-dHGP group (p = 0.001) (Table 1). In multivariable analysis, the use of preoperative chemotherapy remained independently associated with the development of dHGP CRLMs (OR = 2.59; 95%CI: 1.23–5.47; p = 0.013) (Table 1).

Postoperative survival and prognostic factors

In the entire group, after a median follow-up of 86 months (range 56–116 months), 3- and 5-year OS were 59% and 43%, respectively, and 3- and 5-year DFS were 49.5% and 32.5%, respectively (Fig. 4a and b). Among the potential prognostic factors evaluated at the primary tumor level, only mutated *KRAS* status was significant for OS in univariate analysis, remaining significant in multivariable analysis (HR: 1.91; 95%CI: 1.22–2.98; p = 0.005) (Table 2). For DFS, positive lymph node status was significantly associated with an increased risk of recurrence in univariate but not in multivariable analyses (Table 2). Moderately or poorly differentiated tumors were also associated with poorer DFS (Table 2), but this factor was not included in multivariable analysis due to high collinearity with HGP type (VIF: 5.3). *KRAS* mutated status tended to be associated with a poorer DFS (Table 2). Of note, when considered as an isolated factor, presence or grade of PTB was not prognostic for OS or for DFS.

At the liver metastasis level, the synchronicity, the number and size of LMs, and carcinoembryonic antigen (CEA)

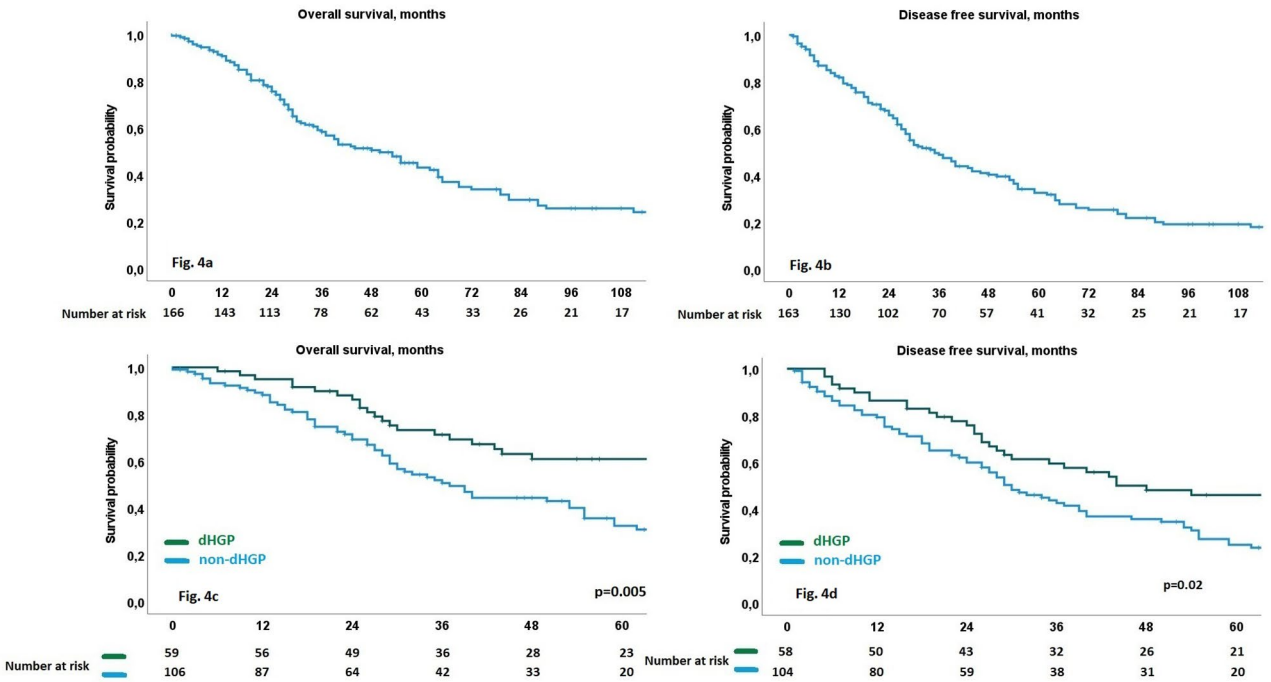

Fig. 4 Kaplan-Meier survival plots of overall survival (OS) and disease-free survival (DFS), in the whole cohort **(a** and **b)**, stratified by histological growth pattern (dHGP and non-dHGP) **(c** and **d)**. dHGP, desmoplastic histological growth pattern

