Analysis of potential response predictors to capecitabine/temozolomide in metastatic pancreatic neuroendocrine tumors

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Abstract

The capecitabine and temozolomide (CAPTEM) regimen is active in the treatment of metastatic pancreatic neuroendocrine tumors (pNETs), with response rates ranging from 30 to 70%. Small retrospective studies suggest that O^6-methylguanine DNA methyltransferase (MGMT) deficiency predicts response to temozolomide. High tumor proliferative activity is also commonly perceived as a significant predictor of response to cytotoxic chemotherapy. It is unclear whether chromosomal instability (CIN), which correlates with alternative lengthening of telomeres (ALT), is a predictive factor. In this study, we evaluated 143 patients with advanced pNET who underwent treatment with CAPTEM for radiographic and biochemical response. MGMT expression ($n=52$), grade ($n=128$) and ALT activation ($n=46$) were investigated as potential predictive biomarkers. Treatment with CAPTEM was associated with an overall response rate (ORR) of 54% by RECIST 1.1. Response to CAPTEM was not influenced by MGMT expression, proliferative activity or ALT pathway activation. Based on these results, no biomarker-driven selection criteria for use of the CAPTEM regimen can be recommended at this time.

Key Words
- predictive factors
- MGMT
- ALT
- DAXX
- ATRX

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Introduction

Chemotherapy regimens containing the oral alkylating agent temozolomide are active in the treatment of metastatic pancreatic neuroendocrine tumors (pNETs), with response rates ranging from 30 to 70% (Kulke *et al.* 2006, Strosberg *et al.* 2011, Chan *et al.* 2012, Fine *et al.* 2013). The cytotoxic activity of temozolomide is related to its ability to induce DNA alkylation/methylation at the O^6 and N^7 positions of guanine, ultimately resulting in DNA mismatch and tumor cell death. The suicide enzyme O^6-methylguanine DNA methyltransferase (MGMT) repairs DNA by removing the O^6-alkylguanine adducts. High levels of MGMT expression contribute to chemoresistance by counteracting the therapeutic effect of alkylating agents (Gerson 2004). Among patients with either advanced glioblastoma or melanoma treated with temozolomide, loss of tumoral MGMT is associated with improved survival (Middleton *et al.* 1998, Hegi *et al.* 2005, Chinot *et al.* 2007). In pNET patients, conflicting results have been reported so far (Ekeblad *et al.* 2007, Kulke *et al.* 2009, Schmitt *et al.* 2014, Walter *et al.* 2015), and it is still unclear whether MGMT deficiency is predictive for clinical benefit from temozolomide.

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Tumor grade, measured by mitotic rate or Ki-67 proliferative index, is often regarded as a significant predictor of response to chemotherapy in pNET patients (Falconi *et al.* 2012, Öberg *et al.* 2012). However, no studies have formally investigated the correlation between proliferative activity and tumor response. Clinically aggressive pNETs are also characterized by chromosomal instability (CIN) (Jonkers *et al.* 2005), which has been recently associated with loss of DAXX/ATRX and activation of the alternative lengthening of telomeres (ALT), a telomerase-independent mechanism of telomere maintenance (Marinoni *et al.* 2014). Although patients with fast-growing, bulky, highly mutated pNETs are deemed to be ideal candidates for chemotherapy (Kunz *et al.* 2015), it is unclear whether DAXX/ATRX loss and ALT activation, as surrogate marker of CIN, predicts response to temozolomide.

In an era in which the therapeutic landscape of pNETs is rapidly evolving and multiple treatment options are available (Cives & Strosberg 2014), providers are faced with the challenge of treatment sequencing. As a result, there is a clear need for identification of predictive biomarkers to enable selection of patients who are likely to benefit from specific therapies. In this study, we investigated MGMT expression, tumor proliferation, DAXX/ATRX status and ALT activation as potential predictors of response to capecitabine/temozolomide (CAPTEM) chemotherapy in patients with advanced pNETs.

