

# Ki-67 index, tumor differentiation, and extent of liver involvement are independent prognostic factors in patients with liver metastases of digestive endocrine carcinomas

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## Abstract

The prognosis remains ill-defined in patients with liver metastases of well-differentiated (WD) digestive endocrine carcinomas (DEC) with high Ki-67 index. The objectives of this study were to determine whether Ki-67 index, tumor differentiation, and extent of liver involvement are independent prognostic factors in patients with DEC, and whether chemotherapy commonly used in patients with poorly differentiated (PD) carcinomas might be applied to those with high Ki-67 index but well-differentiated DEC. Sixty-three patients with DEC metastatic to the liver were retrospectively studied and divided into three prognostic groups. Group 1 comprised patients with well-differentiated carcinomas and Ki-67 index < 15% ( $n=28$ ), group 2 comprised those with well-differentiated carcinomas and Ki-67 index  $\geq 15\%$  ( $n=17$ ), and group 3 comprised those with poorly differentiated carcinomas ( $n=18$ ). Therapeutic strategy was decided in accordance to guidelines, and tumoral response rate was assessed by computed tomography scan (RECIST). Prognostic factors were determined by uni/multivariate analysis. The 5-year survival rates were 89, 36, and 6% in groups 1, 2, and 3 respectively ( $P<0.001$ ). Multivariate analysis showed that Ki-67 index  $\geq 15\%$ , poor tumor differentiation, and large liver tumor burden were independent predictors of poorer survival. Disease control rates after etoposide–cisplatin were 50 and 53% in groups 2 and 3 respectively (NS). In conclusion, Ki-67 index, tumor differentiation, and extent of liver involvement are independent prognostic factors in patients with liver metastases of DEC. Patients with well-differentiated carcinomas with high Ki-67 index ( $\geq 15\%$ ) have intermediate prognosis and a similar response rate to the etoposide–cisplatin combination as those with poorly differentiated carcinomas.

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## Introduction

Digestive endocrine tumors constitute a heterogeneous entity in terms of prognosis. Their individual natural history cannot be easily predicted at the time of diagnosis, although many studies have shown that the presence of liver metastases is a major prognostic factor (Madeira *et al.* 1998, Panzuto *et al.* 2005, Bilimoria *et al.* 2008, Pape *et al.* 2008a, Steinmuller

*et al.* 2008). Even in this latter group of patients, the clinical course is highly variable, depending mainly on tumor differentiation according to the WHO classification (Solcia *et al.* 2000). Furthermore, patients with metastatic well-differentiated carcinomas (WHO group 3, or stage IV of the recently published tumor, nodes, metastases (TNM) staging system) are still very heterogeneous, and several prognostic factors

have been used to improve the therapeutic strategy in these patients (Rindi *et al.* 2006, 2007). They include site and resection of the primary tumor, presence of extra-hepatic metastases, and functional status (Madeira *et al.* 1998, Van Gompel *et al.* 2004, Panzuto *et al.* 2005, Bilimoria *et al.* 2008). Furthermore, the extent of liver tumor involvement seems to be an important prognostic factor with better progression-free survival in patients with midgut digestive endocrine carcinomas (DEC) treated with octreotide and low liver tumor load (Rinke *et al.* 2009). Additional pathological prognostic markers are used, among which the determination of the proliferative index (Ki-67) is the most important, leading recently to the publication and the validation of a grading system under the auspices of the European Neuroendocrine Tumor Society (ENETS; Pelosi *et al.* 1996, Solcia *et al.* 2000, Rindi & Bordi 2005, Rindi *et al.* 2006, 2007, Pape *et al.* 2008a,b).

