Endocrine
DOI 10.1007/s12020-017-1474-3

ORIGINAL ARTICLE

Correlation between MGMT promoter methylation and response to temozolomide-based therapy in neuroendocrine neoplasms: an observational retrospective multicenter study

Davide Campana[1] · Thomas Walter[2] · Sara Pusceddu[3] · Fabio Gelsomino[4] · Emmanuelle Graillot[2] · Natalie Prinzi[3] · Andrea Spallanzani[4] · Michelangelo Fiorentino[5] · Marc Barritault[2] · Filippo Dall'Olio[5] · Nicole Brighi[5] · Guido Biasco[5]

Received: 13 July 2017 / Accepted: 13 November 2017

Abstract
Purpose Temozolomide (TEM) based therapy has been reported being effective in the treatment of metastatic neuroendocrine neoplasms (NEN), with response rates ranging from 30 to 70%. Among patients affected by advanced glioblastoma or melanoma and treated with TEM, loss of tumoral O6-methylguanine DNA methyltransferase (MGMT) is correlated with improved survival. In NEN patients, the role of MGMT deficiency in predicting clinical outcomes of TEM treatment is still under debate.
Methods In this study we evaluated 95 patients with advanced NENs undergoing treatment with TEM-based therapy. *MGMT* promoter methylation status was evaluated with two techniques: methylation specific-polymerase chain reaction or pyrosequencing.
Results Treatment with TEM-based therapy was associated with an overall response rate of 27.4% according to RECIST criteria (51.8% of patients with and 17.7% without *MGMT* promoter methylation). Response to therapy, progression free survival and overall survival was correlated to MGMT status at univariate and multivariate analysis. Methylation of *MGMT* promoter could be a strong predictive factor of objective response and an important prognostic factor of a longer PFS and OS.
Conclusion According to our results, *MGMT* methylation status, evaluated with methylation specific-polymerase chain reaction or pyrosequencing, should have an important role in patients with metastatic NENs, in order to guide therapeutic options. These results need further confirmation with prospective studies.

Keywords Neuroendocrine neoplasms · Temozolomide · MGMT · Chemotherapy · Capecitabine · Carcinoid

Introduction

Neuroendocrine neoplasms (NENs) are tumors arising from neuroendocrine cells. They are most frequently located in the lung, the pancreas and the gastrointestinal (GI) tract. Neuroendocrine tumors (NETs) have a reported incidence of 2.5–5 cases/100,000 people and a prevalence of 35/100,000 people [1]. NENs may be classified by grade (G)—G1 defined by mitotic index < 2 mitoses/10 high power fields (hpf) and Ki-67 ≤ 2%, G2 defined by mitotic index 2–20 mitoses/10 hpf and Ki-67 3–20%, and G3 defined by mitotic index > 20 mitoses/10 hpf and Ki-67 > 20% [2]. Another classification takes into account the expression of somatostatin receptors which can be targeted by somatostatin analogues (SSAs) and may represent a first line systemic treatment for stable or slowly progressive advanced disease. For those tumors not expressing somatostatin receptors and for those expressing them after progression to

✉ Davide Campana
davide.campana@unibo.it

[1] Department of Medical and Surgical Sciences, S.Orsola-Malpighi University Hospital, Bologna, Italy

[2] Hospices Civils de Lyon, University Hospital of Lyon, Lyon, France

[3] Department of Medical Oncology, ENETS Center of Excellence, Fondazione IRCCS 'Istituto Nazionale dei Tumori', Milan, Italy

[4] Department of Oncology and Haematology, Division of Oncology, University Hospital of Modena, Modena, Italy

[5] Department of Experimental, Diagnostic and Specialty Medicine, S.Orsola-Malpighi University Hospital, Bologna, Italy

Published online: 17 November 2017

SSAs, the therapeutic landscape is rapidly evolving and multiple treatment options are currently available. Therefore, sequencing and tailoring the various available treatments is becoming more and more challenging. Randomized prospective studies reported the good clinical outcome of molecular therapies targeting angiogenesis and the mTOR pathway, such as sunitinib [3] and everolimus [4, 5], in patients with advanced progressing NENs. Although targeted therapies are reported to frequently achieve prolonged progression-free survival (PFS) in patients with pNENs, they are rarely associated with objective tumor shrinkage [6].

Response rates ranging from 30 to 70% are reported when TEM-based chemotherapy regimens are used in the treatment of metastatic pNENs [7–10]. TEM is an oral alkylating agent, whose cytotoxic activity is related to DNA alkylation/methylation at the O6 and N7 positions of guanine, resulting in DNA mismatch and tumor cell death. The suicide enzyme O6-methylguanine DNA methyltransferase (MGMT) repairs DNA by removing the O6-alkylguanine adducts. High levels of MGMT, whose expression is regulated by mean of promoter methylation, contribute to chemoresistance by counteracting the therapeutic effect of alkylating agents [11].

A loss of tumoral MGMT is associated with improved survival in patients affected by advanced glioblastoma or melanoma treated with TEM [12–14]. In NEN patients, however, conflicting results have been reported so far [15–20], and the role of MGMT deficiency in predicting the clinical outcome of patients treated with TEM is still under debate. The objective of our study was to evaluate retrospectively the value of MGMT status in predicting treatment efficacy in a large cohort of NEN-patients treated with TEM-based therapy.

Patients and methods

Study design and patients

A multicenter, international retrospective analysis of prospective institutional database was performed. The study included all consecutive patients with histologically proven, locally advanced or metastatic NENs treated with TEM, alone or in combination with capecitabine, in the participating centers (Sant'Orsola Malpighi Hospital, Bologna, Italy; Centre Hospitalier Universitaire de Lyon, Lyon, France; Istituto Nazionale dei Tumori, Milano, Italy; Policlinico di Modena, Modena, Italy) from January 2008 to March 2016. Inclusion criteria were: (a) histologically proven neuroendocrine neoplasms, (b) signed informed consent for participation in clinical research studies; (c) complete follow-up period in one of the participating institutions and (d) known *MGMT* promoter methylation status or availability of tissue material for sequencing. The primary end-point was to evaluate the correlation between objective response and *MGMT* promoter status. The secondary end-point was to evaluate the correlation between *MGMT* promoter status and both PFS and overall survival (OS).