Patients and methods

Patients, treatment and tumor response evaluation

Approval for data collection and analysis was obtained from the Institutional Review Board of the University of South Florida (Tampa, FL, USA). We retrospectively examined 143 consecutive patients with unresectable pNET who received CAPTEM chemotherapy at our institution between 2005 and 2014 and were assessable for radiographic response. Demographic, clinical and pathological information including tumor grade by World Health Organization (WHO) 2010 criteria (Rindi *et al.* 2010), mitotic rate and Ki-67 labeling index were obtained by review of patient medical records. The chemotherapy regimen consisted of oral capecitabine, 750 mg/m^2 twice daily for 14 days (days 1–14), and oral temozolomide, 200 mg/m^2 once daily for 5 days (days 10–14), every 28 days, as described previously (Strosberg *et al.* 2011).

Radiological assessment of tumor responses was separately and independently performed by two investigators (M C and J S) and all discrepancies in response assessment were adjudicated by a radiologist (B M). The nearest pretreatment computed tomography or magnetic resonance imaging scan was used as baseline and compared with subsequent scans, obtained as part of routine clinical care. The Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (Eisenhauer *et al.* 2009) was used for evaluation of radiographic response. Biochemical response was measured based on baseline chromogranin A (CgA) levels obtained before initiation of the temozolomide-based regimen.

Immunohistochemistry

Immunohistochemistry (IHC) was used to evaluate the expression of MGMT, DAXX and ATRX. Sections of 5 µm in thickness were cut from archival paraffin-embedded pathology specimens and subjected to MGMT staining protocol using the Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, AZ, USA) as per manufacturer's protocol with proprietary reagents. Immunolabeling for DAXX and ATRX was performed as described previously (Jiao *et al.* 2011). The mouse mAb against MGMT (MS-470-P1; Thermo Scientific) was used at a 1:20 concentration, whereas the rabbit polyclonal antibodies anti-DAXX (HPA008736; Sigma-Aldrich) or anti-ATRX (HPA001906; Sigma-Aldrich) were used at 1:150 and 1:400 dilution, respectively. After 1 h of incubation at room temperature, the primary antibodies were detected by 16 (MGMT) and 30 min (DAXX, ATRX) of incubation with the respective HRP-labeled secondary antibody. Tissue sections were developed using 3,3′-diaminobenzidine (Sigma-Aldrich) as a substrate and then counterstained with hematoxylin.

The immunostained sections were examined under a light microscope by two independent pathologists (M G and M B) who were blinded to the patient clinical outcome. In case of disagreement, a consensus was reached after joint review at a multihead microscope. MGMT expression was evaluated by three different systems of interpretation (Allred *et al.* 1993, Ekeblad *et al.* 2007, Kulke *et al.* 2009). Criteria for MGMT deficiency determination are listed in Supplementary Table 1, see section on supplementary data given at the end of this article. For DAXX and ATRX, only nuclear labeling was evaluated. Tumors were scored as positive when there was nuclear labeling in at least 50% of tumor cells. Non-neoplastic cells (endothelial cells, stromal cells and islets of Langerhans) served as an internal positive control in all tissue sections.

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Table 1 Patient demographics and clinical characteristics.

Characteristics	*n* of patients (=143)	%
Age (years)		
Median	59	
Range	28–82	
Gender		
Male	91	64
Female	52	36
Race		
White	117	82
Black	17	12
Hispanic	5	3
Asian	4	3
Time from diagnosis (months)		
Median	12	
Range	1–204	
Genetic syndrome		
No	140	98
Yes	3	2
VHL	2	1
MEN1	1	1
Tumor grade (WHO 2010)		
G1	78	55
G2	37	26
G3	13	9
Not available	15	10
Tumor functionality		
No	116	81
Yes	27	19
Gastrinoma syndrome	11	7
VIPoma syndrome	6	4
Glucagonoma syndrome	4	3
Insulinoma syndrome	4	3
Cushing's syndrome	1	1
Carcinoid syndrome	1	1
Sites of metastases		
Locally advanced	10	7
Liver	128	89
Lymph nodes	37	26
Bone	16	11
Lung	5	3
Others[a]	7	5
Elevated baseline chromogranin A (>ULN)		
Yes	89	62
No	54	38
Baseline chromogranin A (nl <15 ng/mL)		
Median	27	
Mean	340	
Range	(1–8000)	
Prior lines of systemic therapy		
0	61	43
1	56	39
2	16	11
3	8	6
4	2	1
Previous systemic therapy		
Octreotide LAR[c]	59	41
Chemotherapy[b]	24	17
Everolimus	11	8
Sunitinib	6	4
PRRT	2	1
Investigational agents	9	6
Concurrent octreotide LAR		
Yes	32	22
No	111	78
No. of temozolomide/capecitabine cycles		
Median	9	
Range	1–28	