Table 2 Univariate and multivariate analysis of prognostic factors for overall and disease-free survival

	Overall survival				Disease-free survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95%)	p	HR (95% CI)	P	HR (95%)	p
Age > 60	1.17 (0.78–1.77)	0.45			1.06 (0.73–1.53)	0.776		
Female sex	1.40 (0.92–2.12)	0.113			1.31 (0.90–1.91)	0.155		
Right colonic	1.31 (0.75–2.26)	0.343			1.41 (0.97–2.29)	0.159		
Rectal	1.30 (0.84–2.01)	0.235			1.30 (0.87–1.93)	0.195		
KRAS mutation (missing: 23)	1.84 (1.19–2.84)	0.006	1.91 (1.22–2.98)	0.005	1.47 (0.99–2.19)	0.054	1.40 (0.92–2.11)	0.113
T3-4	1.49 (0.82–2.68)	0.188			1.34 (0.79–2.25)	0.272)		
Positive Lymph node	1.53 (0.96–2.44)	0.071	1.11 (0.67–1.84)	0.680	1.67 (1.09–2.56)	0.018	1.33 (0.84–2.13)	0.227
Synchronous	1.05 (0.67–1.66)	0.825			1.35 (0.87–2.09)	0.175		
CEA > 200	1.26 (0.65–2.43)	0.497			1.32 (0.74–2.36)	0.344		
Multiple LMs	1.33 (0.83–2.14)	0.236			1.45 (0.94–2.24)	0.094	1.53 (0.91–2.56)	0.110
Diam > 50 mm	1.44 (0.91–2.27)	0.117			1.39 (0.91–2.10)	0.124		
Preoperative chemotherapy	0.83 (0.55–1.27)	0.398			0.96 (0.66–1.40)	0.823		
Differentiation (Well vs. others)	1.45 (0.93–2.24)	0.1			1.57 (1.05–2.35)	0.028		
Tumor Budding (cont)	1.02 (0.99–1.06)	0.203			1.01 (0.97–1.04)	0.65		
TB categories								
Grade 2 (5–9)	0.97 (0.61–1.54)	0.89			1.04 (0.69–1.56)	0.863		
Grade 3 (≥ 10)	1.36 (0.79–2.34)	0.268			1.11 (0.66–1.87)	0.697		
Presence of tumor budding	1.05 (0.64–1.74)	0.842			1.07 (0.68–1.71)	0.762		
Non-dHGP	1.82 (1.19–2.79)	0.006	2.06 (1.27–3.35)	0.003	1.82 (1.19–2.79)	0.006	1.57 (1.03–2.39)	0.038

Abbreviations: CEA = carcinoembryonic antigen; dHGP = desmoplastic histological growth pattern; IQR = interquartile range; KRAS = Kirsten rat sarcoma viral oncogene homolog; LMs, liver metastases; non-dHGP = non desmoplastic histological growth pattern; TB = tumor budding;

level were not significant for OS and DFS (Table 2). The only significant prognostic factor was HGP. In the non-dHGP group, 3- and 5-year OS were 51.7% and 32.2%, respectively, compared with 71.2% and 60.8%, respectively, in the dHGP group (Log-rank-p = 0.005), while 3- and 5-year DFS were 43.7% and 24.6%, respectively, compared with 59.4% and 45.9%, respectively, (Log-rank-p = 0.020) (Fig. 4c and d). In multivariable analysis, non-dHGP remained independently associated with poorer OS and DFS (HR: 2.06; 95%CI: 1.27–3.35; p = 0.003, and HR: 1.57; 95%CI: 1.03–2.39; p = 0.038, respectively). Finally, we observed no prognostic impact of preoperative chemotherapy for OS (HR = 0.83; 95%CI: 0.55–1.27; p = 0.398) and for DFS (HR = 0.96; 95%CI: 0.66–1.40; p = 0.823).