At baseline evaluation, all patients underwent clinical examination, haematological, liver and kidney function tests, a computed tomography scan (CT) and/or magnetic resonance imaging (MRI). Patients received TEM alone or in combination with capecitabine (CAPTEM). TEM was administrated orally for five consecutive days every 28 days at dose set by investigator (not standardized). In the combination, capecitabine was administrated orally every 28 days at dose set by investigator (days 1–14) and TEM as described above (days 10–14). All patients gave their informed written consent to the chemotherapy. The treatment was approved by the local Ethics Committee. Routine blood tests were performed before each cycle of therapy as well as during follow-up visits. The CT scan (or MRI) was repeated every 3 months (±1 month) until disease progression according to RECIST criteria (unless clinical conditions required shorter intervals).

Data analysis

All data were prospectively collected at the center where the patient had been enrolled. A single computerized data sheet was created and data regarding demographic, clinical and pathological features were retrospectively analysed. The histological specimens were examined by an experienced pathologist at each center. When required, an additional centralized revision of the tumor specimen was performed. Tumors were classified according to the 2010 WHO classification (gastro-entero-pancreatic neuroendocrine tumors) or 2004 WHO classification (thoracic neuroendocrine tumors) and the ENETS grading system [2, 21]. Ki-67 proliferation index was expressed as a percentage based on the count of Ki-67-positive cells in 2000 tumor cells in the areas of the highest immunostaining using the MIB1 antibody (DBA, Milan, Italy). The tumors were evaluated according to the RECIST criteria [22]. Objective response rate (ORR) was defined as the proportion of patients who achieved complete response (CR) or partial response (PR) as better response during therapy. Predictive factors for objective response were evaluated at univariate and multivariate analysis using logistic regression. Predictive factors were expressed as odds ratio (OR) [95 % confidence interval (CI)]. The multivariate model was designed using the forward stepwise method after including all variables. PFS was defined as the interval between the start of the therapy and the time of progression of disease (PD).

PFS was measured using the Kaplan-Meier method and the results were compared using the log-rank test. Predictive risk factors for PD were evaluated by univariate and multivariate analysis using the Cox proportional hazards method. Risk factors were expressed as hazard ratios (HR) [95% confidence interval (CI)]. The multivariate model was designed using the forward stepwise method after including all variables. All analyses carried out for predictive and risk factors are listed in the tables. The distribution of the continuous variables was reported as median and interquartile range (IQR, 25th to 75th percentiles). The comparison between the subgroups was performed using Pearson's chi-square test (Fisher's exact test was used when appropriate) or the Mann–Whitney *U* test for continuous variables. The *p* value was considered significant when inferior to 0.05. Statistical analysis was performed using a dedicated software (IBM—SPSS Statistics v. 22).

MGMT promoter methylation

MGMT promoter methylation status was evaluated using two techniques: methylation specific-polymerase chain reaction (MS-PCR) or pyrosequencing (PSQ), obtaining, respectively, qualitative and quantitative information. All samples examined contained more than 80% tumor cells. DNA extraction from formalin-fixed, paraffin-embedded tissue was performed after deparaffinization using a purification kit (MasterPure DNA, Epicentre, Madison, WI, USA). Genomic DNA was modified by bisulfite conversion (EZ DNA Methylation Gold Kit, Zymo, Irvine, CA, USA). For MS-PCR, we used the primers and PCR conditions described by Dong et al. [23].

Pyrosequencing was performed using the commercially available PyroMark Q24 CpG MGMT kit (Qiagen, Hilden, Germany) on a PyroMark Q24 System (Qiagen). Data were analyzed and quantified with the PyroMark Q24 Software 2.0.7 (Qiagen). The mean percentage of the five CpG methylated islands detected by the kit was used for analysis [24, 25]. For pyrosequencing analysis, an 8% cut off was used, accordingly to neuro-oncology clinical practice [25]. *MGMT* was considered methylated if methylated alleles were more than not methylated alleles by at least 8%; otherwise *MGMT* was scored as not methylated.

Results

Demographics and tumor characteristics

One hundred and four patients affected by NENs were enrolled at the participating centers. Nine patients were excluded at statistical analysis because TEM-based therapy was interrupted early (median: 2 cycles, range 1–3) without radiological evidence of progression (due to worsening of clinical conditions in five patients and to drug-related toxicity in four patients). The characteristics of the 95 patients are listed in Table 1. There were 52 men and 43 women with a mean age of 62 years (range 20–84 years). In 43 of the 95 patients (45.3%), the primary lesion was located in the pancreas, in 22 (23.2%) it was in the lung, whereas in 30 (31.6%) it was in: GI tract (21 patients), larynx (1 patient), unknown (6 patients) or adrenal gland (2 patients). According to the 2010 WHO classification, 7

Table 1 General features of the 95 patients treated with TEM-based therapy and according to MGMT status

