Cancer Treatment Reviews 99 (2021) 102261

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Should temozolomide be used on the basis of O^6-methylguanine DNA methyltransferase status in patients with advanced neuroendocrine tumors? A systematic review and meta-analysis

P. Trillo Aliaga [a,b], F. Spada [c], G. Peveri [d], V. Bagnardi [e], C. Fumagalli [f], A. Laffi [c], M. Rubino [c], L. Gervaso [c,g], E. Guerini Rocco [f], E. Pisa [f], G. Curigliano [a,b], N. Fazio [h,*]

[a] Division of New Drugs and Early Drug Development, European Institute of Oncology IRCCS, Milan, Italy
[b] Department of Oncology and Hematology, University of Milan, Milan, Italy
[c] Division of Gastrointestinal Medical Oncology and Neuroendocrine Tumors, European Institute of Oncology IRCCS, Milan, Italy
[d] Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy
[e] Department of Statistics and Quantitative Methods, University of Milan-Bicocca, Milan, Italy
[f] Division of Pathology, European Institute of Oncology IRCCS, Milan, Italy
[g] Molecular Medicine Department, University of Pavia, Pavia, Italy
[h] IEO, European Institute of Oncology, IRCCS, Milan, Italy

ARTICLE INFO

Keywords:
Neuroendocrine tumors
NETs
O^6-methylguanine DNA methyltransferase
MGMT
Temozolomide
TEM
TMZ

ABSTRACT

Background: Temozolomide (TEM) is an active treatment in metastatic neuroendocrine tumors (NETs). Patients affected by glioblastoma multiforme or advanced melanoma treated with TEM who have deficiency of O^6-methylguanine DNA methyltransferase (MGMT) have a better responses and survival. However, the predictive role of MGMT in patients with NETs treated with TEM is still debated.

Methods: We conducted a systematic review of the literature and meta-analysis, based on PRISMA methodology, searching in the main databases (PubMed, Embase, Scopus, Web of Science, Cochrane Library and clinical trial.gov) and the proceedings of the main international congresses, until April 26, 2021.

Results: Twelve out of 616 articles were selected for our analysis, regarding a total of 858 NET patients treated with TEM-based chemotherapy. The status of MGMT had been tested in 513 (60%) patients, using various methods. The pooled overall response rate (ORR) was higher in MGMT-deficient compared with MGMT-proficient NETs, with a risk difference of 0.31 (95% confidence interval, CI: 0.13–0.50; $p < 0.001$; I^2: 73%) and risk ratio of 2.29 (95% CI: 1.34–3.91; $p < 0.001$; I^2: 55%). The pooled progression free survival (PFS) (hazard ratio, HR = 0.56; 95% CI: 0.43–0.74; $p < 0.001$) and overall survival (OS) (HR = 0.41; 95% CI: 0.20–0.62; $p = 0.011$) were longer in MGMT-deficient versus MGMT-proficient NETs.

Conclusions: Our meta-analysis suggested that MGMT status may be predictive of TEM efficacy. However, due to the high heterogeneity of the evaluated studies the risk of biases should be considered. On this hypothesis future homogeneous prospective studies are warranted.

Introduction

Neuroendocrine tumors (NETs) are rare [1] and heterogeneous malignancies that are classified according to their site of origin, morphology, Ki-67 proliferation index, and mitotic count. An updated classification of the World Health Organization (WHO) of digestive neuroendocrine neoplasms (NENs) has been recently published and divides the NENs in neuroendocrine tumors (NET, grade [G]1, G2, G3), neuroendocrine carcinomas (NEC) and mixed non-neuroendocrine and endocrine malignancies (MiNEN) [2].

The management of NETs depends on the biological and morphological characteristics, functional status, and disease stage. Surgery is the preferred option for resectable tumors, whereas in cases of locoregional, unresectable and metastatic disease, the systemic treatment is

* Corresponding author at: Division of Gastrointestinal Medical Oncology and Neuroendocrine Tumors, European Institute of Oncology (IEO), IRCCS, Via Ripamonti 435, 20141, Milan, Italy.
E-mail address: nicola.fazio@ieo.it (N. Fazio).

https://doi.org/10.1016/j.ctrv.2021.102261
Received 8 April 2021; Received in revised form 7 July 2021; Accepted 9 July 2021
Available online 22 July 2021

recommended and the therapeutic options include somatostatin analogs (SSAs) [3], inhibitors of the mammalian target of rapamycin (everolimus) [4], receptor tyrosine kinase inhibitors (sunitinib) [5], peptide receptor radionuclide therapy (PRRT) [6] and chemotherapy [7].

Chemotherapy can be considered in NET's therapeutic strategy for controlling symptoms by reducing the disease burden and for control of tumor growth. Drugs with activity in this setting belong to the class of alkylating agents (streptozotocin [8,9], dacarbazine [10] and temozolomide [11,12]), anti-metabolites (5-fluorouracil [10] or capecitabine [13]) and more recently oxaliplatin [13] and irinotecan [14,15] (for high-grade tumors).