Discussion

There are still no accurate risk factors or models that are able to predict individual outcomes for patients undergoing surgery for CRLMs [3, 23]. As a consequence, the majority of patients who undergo curative-intent resection recur post-operatively, including a significant proportion with rapid and aggressive relapses [4, 5, 24]. Due to its reproducible prognostic value, the HGP of CRLMs represents a promising candidate marker for improving the selection of these patients for surgery. This parameter could also potentially be used to predict the benefit of a second resection in patients who recur after an initial intervention (23). Furthermore, recent data have demonstrated that the HGP of CRLMs may be useful for predicting the benefit of adjuvant chemotherapy after liver resection. In particular, chemo-naive patients with a non-dHGP seem to have a survival advantage when they receive adjuvant chemotherapy while patients with a dHGP do not [12, 25]. However, at the present time, this parameter is not available for therapeutic decision making as it is only assessable on the resected specimen. Therefore, the development of new tools for predicting the HGP of liver metastases before resection and a better understanding of the mechanisms leading to the development of liver metastases with different patterns (i.e., desmoplastic or non-desmoplastic) could represent significant progress for the improvement of the selection of these patients for surgery. One hypothesis is that primary tumor characteristics might influence the HGP of related liver metastases. To analyze these potential interactions, we investigated the role of primary tumor factors that reflect tumor aggressiveness and migratory capacity, such as tumor differentiation, presence

of PTB, and *KRAS* mutational status, in the context of the HGP of the liver metastases.

As a first result, we confirmed the strong prognostic value of HGP. In the present series, we observed almost a 50% decrease in 5-year postoperative OS and DFS in patients who underwent surgery for non-dHGP CRLMs, as compared to those with dHGP CRLMs. Notably, at the liver metastasis level, none of the other factors related to synchronicity, extent, size, or marker level were prognostic for OS or DFS.

At the primary tumor level, the presence of tumor budding has been shown to be associated with a higher risk of nodal spread and distant metastases, independently of primary tumor and nodal stage [26–28]. Furthermore, tumor differentiation has been consistently demonstrated to be an important independent prognostic factor in patients with colorectal cancer [29, 30]. The main observation of our study was that primary tumor differentiation and PTB are associated with the HGP of CRLMs. As compared with well-differentiated primary tumors, moderately and poorly differentiated tumors were independently associated with the development of liver metastases with non-dHGP. Similarly, we found a significant association between the extent of PTB and the HGP of CRLMs, with a higher number of buds observed in patients with non-dHGP as compared to those with dHGP in univariate analysis. In contrast, although *KRAS*-mutated tumors have been previously associated with liver metastases with a more aggressive or infiltrating behavior [31, 32], no association could be observed between *KRAS* mutational status and HGP in the present series. Furthermore, we evaluated whether these primary tumor characteristics remained of independent prognostic value in patients who underwent surgery for CRLMs or whether their influence is related to their association with HGP. Due to a high correlation with HGP, we could not include primary tumor differentiation in the multivariable model for OS and DFS. However, when we replaced HGP with tumor differentiation in this analysis, this parameter retained its prognostic value (data not shown). For PTB, we found no significant association with postoperative outcome. This could suggest that, in the metastatic condition, the prognosis could mainly depend on the biologic behavior of liver metastases, as reflected by their HGP, rather than on the initial characteristics of the primary tumor. In this interplay, the role of *KRAS* mutation remains unclear. Although we found no association between *KRAS* mutation and HGP of liver metastases, potentially related to the limited number of patients and to missing data, this factor remained independently prognostic for OS after resection of CRLMs. These results confirm previous research on the association between HGP of liver metastases and primary tumor characteristics in colorectal cancer [33, 34]. Several recent studies have similarly documented correlations between non-dHGP CRLMs and infiltrating histology of the primary tumor, high rate of PTB, and the presence of poorly differentiated clusters [33, 34]. It has also been shown that the combination of various primary tumor characteristics, such as right colonic location, tumor differentiation, PTB, infiltrating histology, non-mature stroma, lympho-vascular invasion, perineural invasion, presence of Crohn's like relation, and grade of tumor-infiltrating lymphocyte density could lead to new predictive scores for the HGP of related CRLMs [35].