Few studies, however, have hitherto confirmed the influence of Ki-67 index on survival in patients with metastatic well-differentiated endocrine tumors (Pelosi *et al.* 1996, Clarke *et al.* 1997, Gentil Perret *et al.* 1998). Moreover, even if Ki-67 index is often low in patients with well-differentiated carcinomas, a significant proportion presents with high Ki-67 tumors, making therapeutic choices difficult (Solcia *et al.* 1998, Rindi & Bordi 2003, Rindi *et al.* 2006, 2007). Our preliminary experience with such patients suggested that they might be good responders to the etoposide-cisplatin combination used in patients with poorly differentiated endocrine carcinomas (Moertel *et al.* 1991, Mitry *et al.* 1999, Mitry & Rougier 2001).

We thus decided to perform a retrospective study in patients with liver metastases of DEC in order to determine whether Ki-67 index, tumor differentiation, and extent of liver involvement are independent prognostic factors, and to assess the tumoral response rate using etoposide-cisplatin in patients with 'aggressive' (high Ki-67 index) but well-differentiated DEC.

## Patients and methods

Sixty-three consecutive patients with DEC metastatic to the liver and available Ki-67 index diagnosed in the Pancreatology and Gastroenterology Department of Beaujon University Hospital between 08/97 and 07/06 were included in this retrospective study. Median follow-up from the diagnosis of liver metastases was 45 (3–134) months.

The diagnosis of DEC was histologically confirmed by conventional and immunohistochemical techniques (chromogranin A and synaptophysin), performed on

liver biopsy or surgical specimen of liver metastases. Localization of the primary tumor and of distant metastases was achieved in all patients by abdominal and thoracic computed tomography (CT) scan or abdominal MRI and somatostatin receptor scintigraphy. Endoscopic ultrasonography of the duodeno-pancreatic area, upper and lower gastrointestinal endoscopy, laparotomy with preoperative exploration of small bowel by enteroscopy, and positron emission tomography scan were performed when necessary.

## Pathological assessment

Pathological characteristics of the 63 patients were evaluated by a single pathologist blinded to the clinical data. Tumors were classified into two groups according to the WHO 2000 criteria: well-differentiated (WD) group and poorly differentiated (PD) group endocrine carcinomas. Ki-67 scoring method was also evaluated by the same pathologist. Ki-67 protein was detected with murine monoclonal antibody MIB-1 (Dako, Glostrup, Denmark). An automatized technique (Streptavidin peroxidase with an automate; Ventana, Tucson, AZ, USA) was used. Antigen retrieval was conducted by pretreatment with high temperature. The tissue distribution of the staining was identified at a high optical power (25× objective) to identify the hot spots. In these areas, the proliferative index was calculated in at least 2000 consecutive tumor cells and expressed as a percentage.

According to data of the literature available at the time of study initiation, we identified five Ki-67 index level groups categorized as ≤2%, 3–5%, 6–14%, 15–20%, and >20% (Pelosi *et al.* 1996, Solcia *et al.* 2000, Couvelard *et al.* 2006, Rindi *et al.* 2006, 2007).

## Radiological assessment

By a visual quantification of the liver volume infiltrated by the disease, a specialist in DET radiologist categorized the extent of liver involvement as ≤10%, 10–25%, 25–50%, and >50% (Rinke *et al.* 2009).

## Therapeutic strategy

Treatment was decided by the oncological multidisciplinary committee in accordance to available guidelines. Patients with poorly differentiated endocrine carcinomas thus received the etoposide-cisplatin combination (Moertel *et al.* 1991, Mitry *et al.* 1999, Mitry & Rougier 2001). Radical resection of primary tumor and metastases was performed whenever possible in the other two groups of patients (Chamberlain *et al.* 2000, Kianmanesh *et al.* 2008,

Steinmuller *et al.* 2008). When radical resection was not achievable due to the diffusion of metastases, surgery of the primary tumor was proposed for prevention of local complications, especially in patients with small bowel primaries (Eriksson *et al.* 2008).