	Total ($n = 95$)		MGMT + ($n = 27$)		MGMT- ($n = 68$)		
	N	%	N	%	N	%	p
Sex							
Male	52	54.7	17	63.0	35	51.5	0.310
Female	43	45.3	10	37.0	33	48.5	
Primary Site							
Pancreas	43	45.3	13	48.1	30	44.1	0.796
Lung	22	23.2	5	18.5	17	25.0	
Other	30	31.6	9	33.4	21	30.9	
Grade							
Low grade	68	71.6	16	64.0	52	77.6	0.186
High grade	24	25.3	9	36.0	15	22.4	
Not evaluable	3	3.2					
Lines of therapy							
First	20	21.0	4	14.8	16	23.5	0.347
Subsequent	75	78.9	23	85.2	52	76.5	
Previous Chemotherapy							
Yes	37	38.9	15	55.5	22	32.3	**0.036**
No	58	61.1	12	44.5	46	67.7	
Median Ki67	13.0		16.5		12.5		0.824
IQR	6–25		7–30		4–21		
Therapy							
TEM alone	41	43.2	12	44.4	29	42.6	0.873
CAPTEM	54	56.8	15	55.6	39	57.4	
Best Response							
CR+PR	26	27.4	14	51.8	12	17.7	**0.001**
SD+PD	69	72.6	13	48.2	56	82.3	
MGMT determination							
PSQ	53	55.8	15	55.5	38	55.9	0.977
MS-PCR	42	44.2	12	44.5	30	44.1	

MGMT+ MGMT promoter methylated, *MGMT−* MGMT promoter not methylated, *TEM* temozolomide, *CAPTEM* capecitabine + temozolomide, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *PSQ* pyrosequencing, *MS-PCR* methylation specific-polymerase chain reaction

Bold values are statistical significance

patients (7.4%) had a NET G1, 43 (45.3%) had a NET G2 and 20 (21.0%) had a neuroendocrine carcinoma (NEC); according to the 2004 WHO classification, 5 (5.3%) had a typical carcinoid of the lung, 13 (18.9%) an atypical carcinoid and 4 (4.2%) a large cell neuroendocrine carcinoma. Three of the 95 patients presented with a not otherwise specified NEN diagnosis and histological revision was not possible due to the scarcity of the tissue samples. Considering pathological characteristics, patients were divided in low-grade (68 patients with NET G1, NET G2, typical and atypical carcinoids) and high grade (24 patients with NECs and large cell lung NECs). Median Ki67 was 13.0% (IQR: 6–25%). Tumors were metastatic in 94 patients and locally advanced in 1 patient. Ninety-three (97.9%) patients progressed at the time of TEM-based therapy start. TEM-based therapy was first line treatment in 20 (21.0%) patients and a subsequent line in 75 (78.9%). Among these latter 75 cases, 37 (38.9%) patients had been treated previously with another chemotherapy regimen.

MGMT status

MGMT status was evaluated with PSQ in 53 (55.8%) patients and MS-PCR in 42 (44.2%). Twenty-seven patients (28.4%) presented a MGMT promoter methylation (MGMT +), while 68 (71.6%) had MGMT promoter not methylated (MGMT−). As reported in Table 1, there was no difference in MGMT promoter methylation when considering sex, primary site (pancreas, lung, other), grading (low and high grade), Ki67, chemotherapy regimen (TEM alone or CAPTEM) and type of MGMT determination.

Treatment outcome

Patients received a median of six cycles of chemotherapy (range 1–45). Reasons for treatment discontinuation included radiological tumor progression ($n = 48$), maximal response or chemotherapy break (at physician's discretion; $n = 38$), unacceptable toxicity ($n = 1$) and patient decision ($n = 1$). Seven patients were still on treatment at the time of data analysis. All 95 patients were assessable for radiographic response. When best response to therapy was evaluated, 26 (27.4%) patients experienced PR according to RECIST criteria, whereas 42 (44.2%) had stable disease and 27 (28.4%) experienced PD. Fourteen patients MGMT+ (51.8%) and 12 patients MGMT− promoter methylation (17.7%; $p = 0.001$) achieved an objective response (Table 1).

As reported in Table 2, CAPTEM regimen (OR: 3.43, $p = 0.019$), MGMT promoter methylation (OR: 5.03, $p = 0.001$) and pancreatic primary site (OR: 3.85, $p = 0.030$) resulted being predictive factors of objective response at univariate analysis. Multivariate analysis confirmed the role of CAPTEM therapy (OR: 3.87, $p = 0.019$) and MGMT promoter methylation (OR: 6.53, $p = 0.001$).

Table 2 Predictive factors of objective response at univariate and multivariate analysis

	Univariate			Multivariate		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Male gender	1.18	0.47–2.93	0.723	–	–	–
Primary site						
Pancreas vs. other	**3.85**	**1.14–13.06**	**0.030**	–	–	–
Lung vs. other	2.44	0.59–9.98	0.216	–	–	–
High grade	1.39	0.51–3.79	0.522	–	–	–
First line of therapy	1.18	0.40–3.49	0.767	–	–	–
Previous Chemotherapy	0.97	0.38–2.45	0.952	–	–	–
CAPTEM Therapy	**3.43**	**1.23–9.58**	**0.019**	**3.87**	**1.25–11.97**	**0.019**
MGMT+	**5.03**	**1.89–13.38**	**0.001**	**6.53**	**2.23–19.10**	**0.001**

MGMT+ MGMT promoter methylated, *CAPTEM* capecitabine + temozolomide, *OR* odds ratio

Bold values are statistical significance

Progression-free survival

At the time of data cut-off, 74 patients (77.9%) had PD. Median PFS was 10 months (95% CI: 5.6–14.4 months; Fig. 1). Significant differences in PFS were observed according to MGMT promoter methylation with median PFS being 21 and 8 months for MGMT+ and MGMT−, respectively ($p = 0.017$, Fig. 2). Differences in PFS were also correlated with previous chemotherapy (yes/no: 6/17 months, $p = 0.039$) and objective response during treatment (median PFS: 27 vs. 7 months; $p = 0.001$); however, no statistically significant difference was observed when correlating PFS to sex (median in male: 11 months, female: 9 months; $p = 0.881$), grading (median in high grade: 9 months, low grade: 12 months; $p = 0.309$), primary site (median in pancreas: 13 months, lung: 12 months, other: 7 months; $p = 0.212$), regimen (median in CAPTEM: 13 months, TEM: 7 months, $p = 0.758$) and line of therapy (median in first line: 17 months, not first line: 9 months, $p = 0.336$).