[a]Including kidneys, adrenal glands, breasts, adnexa, spleen and peritoneum; [b]Including etoposide/cisplatin (21/143), streptozotocin (1/143), gemcitabine (1/143) and capecitabine (1/143); [c]Octreotide LAR was the only prior therapy in 39 patients (27%).

Cases lacking positive immunostaining in benign elements were considered to be uninformative.

Telomere-specific FISH

Telomere-specific FISH was performed and interpreted as described previously (Heaphy *et al.* 2011). Briefly, deparaffinized slides were hydrated, steamed for 20 min in citrate buffer, dehydrated and hybridized with a Cy3-labeled peptide nucleic acid (PNA) probe complementary to the mammalian telomere repeat sequence ([N-terminus to C-terminus] CCCTAACCCTAACCCTAA). As a positive control for hybridization efficiency, a FITC-labeled PNA probe having specificity for human centromeric DNA repeats (ATTCGTTGGAAACGGGA; CENP-B binding sequence) was also included in the hybridization solution. Following post-hybridization washes, nuclear counterstaining with 4′,6-diamidino-2-phenylindole (DAPI) was conducted. Slides were imaged with a Nikon 50i epifluorescence microscope equipped with X-Cite series 120 illuminator (EXFO Photonics Solutions Inc, Ontario, Canada) and appropriate excitation/emission filters. Gray-scale images were captured using Nikon NIS-Elements software and an attached Photometrics CoolSNAP EZ digital camera, pseudo-colored and merged.

The FISH slides were assessed by A K M Large, ultra-bright telomere repeat DNA aggregates are unique to ALT-positive cell populations and are significantly larger and brighter than the FISH signals emanating from

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normal telomeres in the same cell population. pNETs were classified as ALT-positive if they met the following criteria: (i) the presence of ultra-bright, intranuclear foci of telomere FISH signals and (ii) ALT-associated telomeric DNA foci in ≥1% of neoplastic cells. Tumor samples lacking ALT-associated telomeric foci were considered ALT-negative. In all cases, areas exhibiting necrosis were excluded from consideration.

Statistical analysis

Expression of MGMT, proliferative activity, activation of the ALT pathway and tumor mutational status were correlated with the patients' radiographic or biochemical response using the χ^2 test or Fisher's exact test, as appropriate. The Cohen's kappa coefficient was used to assess the degree of correlation between the different systems adopted for MGMT status interpretation. All time-to-event functions were estimated by the Kaplan–Meier method and compared by the log-rank test. Progression-free survival (PFS) was calculated from initiation of chemotherapy until the date of first progressive disease or death due to any cause. Overall survival (OS) was defined as the time from start of treatment until death as a result of any cause, with patients censored at the date of last follow-up if still alive. Time-to-treatment failure (TTF) was defined as the time from treatment initiation until discontinuation for any reason. Exact 95% CI were calculated for each proportion of interest. All tests were two sided and statistical significance was declared at $P<0.05$. Statistical analysis was performed using MedCal statistical software 12.7 (MedCalc Software bvba, Ostend, Belgium).