Medical treatment was decided in case of tumor-related symptoms and/or liver tumor burden >50%, and/or progression of metastases according to the RECIST criteria on two consecutive imaging procedures performed at 6-month intervals (Skinazi *et al.* 1996, Steinmuller *et al.* 2008, Durante *et al.* 2009). Systemic chemotherapy using doxorubicin–streptozotocin, 5FU–dacarbazine, 5FU–streptozotocin, 5FU–leucovorin–CPT11, or temozolomide was proposed according to the site of primary tumor, previous treatments, and specific contraindications to any cytotoxic drug (Moertel & Hanley 1979, Moertel *et al.* 1994, Ducreux *et al.* 2006, Steinmuller *et al.* 2008, Maire *et al.* 2009). As mentioned above, however, patients with well-differentiated carcinomas and Ki-67 index  $\geq 15\%$  received the etoposide–cisplatin combination.

Hepatic chemoembolization was indicated in patients without extra-hepatic metastases, especially in those with small bowel primary (Dominguez *et al.* 1999, 2000, Marrache *et al.* 2007).

Tumor response rate was assessed by CT scan according to the RECIST criteria every 6 months in patients with well-differentiated endocrine carcinomas and every 4 months in those with high Ki-67 index/poorly differentiated endocrine carcinomas (Therasse *et al.* 2000).

### Statistical analysis

General characteristics were expressed as median and range or percentages. Comparisons of variables were performed using the Kruskal–Wallis test for continuous data and the  $\chi^2$  test or the Fisher's exact test for categorical data. For survival analysis, primary point used was the date of diagnosis of liver metastasis. The end point used was the date of last contact or death. Overall survival of the whole population was estimated by using the Kaplan–Meier method. Survival analysis was performed according to gender, age (<55 vs  $\geq 55$  years), resection of primary tumor (versus no resection), presence of extra-hepatic metastases (versus exclusive hepatic metastases), localization of primary tumor (pancreatic versus other), liver tumor burden ( $\leq 10$  vs 10–25 vs 25–50 vs >50%), tumor differentiation (WD versus PD group), and Ki-67 index (<15 vs  $\geq 15\%$ ). Survival curves were compared with the log-rank test. For the purpose of survival comparisons, three groups of patients were considered: those

with poorly differentiated carcinomas, those with well-differentiated carcinomas with low (<15%) Ki-67 index, and those with well-differentiated carcinomas with high Ki-67 index ( $\geq 15\%$ ). A multidimensional analysis was performed using a Cox regression analysis to search for prognostic factors of death. Dummy variables were created for two variables: extent of liver involvement and Ki-67 index. The stepwise selection option was used. *P* values below 0.20 were considered as significant as level of entry in the model. Data were analyzed with the SAS 9.1 statistical software for Windows (SAS Institute, Inc., Cary, NC, USA). All statistical tests were two-sided. The critical level of statistical significance was set at  $P < 0.05$ .

## Results

### General characteristics

Thirty-three men and 30 women had DEC metastatic to the liver, metachronous in 14% (Table 1). Median age was 54 (30–74) years. Twenty-three (37%) patients had extra-hepatic metastases. Primary tumor was located in the pancreas in 32 (51%) patients. Forty-five (71%) and 18 (29%) patients had well- and poorly differentiated carcinomas respectively. Ki-67 index was  $\leq 2$ , 3–5, 6–14, 15–20, and >20% in 6, 13, 9, 14, and 21 patients respectively. A pancreatic primary was found in 24 (53%) and 8 (44%) patients with well- and poorly differentiated carcinomas respectively.

The repartition of patients according to differentiation/Ki-67 index and according to differentiation/liver tumor burden is reported in Tables 2 and 3 respectively.

**Table 1** Characteristics of 63 patients with liver metastases of digestive endocrine carcinomas

	WD group <sup>a</sup>	PD group <sup>a</sup>	<i>P</i>
Number of patients ( <i>n</i> (%))	45 (71%)	18 (29%)	
Median age (range)	53 (30–74)	58 (33–71)	NS
Sex (M/F)	26/19	7/11	NS
Primary tumor			
Pancreas	24	8	NS
Small bowel	12	1	
Stomach	0	1	
Rectum	2	0	
Unknown	7	8	
Extra-hepatic metastases <sup>b</sup>	20 (44%)	3 (17%)	0.04

<sup>a</sup>WD, well-differentiated carcinomas; PD, poorly differentiated carcinomas.