Risk factors for PFS were reported in Table 3. At univariate analysis, significant risk factors were MGMT- (HR: 1.88, $p = 0.023$), higher Ki67 (HR: 1.01, $p = 0.029$) and previous chemotherapy (HR: 1.60, $p = 0.048$). These risk factors, previously identified at univariate analysis, were also confirmed at multivariate

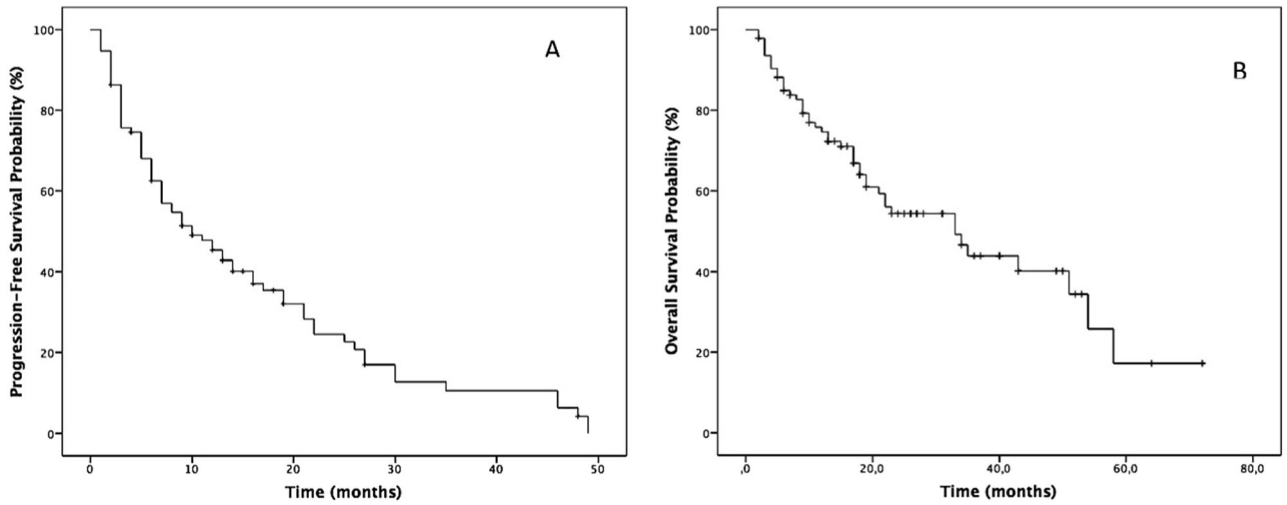

Fig. 1 Kaplan-Meier estimates of progression-free survival **a** and overall survival **b** in 95 patients treated with TEM-based therapy

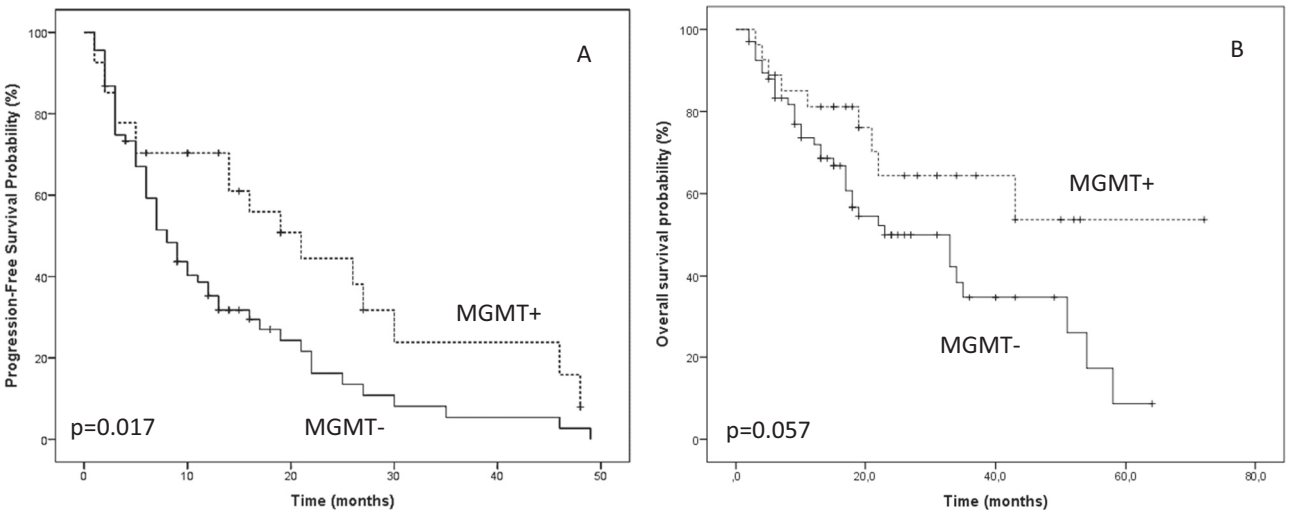

Fig. 2 A. Kaplan-Meier estimates of progression-free survival **a** and overall survival **b** in 95 patients treated with TEM-based therapy according to MGMT status. *MGMT*− MGMT promoter not methylated, *MGMT*+ MGMT promoter methylated

analysis: MGMT- with HR: 2.62 ($p = 0.003$), higher Ki67 with HR: 1.02 ($p = 0.019$) and previous chemotherapy with HR: 1.92 ($p = 0.014$). Sex, primary site, grading, chemotherapy regimen and line of therapy were not statistically significant.