Results

Demographics and tumor characteristics

Demographic variables and clinicopathological characteristics of 143 patients enrolled in the study are listed in Table 1. At treatment onset, median age of the patient population was 59 (28–82) years. The majority (91/143) of patients were males, and more than three quarters (115/143) had grade 1 or 2 pNETs. No large cell or small cell neuroendocrine carcinomas were included in the study. Twenty-seven patients had hormonally functioning tumors, including 11 patients with gastrinoma syndrome, 8 patients with glucagonoma or insulinoma syndrome, 6 patients with VIPoma syndrome, 1 patient with carcinoid syndrome and 1 patient with ectopic ACTH secretion. Tumors were metastatic in 133 patients and locally advanced in 10 patients. Most patients (117/143) were treatment naïve or had received only one prior line of systemic therapy; 59 received prior octreotide long-acting repeatable (LAR), 24 received prior cytotoxic chemotherapy (including etoposide/cisplatin, streptozotocin, gemcitabine and radiosensitizing capecitabine), 11 had prior everolimus, 6 had prior sunitinib, 2 had prior peptide receptor radionuclide therapy (PRRT) and 9 had prior investigational agents (including pasireotide, bevacizumab and ganitumab). The median time from diagnosis until CAPTEM initiation was 12 (1–204) months.

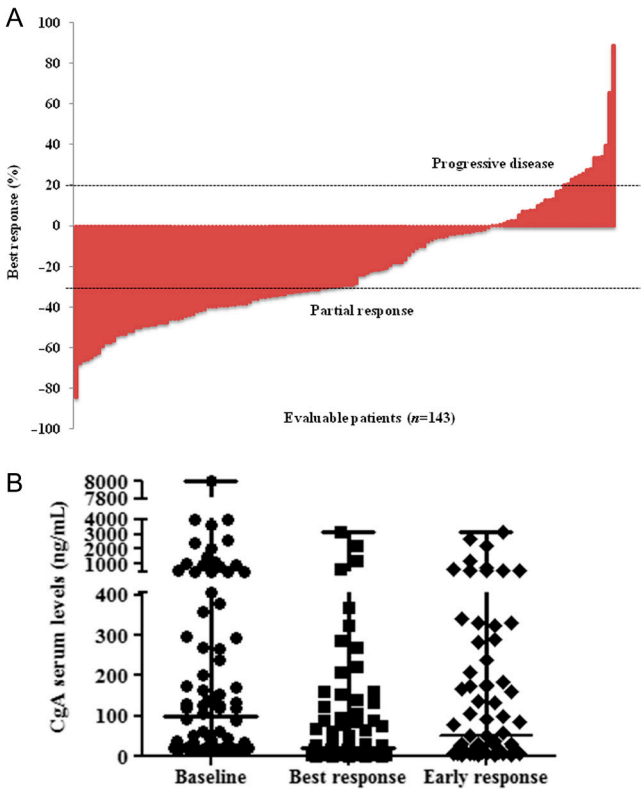

Figure 1
Radiographic and biochemical responses following CAPTEM chemotherapy. (A) Waterfall plot summarizing the maximum percent change from baseline in the sum of longest diameters of target lesions. Two patients with stable disease were classified as progressing by RECIST because of the emergence of new lesions. (B) In patients with elevated baseline CgA, median CgA levels decreased from 99.8 to 20.8 ng/mL. After 3 months of treatment, median CgA concentration was 50.1 ng/mL. Differences were statistically significant by Wilcoxon-matched pairs signed-rank test ($P<0.0001$ and $P=0.0004$, respectively). Pair row values, median change and interquartile range are represented. A full colour version of this figure is available at http://dx.doi.org/10.1530/ERC-16-0147.

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Treatment outcomes

Patients received a median of nine 28-day treatment cycles. Reasons for discontinuation included radiographic tumor progression ($n=47$), maximal response or chemotherapy break (at physician's discretion; $n=66$), unacceptable toxicity ($n=20$) and patient decision ($n=2$). Eight patients remained on treatment at the time of data analysis. Toxicities leading to CAPTEM discontinuation included thrombocytopenia ($n=11$), fatigue ($n=5$), palmoplantar erythrodysesthesia ($n=3$) and neutropenia ($n=1$).