Along with a relatively small number of patients, a significant limitation of our study is due to the fact that a majority of the patients received preoperative chemotherapy. It has been previously shown that the administration of systemic chemotherapy before resection of CRLMs is associated with an increase in the proportion of patients with dHGP [36]. The real significance of this remains unclear, as it has been shown that the prognosis of patients with dHGP CRLMs after chemotherapy remains significantly poorer as compared with chemo-naive patients with (spontaneous) dHGP [36, 37]. However, this might have, at least partially, biased our results and it could be expected that the relationships between primary tumor differentiation and budding and HGP would be stronger in chemo-naive patients. It should be acknowledged that, overall, our observations do not allow conclusions to be made regarding the effect of preoperative chemotherapy. Indeed, in addition to the limited number of patients and the retrospective nature of the study, patients with exquisite response to preoperative chemotherapy, as indicated by a complete pathological response, were excluded as HGP was not assessable in these cases. This could also have influenced the evaluation of the association between *KRAS* mutation and HGP that should be further verified in larger populations including patients without preoperative chemotherapy.

Taken together, these results confirm recent studies that have shown the role of primary tumor characteristics in driving the development of subsequent CRLMs with distinct HGPs. In the future, the combination of these factors into multiparametric models, including other characteristics of the primary tumor and data derived from liver metastasis imaging and radiomic algorithms, should be considered to develop new predictive scores that aim to improve the prognostication and selection of patients for CRLM surgery [11, 14–16]. Furthermore, this information could help to adapt preoperative strategies, for example to prioritize chemotherapy and avoid anti-angiogenic drugs in patients who are susceptible to having a non-dHGP CRLM phenotype.

Acknowledgements The authors acknowledge the contribution of a medical writer, Sandy Field, PhD, for English language editing of the manuscript.

Author contributions The authors confirm contribution to the paper as follows: study conception and design: AB, CT, PV, PD, VD; data collection: AB, CT, SV, LC, PD; analysis and interpretation of results: AB, VL, FB, JV, AH, DL, PV ; draft manuscript preparation: AB, CT,PV, PD, VD. All authors reviewed the results and approved the final version of the manuscript.

Funding This work has been funded by "Le Fonds Ithier" and "Les Amis de Bordet". The funding source had no role in preparation of this manuscript.

Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors have no conflicts of interest to disclose.

Ethics approval and consent to participate The study was approved by the Ethics Committee of Institute Jules Bordet (CE2953) and Hôpital Erasme P2019/232, and was in accordance with the Declaration of Helsinki, the International Conference on Harmonisation-Good Clinical Practice and local laws.

Consent for publication Not applicable.