Overall survival

A total of 46 (48.9%) out of 95 patients died during follow-up. Median OS was 33 months (95% CI: 19.9–46.1 months; Fig. 1b). Differences in OS were observed when stratified according to MGMT promoter methylation with median OS being "not reached" and 23 months for MGMT+ and MGMT−, respectively ($p = 0.057$, Fig. 2b). Differences in OS were also correlated with objective response during therapy (median OS: 54 vs. 19 months; $p = 0.001$), grading (median OS in high grade: 17 months, low grade: 35 months; $p = 0.017$) and previous chemotherapy (median OS: yes/no 19/54 months; $p = 0.001$). However, no statistically significant difference was observed when correlating OS with sex (median in male: 33 months, female: 23 months; $p = 0.971$), primary site (median in pancreas: 35 months, lung: 58 months, other: 17 months; $p = 0.095$), regimen (median in CAPTEM: 33 months, TEM: 34 months, $p = 0.497$) and line of therapy ($p = 0.106$). Risk factors for OS were reported in Table 4. At multivariate analysis MGMT− (HR: 2.87, $p = 0.003$), higher Ki67 (HR: 1.02, $p = 0.001$) and previous chemotherapy (HR: 2.87, $p = 0.003$) were risk factors for poorer OS.

Table 3 Risk factors of progression free survival during follow-up after univariate and multivariate analysis

	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Male gender	0.97	0.60–1.54	0.885	–	–	–
Primary site						
Other vs. Lung	1.58	0.84–2.97	0.154	–	–	–
Other vs. Pancreas	1.49	0.88–2.52	0.141	–	–	–
High grade	1.31	0.76–2.23	0.327	–	–	–
First line of therapy	0.75	0.41–1.37	0.355	–	–	–
Previous Chemotherapy	**1.60**	**1.01–2.54**	**0.048**	**1.92**	**1.14–3.22**	**0.014**
CAPTEM Therapy	0.93	0.58–1.49	0.766	–	–	–
MGMT-	**1.88**	**1.09–3.24**	**0.023**	**2.62**	**1.38–5.00**	**0.003**
Ki67*	**1.01**	**1.00–1.03**	**0.029**	**1.02**	**1.00–1.03**	**0.019**

MGMT– MGMT promoter not methylated, *CAPTEM* capecitabine + temozolomide, *HR* hazard ratio
*Continuous variable

Bold values are statistical significance

Table 4 Risk factors of overall survival related during follow-up after univariate and multivariate analysis

	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Male gender	1.01	0.56–1.84	0.971	–	–	–
Primary site						
Other vs. Lung	1.77	0.79–3.98	0.168	–	–	–
Other vs. Pancreas	**1.99**	**1.02–3.87**	**0.042**	–	–	–
High grade	**2.16**	**1.12–4.15**	**0.021**	–	–	–
First line of therapy	**0.49**	**0.21–1.19**	**0.016**	–	–	–
Previous Chemotherapy	**2.66**	**1.44–4.91**	**0.002**	**2.87**	**1.44–5.75**	**0.003**
CAPTEM Therapy	0.82	0.45–1.47	0.501	–	–	–
MGMT-	2.00	0.96–4.16	0.064	**2.91**	**1.24–6.86**	**0.014**
Ki67*	**1.03**	**1.01–1.04**	**<0.001**	**1.02**	**1.01–1.04**	**0.001**

MGMT– MGMT promoter not methylated, *CAPTEM* capecitabine + temozolomide, *HR* hazard ratio
*Continuous variable

Bold values are statistical significance

Discussion

In this series, we have evaluated retrospectively the predictive value of MGMT methylation status by MS-PCR or PSQ in a large cohort of NEN patients treated with TEM-based chemotherapy. We observed that MGMT promoter methylation was a strong predictive factor for objective response and an important prognostic factor of longer PFS and OS.

It has been widely reported that MGMT status is a powerful predictor of response to TEM for newly diagnosed glioblastoma patients [26]. MGMT status is commonly assessed by immunohistochemistry (IHC), MS-PCR, quantitative MS-PCR (qMS-PCR), and/or PSQ. Immunohistochemistry stain for MGMT protein expression was the easiest method to perform, but it had a lower predictive power for PFS and OS. On the contrary, PSQ, MS-PCR, and qMS-PCR represent the gold standard methods to evaluate MGMT status [27]. In the last 10 years, evidences suggesting the efficacy of TEM in metastatic NENs are mounting [7–10, 15, 18, 19]. Therefore, the use of TEM, either as mono- or combination therapy with capecitabine, has been reported for the first time in the most recently updated ENETS guidelines [28]. Nevertheless, previous studies concerning the role of MGMT status in NENs reported contrasting results. In two previous studies [15, 19, 29–31] no correlation was found between MGMT status and response to TEM. In three other studies [16, 18, 20], a predictive value was found only in the sub-group of pancreatic NENs. In all these series, the evaluation of MGMT status was observed in a scarce number of cases and only in three series MS-PCR or PSQ techniques were used [18, 29, 30].

The primary end-point of our study was the identification of a correlation between MGMT promoter methylation

(evaluated with MS-PCR and PSQ) and objective response in patients treated with TEM-based therapy. Our data strongly suggest that MGMT methylated tumors are more sensible to TEM-based chemotherapy. In fact, we showed a ORR of 51.8 and 17.7% in patients with or without MGMT promoter methylation, respectively. The presence of MGMT promoter methylation represented a strong predictive factor for response at univariate and multivariate analysis (OR: 8.67, $p < 0.001$). In 2015, Walter et al. reported the association of MGMT status (evaluated by MSP and PSQ) and ORR in 53 patients affected by GI and lung NENs [17]. The association of capecitabine and TEM compared to TEM alone (OR: 4.5, $p = 0.015$) resulted being a further independent factor for ORR. This data was not previously reported and a randomized phase II study comparing TEM vs. CAPTEM is currently ongoing (ClinicalTrials.gov Identifier: NCT01824875) to prospectively demonstrate this data. This evidence, if confirmed, could have a strong clinical relevance in all situations in which tumor shrinkage is needed, such as in presence of a high, symptomatic tumor burden, or when a down-staging for borderline surgically resectable tumors is needed to allow a surgical approach. Our results suggest that the presence of *MGMT* promoter methylation and the combination of capecitabine and TEM could be effective to achieve a disease down-staging.