All 143 patients were assessable for radiographic response. When best response to therapy was evaluated, 54% (77/143) of patients experienced partial response according to RECIST criteria, whereas 35% (50/143) had stable disease and 11% (16/143) experienced progressive disease. The waterfall plot analysis (Fig. 1A) showed some degree of tumor shrinkage in 78% (112/143) of evaluable patients and continued tumor growth in 22% (31/143) of the cohort. Among 89 patients with baseline-elevated (>ULN) serum CgA levels, 54 patients (61%) experienced major reductions (>50%) or normalization of the tumor marker. This biochemical response was observed within 3 months of treatment initiation in 28 patients (31%). Differences between the median baseline CgA concentration and its lowest and 3-month value following initiation of treatment were statistically significant ($P<0.0001$ and $P=0.0004$, respectively; Fig. 1B).

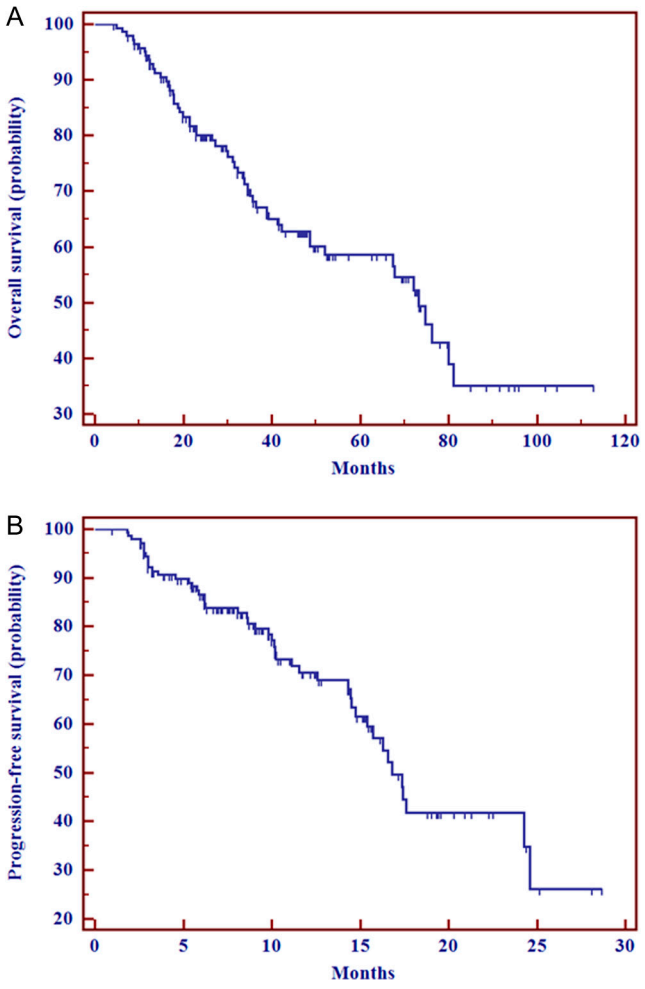

Figure 2
Kaplan–Meier estimates of overall survival (A) and progression-free survival (B). A full colour version of this figure is available at http://dx.doi.org/10.1530/ERC-16-0147.

At the time of data cutoff, 54 patients had died and 89 patients were alive, with median follow-up duration of 34 months (range: 4–113 months). As depicted in Fig. 2A, the median OS was 73.2 months (95% CI, 51.9–81.1 months), and the 5-year survival rate was 58.6% (±4.9%). The median PFS was 17 months (95% CI: 15–25 months; Fig. 2B). At 1 and 2 years, estimated rates of PFS were 70.6% (±4.6%) and 41.8% (±6.9%), respectively. Among responding patients, the median duration of response was 19 months (95% CI: 9–28 months). The median TTF was 9 months (95% CI: 7.8–10.2 months).