References

1. Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D et al (2016 Aug) ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol [Internet] 27(8):1386–1422
2. LiverMetSurvey ARCAD [Internet]
3. Ayez N, Lalmahomed ZS, van der Pool AEM, Vergouwe Y, van Montfort K, de Jonge J et al (2011 Oct) Is the clinical risk score for patients with colorectal liver metastases still useable in the era of effective neoadjuvant chemotherapy? Ann Surg Oncol [Internet] 18(10):2757–2763
4. Viganò L, Capussotti L, Lapointe R, Barroso E, Hubert C, Giuliante F et al (2014) Early recurrence after liver resection for colorectal metastases: risk factors, prognosis, and treatment. A LiverMetSurvey-based study of 6,025 patients. Ann Surg Oncol 21(4):1276–1286
5. Viganò L, Gentile D, Galvanin J, Corleone P, Costa G, Cimino M et al (2022) Very Early Recurrence After Liver Resection for Colorectal Metastases: Incidence, Risk Factors, and Prognostic Impact. J Gastrointest Surg [Internet]. Mar 1;26(3):570–82
6. Ecker BL, Lee J, Saadat LV, Aparicio T, Buisman FE, Balachandran VP et al (2022) Sep Recurrence-free survival versus overall survival as a primary endpoint for studies of resected colorectal liver metastasis: a retrospective study and meta-analysis. Lancet Oncol [Internet] ;0(0)
7. Vermeulen PB, Colpaert C, Salgado R, Royers R, Hellemans H, Van den Heuvel E et al (2001 Oct) Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. J Pathol [Internet] 195(3):336–342
8. Latacz E, Höppener D, Bohlok A, Leduc S, Tabariès S, Fernández Moro C et al (2022) Histopathological growth patterns of liver metastasis: updated consensus guidelines for pattern scoring, perspectives and recent mechanistic insights. Br J Cancer 2022 1276 [Internet]. Jun 1;127(6):988–1013
9. Galjart B, Nierop PMH, van der Stok EP, van den Braak RRJC, Höppener DJ, Daelemans S et al (2019) Angiogenic desmoplastic histopathological growth pattern as a prognostic marker of good outcome in patients with colorectal liver metastases. Angiogenesis [Internet]. May 12;22(2):355–68
10. Höppener DJ, Nierop PMH, Herpel E, Rahbari NN, Doukas M, Vermeulen PB et al (2019 Aug) Histopathological growth patterns of colorectal liver metastasis exhibit little heterogeneity and can be determined with a high diagnostic accuracy. Clin Exp Metastasis [Internet] 15(4):311
11. Buisman FE, Giardiello D, Kemeny NE, Steyerberg EW, Höppener DJ, Galjart B et al (2022 Jun) Predicting 10-year survival after resection of colorectal liver metastases; an international study including biomarkers and perioperative treatment. Eur J Cancer [Internet] 1:168:25–33
12. Bohlok A, Inchiostro L, Lucidi V, Vankerckhove S, Hendlisz A, Van Laethem JL et al (2022) Aug Tumor biology reflected by histological growth pattern is more important than surgical margin for the prognosis of patients undergoing resection of colorectal liver metastases. Eur J Surg Oncol.
13. Eefsen RL, Vermeulen PB, Christensen IJ, Laerum OD, Mogensen MB, Rolff HC et al (2015 Apr) Growth pattern of colorectal liver metastasis as a marker of recurrence risk. Clin Exp Metastasis [Internet] 32(4):369–381
14. Han Y, Chai F, Wei J, Yue Y, Cheng J, Gu D et al (2020 Aug) Identification of predominant histopathological growth patterns of colorectal liver metastasis by Multi-Habitat and Multi-Sequence Based Radiomics Analysis. Front Oncol [Internet] 14:10:1363
15. Latacz E, van Dam PJ, Vanhove C, Llado L, Descamps B, Ruiz N et al (2020) Can medical imaging identify the histopathological growth patterns of liver metastases? Semin Cancer Biol [Internet].
16. Cheng J, Wei J, Tong T, Sheng W, Zhang Y, Han Y et al (2019) Prediction of Histopathologic Growth Patterns of Colorectal Liver Metastases with a Noninvasive Imaging Method. Ann Surg Oncol [Internet]. Dec 1;26(13):4587–98
17. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH (1999 Sep) Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg [Internet] 230(3):309–318 discussion 318 – 21
18. Tomlinson JS, Jarnagin WR, DeMatteo RP, Fong Y, Kornprat P, Gonen M et al Actual 10-Year Survival after Resection of Colorectal Liver Metastases defines cure. J Clin Oncol [Internet] 2007 Oct 10;25(29):4575–80
19. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P et al (2020) The 2019 WHO classification of tumours of the digestive system. Histopathology [Internet]. Jan 1;76(2):182
20. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H et al (2017) Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol. Sep 1;30(9):1299–311
21. Miles J, Shevlin M (2001 Mar) Applying regression and correlation: a guide for students and researchers. Sage, p 8
22. Kutner MH, Nachtsheim CJ, Neter J, Li W et al (2005) McGraw-Hili wa, Burr RIdge B,. Applied Linear Statistical Models Fifth Edition t:a Irwin.
23. Massaut E, Bohlok A, Lucidi V, Hendlisz A, Klastersky JA, Donckier V (2018) The concept of oligometastases in colorectal cancer: from the clinical evidences to new therapeutic strategies. Current Opinion in Oncology, vol 30. Lippincott Williams and Wilkins, pp 262–268