When considering the efficacy of TEM-based treatment, we observed complete and partial response in 27.4% of patients. Our data was similar to what had been previously reported. In literature, ORR ranged from 20 to 70% [8, 17–19, 30]. Walter et al. reported an ORR of 20% in a cohort of pancreatic and GI NENs [17]. On the other hand, Cives et al. in 2016 reported a ORR of 54% in a large cohort of pancreatic NENs [19]. Our data was similar to what reported by Walter and, if considering only pancreatic NENs, we found a lower ORR (34%) compared to the data reported by Cives. These differences could be explained considering the different therapeutic approaches used. In fact, Cives reported the use of a specific CAPTEM schedule while we used TEM alone (31.9% of pancreatic NENs) or associated with capecitabine (68.1% of pancreatic NENs) in different and not standardized schedules.

Considering the secondary end-point, in our series we observed a significant difference in PFS after TEM-based therapy according to MGMT status. Median PFS was found to be significantly longer in patients with MGMT promoter methylation (21.0 vs. 8.0, $p = 0.017$). The analysis of prognostic factors for disease progression showed that the absence of MGMT promoter methylation increased the risk of disease progression 2.5 times at multivariate analysis. Our data were similar to those published by Walter et al, reporting a 16 months' difference in median PFS between methylated and not methylated patients (26.0 vs. 10.8 months) [17]. Recently, Raj et al. did not report any correlation with MGMT status nor response to alkylating agent therapy in a cohort of 46 patients with pancreatic NETs [30]. In this paper, PSQ was performed only in 28/56 patients and only a descriptive statistics analysis is reported.

In our series, a higher Ki67 value and previous chemotherapy represented another independent prognostic factors of disease progression. The role of Ki67 as a prognostic factor in NENs is well known and widely demonstrated [32–34]. No differences were found when considering the primary site and the association between capecitabine and TEM.

Overall survival with TEM-based therapy was significantly longer in patients with MGMT promoter methylation (not reached vs. 23 months, $p = 0.057$) and MGMT status, Ki67 and previous chemotherapy were prognostic factors for death at multivariate analysis. Walter et al. described similar result in 2015 with a median OS of 77 and 43 months in patients with and without MGMT promoter methylation [17]. Our results confirmed the role of MGMT status in OS in a larger cohort of patients treated with TEM-based therapy.

However, our results must be interpreted with some caution due to the retrospective design of our study, due to the use of two different technical methods to evaluate MGMT status (PSQ and MS-PCR) and to the fact that TEM-based therapy has been used with different schedules, making it difficult to properly assess its role in treatment efficacy. Prospective studies based on the assessment of MGMT status which could confirm our results are ongoing: an Italian phase II study testing TEM and lanreotide in bronchial carcinoid tumors (ClinicalTrials.gov Identifier: NCT02698410) and an American randomized phase II study comparing TEM-capecitabine vs. TEM alone in pancreatic NET (ClinicalTrials.gov Identifier: NCT01824875).

In conclusion, our data suggests a role of MGMT status, evaluated with MS-PCR or PSQ, in patients treated with TEM-based therapy. Methylation of MGMT promoter could be considered a predictive factor for objective response and a prognostic factor for a longer PFS and OS. Prospective studies to confirm this evidence are required but, currently, the status of MGMT should have an important role in the decision making for patients with metastatic NENs, in order to plan the best therapeutic approach.