MGMT expression as a predictor of response

MGMT expression was evaluated in 65 pNET patients with available tissue. The IHC staining was not interpretable in 13 cases because of lack of positive internal controls or paucity of tumor cells. Among 52 assessable cases, 15 (29%) were MGMT deficient, when the deficiency was defined by the complete absence of staining in all tumor cells (Kulke *et al.* 2009). When MGMT deficiency was defined by the lack of nuclear staining in ≥10% of tumor cells (Ekeblad *et al.* 2007) or by an Allred score <4 (Allred *et al.* 1993), we interpreted as deficient 20 (38%) and 19 (36%) cases, respectively. Interobserver agreement rate was 77%. By Cohen's test, there was a high degree of correlation between the two latter methods of MGMT staining interpretation ($\kappa=0.96 \pm 0.04$). The concordance rates between the interpretation system proposed by Kulke *et al.* (2009) and the other two methods were slightly lower ($\kappa=0.49\pm0.12$ and $\kappa=0.53\pm0.12$, respectively). As detailed in Table 2, patients harboring MGMT-intact or MGMT-deficient pNETs exhibited similar overall response rates (ORR) following CAPTEM treatment. MGMT expression

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Table 2 Candidate biomarkers in pNETs.

Criteria of stratification	Interpretable cases (*n*)	ORR (%)	*P*	Major biochemical response (%)	*P*	PFS, 95% CI (months)	*P*	OS, 95% CI (months)	*P*
MGMT (intact: nuclear staining in any tumor cells)	52		0.10		0.66		0.25		0.40
MGMT intact		65		67		16.8 (14.5–16.8)		NR	
MGMT deficient		40		50		14.5 (6.2–14.5)		81.1 (73.2–81.1)	
MGMT (intact: nuclear staining in ≥10% of tumor cells)	52		0.37		0.56		0.62		0.41
MGMT intact		63		47		14.5 (12.6–24.3)		73.2 (36.4–73.2)	
MGMT deficient		50		35		16.6 (15.4–17.4)		81.1 (48.7–81.1)	
MGMT (intact: Allred score ≥4)	52		0.25		0.57		0.54		0.27
MGMT intact		64		45		17.4 (12.6–24.3)		73.2 (36.4–73.2)	
MGMT deficient		47		37		16.6 (15.4–16.6)		81.1 (39.2–81.1)	
Grade	128		0.29		0.7		0.83		0.84
Low grade		65		64		16.8 (15.4–24.3)		72.1 (48.6–81.1)	
Intermediate grade		52		72		14.5 (10–14.5)		67.4 (35.2–73.2)	
High grade		69		78		24.6 (22.6–24.6)		76.2 (17.8–76.2)	
Mitotic count/10 HPF	96		0.93		0.03		0.58		0.007
<2		54		64		17.4 (15.4–24.3)		74.8 (36.4–81.1)	
2 <MC<20		50		74		16.8 (9.8–24.6)		73.2 (31.5–73.2)	
>20		50		100		NR		14.6 (11.4–14.6)	
Ki-67 labeling index	80		0.38		0.74		0.27		0.61
<3%		65		90		NR		35.2 (33.6–35.7)	
Between 3 and 20%		50		67		NR		73.2 (42.2–81.1)	
>20%		42		71		14.5 (10–24.6)		76.2 (17.9–76.2)	
ALT status	46		0.37		0.66		0.38		0.02
ALT-positive		63		77		NR		NR	
ALT-negative		47		70		14.5 (10.2–16.8)		36.4 (30.1–81.1)	
DAXX/ATRX status	31		0.34		0.27		0.35		0.13
DAXX/ATRX-positive		52		53		16.3 (14.5–16.8)		48.7 (34.5–48.7)	
DAXX/ATRX-negative		69		75		NR		NR	

NR, not reached.

by IHC had no significant influence on biochemical response rate, OS or PFS.

Tumor proliferation as a predictor of response

Tumor grade, mitotic rate and Ki-67 labeling index were assessable in up to 128/143 patients. As detailed in Table 2, response to CAPTEM in patients with pNETs was not significantly influenced by tumor proliferation, even when the lower Ki-67% threshold was set up at 5, 10 or 55%. High mitotic rate (>20 mitoses/10 high-power fields) was associated with poor prognosis.