<sup>b</sup>Peritoneum, bone, lymph node.

**Table 2** Tumor differentiation and Ki-67 index in 63 patients with liver metastases of digestive endocrine carcinomas

	WD group <sup>a</sup>	PD group <sup>a</sup>
Number of patients (n (%))	45 (71%)	18 (29%)
Ki-67		
≤2%	6	0
3–5%	13	0
6–14%	9	0
15–20%	14	0
>20%	3 <sup>b</sup>	18 <sup>c</sup>

<sup>a</sup>WD, well-differentiated carcinomas; PD, poorly differentiated carcinomas.

<sup>b</sup>Ki-67: 25%, n=2; 30%, n=1.

<sup>c</sup>Ki-67: 21%, n=1; 25%, n=2; 30%, n=4; 40%, n=3; 50%, n=5; 80%, n=2; 90%, n=1.

The repartition of treatments of metastatic DEC according to tumor differentiation is presented in Table 4.

Fourteen (31%) and seventeen (94%) patients received etoposide–cisplatin in the WD and PD groups respectively as first, second, or third line therapy. Twenty-five (56%) and three (17%) patients had a surgical resection of their primary tumor in the WD and PD groups respectively.

### Survival analysis

A total of 31 (49%) patients died of their disease after a median duration of 45 (3–134) months. The 1-, 3-, and 5-year survival rates for the whole cohort were 86, 61, and 52% respectively. The 5-year survival rates were 70 and 6% in patients with well- and poorly differentiated carcinomas respectively (Fig. 1,  $P < 0.0001$ ).

Survival of patients with high Ki-67 index was statistically lower than that of patients with lower proliferative index. The 5-year survival rates were 10, 35, 73, 92, and 100% in patients with Ki-67 index >20, 15–20, 6–14, 3–5, and ≤2% respectively ( $P < 0.0001$ ).

**Table 3** Tumor differentiation and extent of liver involvement in 63 patients with liver metastases of digestive endocrine carcinomas

	WD group <sup>a</sup>	PD group <sup>a</sup>
Number of patients (n (%))	45 (71%)	18 (29%)
≤10%	21	1
10–25%	11	0
25–50%	9	9
>50%	4	8

<sup>a</sup>WD, well-differentiated carcinomas; PD, poorly differentiated carcinomas.

In patients with well-differentiated carcinomas, the survival rates of those with high Ki-67 index were significantly lower than that of those with lower Ki-67 index. The 5-year survival rates were 33, 35, 73, 92, and 100% respectively (Fig. 2,  $P = 0.01$ ).

As far as prognosis is concerned, three groups were thus constituted. Group 1 comprised patients with well-differentiated carcinomas and low Ki-67 index <15% ( $n = 28$ , 44%), group 2 comprised those with well-differentiated carcinomas and high Ki-67 index (≥15%) ( $n = 17$ , 27%), and group 3 comprised those with poorly differentiated carcinomas ( $n = 18$ , 29%).

The 5-year survival rates were 88.7 and 35.8% in groups 1 and 2 respectively, while it was 6% in group 3 (median overall survival = 14 months; Fig. 3,  $P < 0.001$ ).

### Prognostic factors

Univariate Cox regression analysis showed that Ki-67 level ( $P < 0.0001$ ), tumor differentiation ( $P < 0.0001$ ), resection of the primary ( $P < 0.0001$ ), extent of liver involvement ( $P < 0.0001$ ), and gender (male > female,  $P = 0.03$ ) were significantly associated with survival, whereas primary site (pancreas versus others), extra-hepatic metastases, and age ≥55 years were not. Multivariate analysis showed that high Ki-67 index (≥15%) odds ratio (OR) 5.8; 95% confidence interval (CI) (1.67–20.16), poor tumor differentiation OR 3.38; 95% CI (1.38–8.22), and extent of liver involvement OR 1.67; 95% (1.11–2.5) were independent predictors of poorer survival. Male gender OR 0.3; 95% CI (0.16–0.79) was an independent predictor of better survival.