Funding This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. J.C. Yao, M. Hassan, A. Phan, C. Dagohoy, C. Leary, J.E. Mares, E.K. Abdalla, J.B. Fleming, J.N. Vauthey, A. Rashid, D.B. Evans, One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J. Clin. Oncol. **26**(18), 3063–3072 (2008)
2. G. Rindi, G. Kloppel, A. Couvelard, P. Komminoth, M. Korner, J. M. Lopes, A.M. McNicol, O. Nilsson, A. Perren, A. Scarpa, J.Y. Scoazec, B. Wiedenmann, TNM staging of midgut and hindgut (neuro) endocrine tumors: a consensus proposal including a grading system. Virchows Arch. **451**(4), 757–762 (2007)
3. E. Raymond, L. Dahan, J.L. Raoul, Y.J. Bang, I. Borbath, C. Lombard-Bohas, J. Valle, P. Metrakos, D. Smith, A. Vinik, J.S. Chen, D. Horsch, P. Hammel, B. Wiedenmann, E. Van Cutsem, S. Patyna, D.R. Lu, C. Blanckmeister, R. Chao, P. Ruszniewski, Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N. Engl. J. Med. **364**(6), 501–513 (2011)
4. J.C. Yao, M.H. Shah, T. Ito, C.L. Bohas, E.M. Wolin, E. Van Cutsem, T.J. Hobday, T. Okusaka, J. Capdevila, E.G. de Vries, P. Tomassetti, M.E. Pavel, S. Hoosen, T. Haas, J. Lincy, D. Lebwohl, K. Oberg, Rad001 in advanced neuroendocrine tumors, T.T. S.G.: Everolimus for advanced pancreatic neuroendocrine tumors. N. Engl. J. Med. **364**(6), 514–523 (2011)
5. J.C. Yao, N. Fazio, S. Singh, R. Buzzoni, C. Carnaghi, E. Wolin, J. Tomasek, M. Raderer, H. Lahner, M. Voi, L.B. Pacaud, N. Rouyrre, C. Sachs, J.W. Valle, G. Delle Fave, E. Van Cutsem, M. Tesselaar, Y. Shimada, D.Y. Oh, J. Strosberg, M.H. Kulke, M.E. Pavel, Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. Lancet **387**(10022), 968–977 (2016)
6. L. de Mestier, C. Dromain, G. d'Assignies, J.Y. Scoazec, N. Lassau, R. Lebtahi, H. Brixi, E. Mitry, R. Guimbaud, F. Courbon, M. d'Herbomez, G. Cadiot, Evaluating digestive neuroendocrine tumor progression and therapeutic responses in the era of targeted therapies: state of the art. Endocr. Relat. Cancer **21**(3), R105–R120 (2014)
7. M.H. Kulke, K. Stuart, P.C. Enzinger, D.P. Ryan, J.W. Clark, A. Muzikansky, M. Vincitore, A. Michelini, C.S. Fuchs, Phase II study of temozolomide and thalidomide in patients with metastatic neuroendocrine tumors. J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. **24**(3), 401–406 (2006)
8. J.R. Strosberg, R.L. Fine, J. Choi, A. Nasir, D. Coppola, D.T. Chen, J. Helm, L. Kvols, First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. Cancer **117**(2), 268–275 (2011)
9. J.A. Chan, K. Stuart, C.C. Earle, J.W. Clark, P. Bhargava, R. Miksad, L. Blaszkowsky, P.C. Enzinger, J.A. Meyerhardt, H. Zheng, C.S. Fuchs, M.H. Kulke, Prospective study of bevacizumab plus temozolomide in patients with advanced neuroendocrine tumors. J. Clin. Oncol. **30**(24), 2963–2968 (2012)
10. R.L. Fine, A.P. Gulati, B.A. Krantz, R.A. Moss, S. Schreibman, D.A. Tsushima, K.B. Mowatt, R.D. Dinnen, Y. Mao, P.D. Stevens, B. Schrope, J. Allendorf, J.A. Lee, W.H. Sherman, J.A. Chabot, Capecitabine and temozolomide (CAPTEM) for metastatic, well-differentiated neuroendocrine cancers: The Pancreas Center at Columbia University experience. Cancer Chemother. Pharmacol. **71**(3), 663–670 (2013)
11. S.L. Gerson, MGMT: its role in cancer aetiology and cancer therapeutics. Nat. Rev. Cancer **4**(4), 296–307 (2004)
12. M.R. Middleton, J.M. Lunn, C. Morris, G. Rustin, S.R. Wedge, M.H. Brampton, M.J. Lind, S.M. Lee, D.R. Newell, N.M. Bleehen, E.S. Newlands, A.H. Calvert, G.P. Margison, N. Thatcher, O6-methylguanine-DNA methyltransferase in pretreatment tumour biopsies as a predictor of response to temozolomide in melanoma. Br. J. Cancer **78**(9), 1199–1202 (1998)
13. M.E. Hegi, A.C. Diserens, T. Gorlia, M.F. Hamou, N. de Tribolet, M. Weller, J.M. Kros, J.A. Hainfellner, W. Mason, L. Mariani, J. E. Bromberg, P. Hau, R.O. Mirimanoff, J.G. Cairncross, R.C. Janzer, R. Stupp, MGMT gene silencing and benefit from temozolomide in glioblastoma. N. Engl. J. Med. **352**(10), 997–1003 (2005)
14. O.L. Chinot, M. Barrie, S. Fuentes, N. Eudes, S. Lancelot, P. Metellus, X. Muracciole, D. Braguer, L. Ouafik, P.M. Martin, H. Dufour, D. Figarella-Branger, Correlation between O6-methylguanine-DNA methyltransferase and survival in inoperable newly diagnosed glioblastoma patients treated with neoadjuvant temozolomide. J. Clin. Oncol. **25**(12), 1470–1475 (2007)
15. S. Ekeblad, A. Sundin, E.T. Janson, S. Welin, D. Granberg, H. Kindmark, K. Dunder, G. Kozlovacki, H. Orlefors, M. Sigurd, K. Oberg, B. Eriksson, B. Skogseid, Temozolomide as monotherapy is effective in treatment of advanced malignant neuroendocrine tumors. Clin. Cancer Res. **13**(10), 2986–2991 (2007)
16. A.M. Schmitt, M. Pavel, T. Rudolph, H. Dawson, A. Blank, P. Komminoth, E. Vassella, A. Perren, Prognostic and predictive roles of MGMT protein expression and promoter methylation in sporadic pancreatic neuroendocrine neoplasms. Neuroendocrinology **100**(1), 35–44 (2014)
17. T. Walter, B. van Brakel, C. Vercherat, V. Hervieu, J. Forestier, J. A. Chayvialle, Y. Molin, C. Lombard-Bohas, M.O. Joly, J.Y. Scoazec, O6-Methylguanine-DNA methyltransferase status in neuroendocrine tumours: prognostic relevance and association with response to alkylating agents. Br. J. Cancer **112**(3), 523–531 (2015)
18. J. Cros, O. Hentic, V. Rebours, M. Zappa, N. Gille, N. Theou-Anton, D. Vernerey, F. Maire, P. Levy, P. Bedossa, V. Paradis, P. Hammel, P. Ruszniewski, A. Couvelard, MGMT expression predicts response to temozolomide in pancreatic neuroendocrine tumors. Endocr. Relat. Cancer **23**(8), 625–633 (2016)
19. M. Cives, M. Ghayouri, B. Morse, M. Brelsford, M. Black, A. Rizzo, A. Meeker, J. Strosberg, Analysis of potential response predictors to capecitabine/temozolomide in metastatic pancreatic neuroendocrine tumors. Endocr. Relat. Cancer **23**(9), 759–767 (2016)
20. M.H. Kulke, J.L. Hornick, C. Frauenhoffer, S. Hooshmand, D.P. Ryan, P.C. Enzinger, J.A. Meyerhardt, J.W. Clark, K. Stuart, C.S. Fuchs, M.S. Redston, O6-methylguanine DNA methyltransferase deficiency and response to temozolomide-based therapy in patients with neuroendocrine tumors. Clin. Cancer Res. **15**(1), 338–345 (2009)
21. G. Rindi, G. Kloppel, H. Alhman, M. Caplin, A. Couvelard, W.W. de Herder, B. Erikssson, A. Falchetti, M. Falconi, P. Komminoth, M. Korner, J.M. Lopes, A.M. McNicol, O. Nilsson, A. Perren, A. Scarpa, J.Y. Scoazec, B. Wiedenmann; all other Frascati Consensus Conference, p., European Neuroendocrine Tumor, S., TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. Virchows Arch. **449**(4), 395–401 (2006)