ALT activation as a predictor of response

Among 61 pNET samples analyzed by telomeric FISH to detect ALT activation, 15 were not interpretable due to nonspecific background staining or paucity of tumor cells. Twenty-seven (59%) tumors had ultra-bright telomere FISH signals, a nearly universal feature of ALT (Fig. 3). Histomorphologically, ALT-positive samples were characterized by atypical cytology, defined as chromatin density heterogeneity and variation in nuclear size. ALT activation did not predict response to CAPTEM but was associated with improved survival (P=0.02; Table 2). Given the reported association between ALT activation and DAXX/ATRX loss (Heaphy *et al.* 2011, Marinoni *et al.* 2014), we evaluated using IHC the expression of both proteins in pNETs. In ALT-positive tumors, DAXX/ATRX were deficient in 16 (59%) cases, positive in 1 (4%) and unevaluable in 10 (37%) tumors because of the lack of positive internal controls. In ALT-negative tumors, we observed the loss of DAXX or ATRX in 4 (21%) tumors. Overall, there was an inverse correlation between ALT activation and loss of DAXX/ATRX (P<0.0001). The expression of DAXX/ATRX was neither predictive of response to CAPTEM, nor affected patient prognosis (Table 2). The overall cohort and the cohorts evaluated in MGMT expression, tumor proliferation and ALT status were not different in terms of baseline characteristics, nor therapeutic outcomes.

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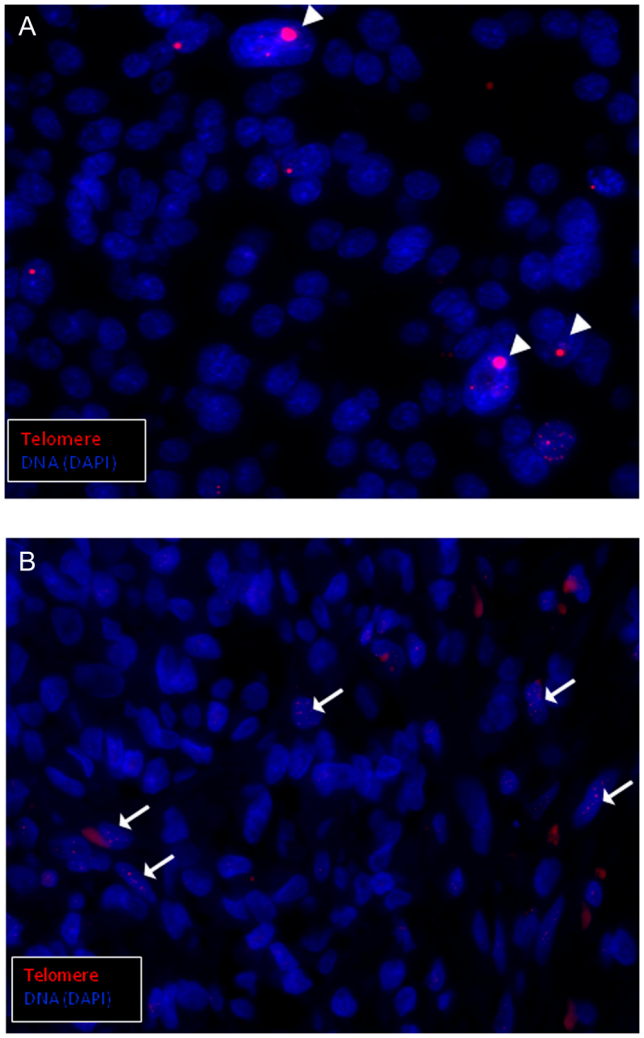

Figure 3
Representative images of ALT-positive (A) and ALT-negative (B) pNETs. Large, ultra-bright telomere FISH signals indicative of ALT are marked (arrowheads). Benign stromal or endothelial cells (arrows) served as positive controls. A full colour version of this figure is available at http://dx.doi.org/10.1530/ERC-16-0147.

Discussion

This is the largest reported cohort of pNET patients treated with CAPTEM chemotherapy. We observed an ORR of 54%, a median OS of 73.2 months and a median PFS of 17 months. We also found that MGMT expression as measured by IHC, proliferative activity and ALT pathway activation did not predict response to CAPTEM.