### Tumor response rate

Tumor response rates to etoposide–cisplatin in patients with well-differentiated carcinomas and Ki-67 index ≥15% (group 2) and with poorly differentiated carcinomas (group 3) are presented in Table 5. Disease control rates after etoposide–cisplatin (partial response and stabilization) were 50 and 53% in groups 2 and 3 respectively (NS).

### Discussion

In patients with DEC, differentiation and liver metastases are the two major prognostic factors influencing survival (Madeira et al. 1998, Panzuto et al. 2005, Bilimoria et al. 2008, Pape et al. 2008a, Steinmuller et al. 2008). Several studies have shown that Ki-67 index is an important prognostic marker correlated with survival for primary endocrine tumors,



**Table 4** Treatments of 63 patients with liver metastases of digestive endocrine carcinomas according to tumor differentiation

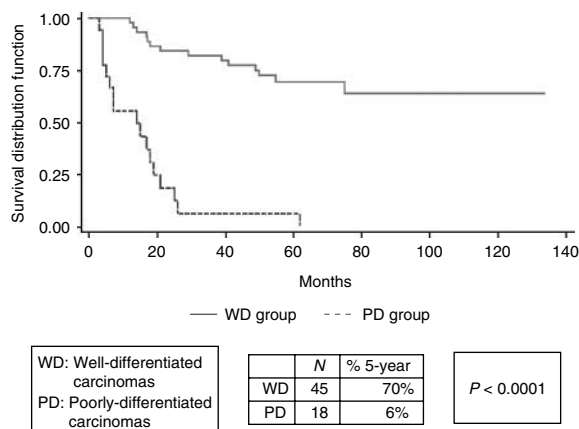
	WD group <sup>a</sup>	PD group <sup>a</sup>
Number of patients (n (%))	45 (71%)	18 (29%)
Surgical resection of liver metastases	15	1
Etoposide–cisplatin <sup>b</sup>	14	17
Other chemotherapy regimen	14	1
Resection of primary tumor	25	3
Chemoembolization	9	0
No treatment	1	0

<sup>a</sup>WD, well-differentiated carcinomas; PD, poorly differentiated carcinomas.

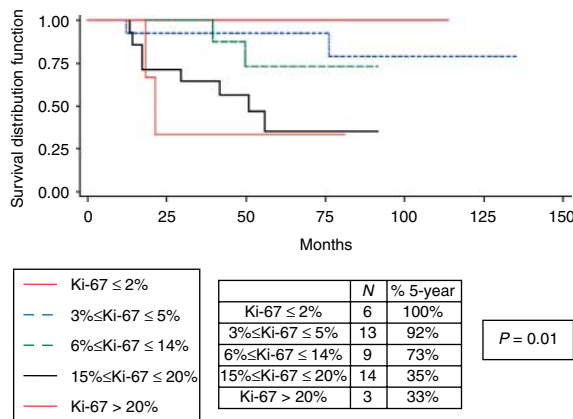
<sup>b</sup>As first-, second-, or third-line treatment.

but its role in the management of patients with liver metastases of well-differentiated DEC remains ill-defined (Pelosi *et al.* 1996, Clarke *et al.* 1997, Gentil Perret *et al.* 1998). Furthermore, all these patients are classified as WHO group 3 despite variable prognosis (Solcia *et al.* 2000). The recently proposed TNM classification clearly identified the prognostic value of metastases (stage M) and coined the importance of cell proliferation rate (grading system; Rindi *et al.* 2006, 2007). In the recently published PROMID study, the extent of liver involvement seemed to be an important prognostic factor with better progression-free survival in patients with midgut DEC treated with octreotide LAR and low liver tumor load ( $\leq 10\%$ ; Rinke *et al.* 2009).