22. P. Therasse, S.G. Arbuck, E.A. Eisenhauer, J. Wanders, R.S. Kaplan, L. Rubinstein, J. Verweij, M. Van Glabbeke, A.T. van Oosterom, M.C. Christian, S.G. Gwyther, New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J. Natl. Cancer Inst. **92**(3), 205–216 (2000)
23. S.M. Dong, E.J. Lee, E.S. Jeon, C.K. Park, K.M. Kim, Progressive methylation during the serrated neoplasia pathway of the colorectum. Mod. Pathol. **18**(2), 170–178 (2005)
24. L. Karayan-Tapon, V. Quillien, J. Guilhot, M. Wager, G. Fromont, S. Saikali, A. Etcheverry, A. Hamlat, D. Loussouarn, L. Campion, M. Campone, F.M. Vallette, C. Gratas-Rabbia-Re, Prognostic value of O6-methylguanine-DNA methyltransferase status in glioblastoma patients, assessed by five different methods. J. Neurooncol. **97**(3), 311–322 (2010)
25. V. Quillien, A. Lavenu, L. Karayan-Tapon, C. Carpentier, M. Labussiere, T. Lesimple, O. Chinot, M. Wager, J. Honnorat, S. Saikali, F. Fina, M. Sanson, D. Figarella-Branger, Comparative assessment of 5 methods (methylation-specific polymerase chain reaction, MethyLight, pyrosequencing, methylation-sensitive high-resolution melting, and immunohistochemistry) to analyze O6-methylguanine-DNA-methyltranferase in a series of 100 glioblastoma patients. Cancer **118**(17), 4201–4211 (2012)
26. R. Stupp, M.E. Hegi, W.P. Mason, M.J. van den Bent, M.J. Taphoorn, R.C. Janzer, S.K. Ludwin, A. Allgeier, B. Fisher, K. Belanger, P. Hau, A.A. Brandes, J. Gijtenbeek, C. Marosi, C.J. Vecht, K. Mokhtari, P. Wesseling, S. Villa, E. Eisenhauer, T. Gorlia, M. Weller, D. Lacombe, J.G. Cairncross, R.O. Mirimanoff; European Organisation for, R., Treatment of Cancer Brain, T., Radiation Oncology, G., National Cancer Institute of Canada Clinical Trials, G., Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. **10**(5), 459–466 (2009)
27. C.Y. Hsu, H.L. Ho, S.C. Lin, M.H. Chen, S.P. Hsu, Y.S. Yen, W. Y. Guo, D.M. Ho, Comparative assessment of four methods to analyze MGMT status in a series of 121 glioblastoma patients. Appl. Immunohistochem. Mol. Morphol. **25**(7), 497–504 (2017)
28. M. Pavel, D. O'Toole, F. Costa, J. Capdevila, D. Gross, R. Kianmanesh, E. Krenning, U. Knigge, R. Salazar, U.F. Pape, K. Oberg; Vienna Consensus Conference, p., ENETS consensus guidelines update for the management of distant metastatic disease of intestinal, pancreatic, bronchial neuroendocrine neoplasms (NEN) and NEN of unknown primary site. Neuroendocrinology **103**(2), 172–185 (2016)
29. P. Girot, C. Dumars, J.F. Mosnier, L. Muzellec, H. Senellart, F. Foubert, F.X. Caroli-Bosc, E. Cauchin, N. Regenet, T. Matysiak-Budnik, Y. Touchefeu, Evaluation of O6-methylguanine-DNA methyltransferase as a predicting factor of response to temozolomide-based chemotherapy in well-differentiated metastatic pancreatic neuroendocrine tumors. Eur. J. Gastroenterol. Hepatol. **29**(7), 826–830 (2017)
30. N. Raj, D.S. Klimstra, N. Horvat, L. Zhang, J.F. Chou, M. Capanu, O. Basturk, R.K.G. Do, P.J. Allen, D. Reidy-Lagunes, O6-Methylguanine DNA methyltransferase status does not predict response or resistance to alkylating agents in well-differentiated pancreatic neuroendocrine tumors. Pancreas **46**(6), 758–763 (2017)
31. S. Krug, M. Boch, P. Rexin, T.M. Gress, P. Michl, A. Rinke, Impact of therapy sequence with alkylating agents and MGMT status in patients with advanced neuroendocrine tumors. Anticancer. Res. **37**(5), 2491–2500 (2017)
32. F. Panzuto, E. Merola, M.E. Pavel, A. Rinke, P. Kump, S. Partelli, M. Rinzivillo, V. Rodriguez-Laval, U.F. Pape, R. Lipp, T. Gress, B. Wiedenmann, M. Falconi, G. Delle Fave, Stage IV gastro-entero-pancreatic neuroendocrine neoplasms: A risk score to predict clinical outcome. Oncologist **22**(4), 409–415 (2017)
33. F. Panzuto, L. Boninsegna, N. Fazio, D. Campana, M.P. Brizzi, G. Capurso, A. Scarpa, F. De Braud, L. Dogliotti, P. Tomassetti, G. Delle Fave, M. Falconi, Metastatic and locally advanced pancreatic endocrine carcinomas: Analysis of factors associated with disease progression. J. Clin. Oncol. **29**(17), 2372–2377 (2011)
34. F. Panzuto, D. Campana, N. Fazio, M.P. Brizzi, L. Boninsegna, F. Nori, G. Di Meglio, G. Capurso, A. Scarpa, L. Dogliotti, F. De Braud, P. Tomassetti, G. Delle Fave, M. Falconi, Risk factors for disease progression in advanced jejunoileal neuroendocrine tumors. Neuroendocrinology **96**(1), 32–40 (2012)