There remains considerable controversy regarding the optimal method of MGMT detection in tumor samples. In pNETs, both methyl-specific PCR and pyrosequencing have been used to evaluate *MGMT* promoter methylation status as a surrogate of MGMT activity (Schmitt *et al*. 2014, Walter *et al*. 2015). Direct measurement of MGMT protein expression by IHC is the most convenient technique to measure MGMT status in the clinical setting, despite pitfalls in the interpretation of the staining that have been described (Kulke *et al*. 2009, Walter *et al*. 2015). In the absence of formal recommendations or uniformly used criteria for the interpretation of MGMT immunostaining (Ekeblad *et al*. 2007, Kulke *et al*. 2009, Schmitt *et al*. 2014, Walter *et al*. 2015), we defined the MGMT status according to different systems and found that MGMT was undetectable in 29–38% of tumors. These rates are slightly lower than previously reported for pNETs (36–66%) (Ekeblad *et al*. 2007, Kulke *et al*. 2009, Schmitt *et al*. 2014, Walter *et al*. 2015). Sample bias, sampling issues, interobserver variability and/or IHC technical differences (including the use of different antibodies against MGMT) might account for this difference. Although small studies have identified MGMT deficiency by IHC as a predictor of response to temozolomide in pNETs (Kulke *et al*. 2009, Walter *et al*. 2015), this biomarker was neither predictive nor prognostic in our series. A possible explanation is that concurrent capecitabine may counteract MGMT-associated resistance to temozolomide (Fine *et al*. 2013). Alternatively, variations in quality of tissue samples and in interpretation of IHC data may have attenuated the predictive power of this assay. High proliferative activity and rapid pace of disease progression are commonly regarded as major determinants of sensitivity to cytotoxic drugs in patients with pNETs. However, no correlation between tumor grade, mitotic rate or Ki-67 labeling index and tumor response to CAPTEM was observed in our series. This finding might be related to the fact that the cytotoxic activity of temozolomide is not confined to mitosis, but spans the whole cell cycle (Gerson 2004). Moreover, it emphasizes the concept that tumor proliferative activity, measured on needle biopsy or resected primary tumor specimen, may not always reflect the clinical aggressiveness of a metastatic pNET.

Consistent with previous studies (Heaphy *et al*. 2011, Marinoni *et al*. 2014), we found that 59% of analyzed pNETs were ALT-positive. ALT activation was negatively associated with DAXX/ATRX expression ($P<0.0001$) and prognostic for improved survival ($P=0.02$) in a population of patients with advanced/metastatic pNETs. This association has been observed in other series of metastatic pNETs (Jiao *et al*. 2011). Both ALT status and DAXX/ATRX expression, as surrogate markers of CIN (Marinoni *et al*. 2014), were not able to predict response to CAPTEM.

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Limitations of this study included its retrospective design and paucity of tissue in a large fraction of cases. Tissue limitations precluded assessment of MGMT promoter methylation assays and other potential molecular biomarkers and reduced our ability to detect meaningful differences in the tested potential predictors. Moreover, it is important to emphasize that even though mitotic rate and Ki-67 index were not predictive of response in this series, nearly all tumors were clinically aggressive (i.e. symptomatic, rapidly progressive or widespread). Thus, the response rates observed in this series should not be assumed to reflect the activity of CAPTEM in patients with indolent, low-grade pNETs.

In conclusion, CAPTEM is associated with very encouraging treatment outcomes and survival durations in patients with advanced pNETs. Although MGMT status has been postulated to be a predictive factor for response based on small retrospective studies and has been used in clinical practice, we have not observed any correlation between protein expression and radiographic response in this series of patients treated with CAPTEM. Moreover, despite the common perception that chemotherapy is particularly active in highly proliferative tumors, we were unable to observe any correlation between mitotic activity or Ki-67 index and response. Although we cannot recommend any biomarker-driven selection criterion for use of the CAPTEM regimen, future studies, including a prospective randomized trial of temozolomide alone or in combination with capecitabine (NCT01824875), may provide further insight into the predictive validity of MGMT promoter methylation, among other assays.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-16-0147.

Declaration of interest

Dr Strosberg has performed consultation for Novartis and Ipsen within institutional conflict of interest payment guidelines. All remaining authors have declared no conflicts of interest.

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