We aimed to look at prognostic factors in 63 patients with DEC metastatic to the liver to determine the respective role of tumor differentiation, Ki-67 proliferation index, and extent of liver involvement. Owing to the recruitment specificities of our center, the primary tumor was located in the pancreas in 32 of 63 patients,



**Figure 1** Survival in patients according to tumor differentiation.



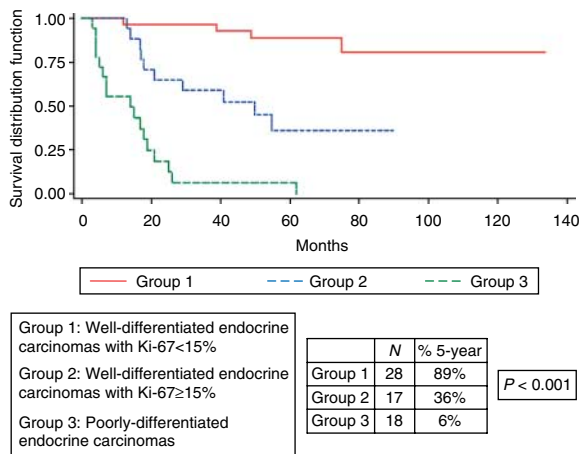
**Figure 2** Survival in patients with well-differentiated carcinomas according to Ki-67 index.

in contrast with other series where it was more often located in the small bowel (Modlin *et al.* 2003, Van Gompel *et al.* 2004).

We found a strong correlation between the 5-year survival rate and tumor differentiation on one hand (70 and 6% in patients with well- and poorly differentiated carcinomas respectively), as well as with Ki-67 index on the other hand (100, 92, 73, 35, and 10% in patients with Ki-67 index  $\leq 2$ , 3–5, 6–14, 15–20, and  $> 20\%$  respectively).

The 5-year survival rate of 6% and the median survival duration of 14 months in patients with poorly differentiated tumors (in whom Ki-67 index always exceeds 20%) are in agreement with that previously reported (Moertel *et al.* 1991, Mitry *et al.* 1999, Mitry & Rougier 2001). Noticeably, the 5-year survival rate in patients with well-differentiated metastatic endocrine carcinomas was higher than that generally reported (about 40% at 5 years; Madeira *et al.* 1998, Modlin *et al.* 2003). This could be due to our aggressive approach of liver metastases by intra-arterial hepatic chemoembolization and surgical resection in 24 and 30% of the patients respectively. The 5-year survival rate might indeed reach 70% in the latter group (Chamberlain *et al.* 2000, Elias *et al.* 2003, Kianmanesh *et al.* 2008, Steinmuller *et al.* 2008).

Concerning Ki-67 index, we decided to consider ENETS cut-off points of 2 and 20%, while the 5 and 15% values also seemed to be very useful in this group of patients for clinical and epidemiological considerations (only 3/45 patients in the WD group fell in the  $> 20\%$  Ki-67 index subgroup; Rindi *et al.* 2006, 2007). Indeed, an important proportion (17/45, 38%) of the patients with well-differentiated carcinomas had a Ki-67 index  $\geq 15\%$ . Most of them had a pancreatic primary tumor (15/17). Such patients had a poorer



**Figure 3** Survival in patients according to prognostic group (group 1 versus group 2 versus group 3).

prognosis than those with lower Ki-67 values. It is known that a proportion of well-differentiated carcinomas have a less predictable, moderately aggressive course, progressing rapidly at imaging. The evaluation of the proliferation index in well-differentiated carcinomas allows separating them from those that grow slowly, whereas their histological appearance can be very close.

We thus proposed to consider three groups with different prognosis. Group 1 comprised patients with well-differentiated, low Ki-67 (<15%) carcinomas. Group 2 comprised patients with well-differentiated, high Ki-67 carcinomas (≥15%). Group 3 comprised patients with poorly differentiated carcinomas. The 5-year survival rate in group 2 patients (35.8%) was significantly different from that of patients in groups 1 and 3 (88.7 and 6% respectively).