24. Viganò L, Ferrero A, Lo Tesoriere R, Capussotti L (2008 Sep) Liver surgery for colorectal metastases: results after 10 years of follow-up. Long-term survivors, late recurrences, and prognostic role of morbidity. Ann Surg Oncol [Internet] 15(9):2458–2464
25. Buisman FE, van der Stok EP, Galjart B, Vermeulen PB, Balachandran VP, van den Coebergh RRJ et al (2020) Histopathological growth patterns as biomarker for adjuvant systemic chemotherapy in patients with resected colorectal liver metastases. Clin Exp Metastasis [Internet]. Oct 1;37(5):593–605
26. Ueno H, Mochizuki H, Hashiguchi Y, Shimazaki H, Aida S, Hase K et al (2004) Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterol [Internet] 127(2):385–394
27. Ueno H, Kajiwara Y, Shimazaki H, Shinto E, Hashiguchi Y, Nakanishi K et al (2012 Feb) New criteria for histologic grading of colorectal cancer. Am J Surg Pathol [Internet] 36(2):193–201
28. Ueno H, Hase K, Hashiguchi Y, Shimazaki H, Yoshii S, Kudo S, ei et al (2014 Sep) Novel risk factors for lymph node metastasis in early invasive colorectal cancer: a multi-institution pathology review. J Gastroenterol [Internet] 25(9):1314–1323
29. Wiggers T, Arends JW, Volovics A (1988 Jan) Regression analysis of prognostic factors in colorectal cancer after curative resections. Dis Colon Rectum [Internet] 31(1):33–41
30. Newland RC, Dent OF, Lyttle MNB, Chapuis PH, Leslie Bokey E Pathologic determinants of Survival Associated with Colorectal Cancer with Lymph Node Metastases a multivariate analysis of 579 patients
31. Brudvik KW, Mise Y, Chung MH, Chun YS, Kopetz SE, Passot G et al RAS Mutation Predicts Positive Resection Margins and Narrower Resection Margins in Patients Undergoing Resection of Colorectal Liver Metastases. Ann Surg Oncol [Internet]. 2016 Aug 1;23(8):2635–43
32. Brudvik KW, Kopetz SE, Li L, Conrad C, Aloia TA, Vauthey JN (2015) Meta-analysis of KRAS mutations and survival after resection of colorectal liver metastases [Internet]. British Journal of Surgery, vol 102. John Wiley and Sons Ltd, pp 1175–1183
33. Wu JB, Sarmiento AL, Fiset PO, Lazaris A, Metrakos P, Petrillo S et al (2019 Jul) Histologic features and genomic alterations of primary colorectal adenocarcinoma predict growth patterns of liver metastasis. World J Gastroenterol [Internet] 14(26):3408–3425
34. Abe H, Yasunaga Y, Yamazawa S, Nakai Y, Gonoi W, Nishioka Y et al (2022 May) Histological growth patterns of colorectal cancer liver metastases: a strong prognostic marker associated with invasive patterns of the primary tumor and p53 alteration. Hum Pathol 1:123:74–83
35. Höppener DJ, Stook JLPL, Galjart B, Nierop PMH, Nagtegaal ID, Vermeulen PB et al (2022) The relationship between primary colorectal cancer histology and the histopathological growth patterns of corresponding liver metastases. BMC Cancer [Internet]. Dec 1;22(1):911
36. Nierop PMH, Höppener DJ, Buisman FE, van der Stok EP, Galjart B, Balachandran VP et al (2022) Preoperative systemic chemotherapy alters the histopathological growth patterns of colorectal liver metastases. J Pathol Clin Res [Internet]. Jan 1;8(1):48–64
37. Galjart B, Nierop PMH, van der Stok EP, van den Braak RRJC, Höppener DJ, Daelemans S et al (2019) Angiogenic desmoplastic histopathological growth pattern as a prognostic marker of good outcome in patients with colorectal liver metastases. Angiogenesis [Internet]. May 12;22(2):355–68

Authors and Affiliations

Ali Bohlok[1] · Camille Tonneau[1] · Sophie Vankerckhove[1] · Ligia Craciun[2] · Valerio Lucidi[3] · Fikri Bouazza[1] · Alain Hendlisz[4] · Jean Luc Van Laethem[5] · Denis Larsimont[2] · Peter Vermeulen[6] · Vincent Donckier[1] · Pieter Demetter[2]

✉ Vincent Donckier
vincent.donckier@bordet.be

[1] Surgical Oncology, Institut Jules Bordet, Université Libre de Bruxelles (ULB), Brussels, Belgium

[2] Pathology, Institut Jules Bordet, Université Libre de Bruxelles (ULB), Brussels, Belgium

[3] Abdominal Surgery, Hôpital Erasme, Université Libre de Bruxelles (ULB), Brussels, Belgium

[4] Digestive Oncology, Institut Jules Bordet, Université Libre de Bruxelles (ULB), Brussels, Belgium

[5] Hepato-Gastroenterology, Hôpital Erasme, Université Libre de Bruxelles (ULB), Brussels, Belgium

[6] Translational Cancer Research Unit, Gasthuiszusters Antwerpen Hospitals and University of Antwerp (CORE, MIPRO), Wilrijk, Antwerp, Belgium