At multivariate analysis, high Ki-67 index (≥15%), poor differentiation, and large liver tumor burden were associated with a poorer survival. The first two parameters had the strongest prognostic value. The importance of tumor differentiation is well established

**Table 5** Tumor response rate to the etoposide–cisplatin combination in groups 2 and 3

	Group 2 <sup>a</sup>	Group 3 <sup>a</sup>	P
Number of patients (n (%))	20	18	
Number of patients treated	14 (70%)	17 (94%)	
Partial response	2	5	
Stabilization	5	4	
Disease control rate <sup>b</sup>	7 (50%)	9 (53%)	NS
Progression	6	8	

<sup>a</sup>Group 2, well-differentiated endocrine carcinomas with Ki-67 ≥ 15%; Group 3, poorly differentiated endocrine carcinomas.  
<sup>b</sup>Partial response and stabilization.

and addressed in the WHO classification (Madeira et al. 1998, Solcia et al. 2000, Panzuto et al. 2005). The most important finding in our study is that Ki-67 index is the best predictor of survival. Other studies already reported the interest of this parameter especially in pancreatic endocrine tumors, but the cut-off value varied from 2 to 20% (Pelosi et al. 1996, Clarke et al. 1997, Gentil Perret et al. 1998, Panzuto et al. 2005, Bettini et al. 2008, Faggiano et al. 2008, Pape et al. 2008a). Few of them considered only patients with liver metastases (Pelosi et al. 1996, Clarke et al. 1997, Gentil Perret et al. 1998). The extent of liver burden is also an independent prognostic factor as suggested by Rinke et al. (2009) in the PROMID study. This result can account for the fact that resection of the primary does not remain significant in multivariate analysis as previously published (Madeira et al. 1998).

Our results suggest that in patients with well-differentiated endocrine carcinomas with liver metastases, Ki-67 is the major prognostic factor. The WHO classification is thus probably not accurate enough in this respect. Hochwald et al. (2002) proposed a prognostic classification for pancreatic endocrine carcinomas resembling that used for endocrine carcinomas of the lung, adding grade and necrosis to WHO parameters. The ENETS grading system introduced in 2006 and 2007 considered digestive endocrine tumors according to Ki-67 values: G<sub>1</sub> (Ki-67 <2%), G<sub>2</sub> (Ki-67 2–20%), and G<sub>3</sub> (Ki-67 >20%; Rindi et al. 2006, 2007). The novel TNM classification and grading system have been recently validated by Pape et al. (2008a) and Strosberg et al. (2009). A lower Ki-67 threshold value was used in the current study (15%) for clinical and epidemiological reasons. It is difficult to assess the precise cut-off value above which one might consider a poorer prognosis in patients with well-differentiated metastatic DEC, which is probably between 15 and 20%. We believe that it is important to keep a sufficiently high Ki-67 index knowing the potential imprecision when measuring the percentage of cells stained and the intra-tumoral variations due to tissue heterogeneity (Couvelard et al. 2009). Twenty-eight of the 63 patients had a resection of their primary tumor. Among these 28 patients, the Ki-67 index was known in 21. In only 5 patients among the latter did the Ki-67 index differ between primary tumor and liver metastases. Only 4 patients had a higher Ki-67 index in metastases than in the primary tumor.

Finally, the disease control rates with the etoposide–cisplatin combination were similar in groups 2 and 3 (around 50%). This argues in favor of the major importance of cell proliferation rate in the choice of the chemotherapy regimen in patients

with DEC. This factor could be at least as important as tumor differentiation. The choice of a chemotherapy regimen used for poorly differentiated carcinomas in patients with well-differentiated tumors showing a high Ki-67 index could be a valuable therapeutic option, which should be validated by a prospective therapeutic trial.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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