Temozolomide (TEM) -based chemotherapy is currently a valid treatment for metastatic NETs, having exhibited promising overall response rates (ORRs) that range between 30% and 70% [7,16]. TEM is an alkylating agent, that produces anti-tumor activity by inducing DNA methylation at the O^6 and N^7 positions of guanine, which leads to DNA mismatch and tumor cell death [17]. DNA repair enzyme O^6-methylguanine DNA methyltransferase (MGMT) is crucial for genome stability, as it repairs DNA mismatch and transcription errors. MGMT removes mutagenic alkyl groups from O^6-guanine through a covalent transfer to an internal cysteine residue in the conserved active site, resulting in MGMT itself inactivation ("suicidal enzyme") [18]. Preclinical studies have shown that diminished MGMT expression increases the carcinogenic risk in mice exposed to alkylating agents [19,20] whereas high MGMT expression counteracts the therapeutic effect of alkylating agents, thus contributing to chemoresistance [21].

The relationship between MGMT status and response to alkylating agents has been extensively investigated in patients with glioblastoma multiforme (GBM), where MGMT deficiency is a predictive biomarker for TEM response and is also a prognostic biomarker of improved survival [22,23]. Similarly, in metastatic melanoma better overall response rate (ORR) to TEM in patients with MGMT promoter methylation [24] has been reported. However, the role of MGMT status in predicting TEM tumor response in patients with NET is still controversial. In this context, we performed a systematic review and meta-analysis of the available literature in order to clarify the role of MGMT status in predicting the benefits of TEM-based chemotherapy in patients with NET.

Materials and methods

Literature search strategy

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to perform our systematic review and meta-analysis [25,26] (Supplementary Table 1). We formulated a search strategy (NF, FS and PTA) using an organizing framework to identify the search terms, focusing on Population, Intervention, Comparison, and quantitative Outcomes of the articles (PICO) (Supplementary Table 2). We systematically searched PubMed/Medline, Embase, Scopus, Web of Science and Cochrane Library for English language articles published from the database inception to April 26, 2021. We also reviewed the abstracts presented at all major international conferences such as European Neuroendocrine Tumor Society (ENETs), North American Neuroendocrine Tumor Society (NANETs), American Society of Clinical Oncology (ASCO) and European Society for Medical Oncology (ESMO) to enhance our research performance. The following keywords were used in our search strategy ("O-6-methylguanine DNA methyltransferase" OR "MGMT") AND (temozolomide OR ''TEM'' OR TMZ) AND (neuroendocrine tumors [Mesh] OR carcinoid). Each article was reviewed independently by two authors (PTA and FS). If differences in opinion arose between these two authors, then the articles were discussed with a third author (NF) for reconciliation. For duplicated publications, investigators selected the most relevant and/or comprehensive, and possibly the most recent. The reference lists of the major articles were searched and screened to ensure that no studies were overlooked.

Selection criteria

Inclusion criteria

(1) retrospective or prospective studies; (2) patients with histopathologic diagnosis of NET (well differentiated) from the gastroenteropancreatic (GEP) and/or thoracic tract and/or other sites of origin was allowed with a lower prevalence; (3) locoregional unresectable or metastatic tumors; (4) patients treated with TEM-based chemotherapy; (5) studies that reported data of MGMT expression levels and/or MGMT promoter methylation status; (6) correlation of MGMT status with activity (overall response rate) or efficacy (progression free survival and/or time to progression and overall survival); (7) if the same patient population was reported in several publications, only the most complete study was included; (8) only articles with full-text were included.

Exclusion criteria

(1) case reports and case series with less than 10 patients; (2) studies that included only patients with neuroendocrine carcinoma (NEC) (3) studies that include more than 25% of patients with NEC; (4) studies in which MGMT status was not evaluated or was not available; (5) studies with chemotherapy including TEM in which correlation between MGMT and TEM was not extractable.

Data extraction

The following data were collected from each study: first author's name, study design, investigation's country, publication year, number of patients, demographic and clinical characteristics of patients, anatomical primitive site of NET, grade and Ki67 index, methodology of MGMT analysis, type of TEM-based chemotherapy, ORR, progression free survival (PFS), time to progression (TTP) and overall survival (OS). For every study, we synthetized the principal findings.

The endpoints of our interest were ORR, PFS, TTP and OS in patients with NET with MGMT evaluation. Data extraction was performed by two reviewers (PTA and CF) and independently checked by other two authors (VB and GP).

Statistical analysis

A meta-analytic approach was used to pool evidence from different studies. For each study, we extracted, or derived from raw data, the overall response rates (ORR) for the deficient MGMT group (including low MGMT expression by immunohistochemistry (IHC) and/or hypermethylated MGMT by methylation promoter methods) and for the proficient MGMT group (including high MGMT expression and/or unmethylated MGMT), and the hazard ratios (HR) and their 95% confidence intervals (CI) for the deficient vs proficient MGMT groups. When the HR was not reported in a publication, we estimated it from other published data, using the following hierarchical strategy: i) fitting a Cox proportional-hazard model on individual patient data (IPD), when available, or on pseudo-IPD reconstructed from Kaplan-Meier survival curves [27]; ii) by the ratio of median survival times [28]. Studies without any of these pieces of information were excluded from the meta-analysis.

The measures we meta-analyzed were progression-free survival (PFS) HRs, overall survival (OS) HRs, risk differences (RD) of ORR, and relative risks (RR) of ORR. If more than one MGMT deficiency evaluation methods was used in a study, for each meta-analysis we kept measures obtained stratifying patients by methylation techniques instead of IHC, but also conducted a sub-analysis for each method separately.

Since we could not assume *a priori* homogeneity between the measures of effect of the included studies, the pooled PFS HR, OS HR, RD and RR were calculated using random-effects model [29]. The Q-test was performed to assess between-study heterogeneity, and the I^2 statistics, which express the percentage of the total observed variability due to

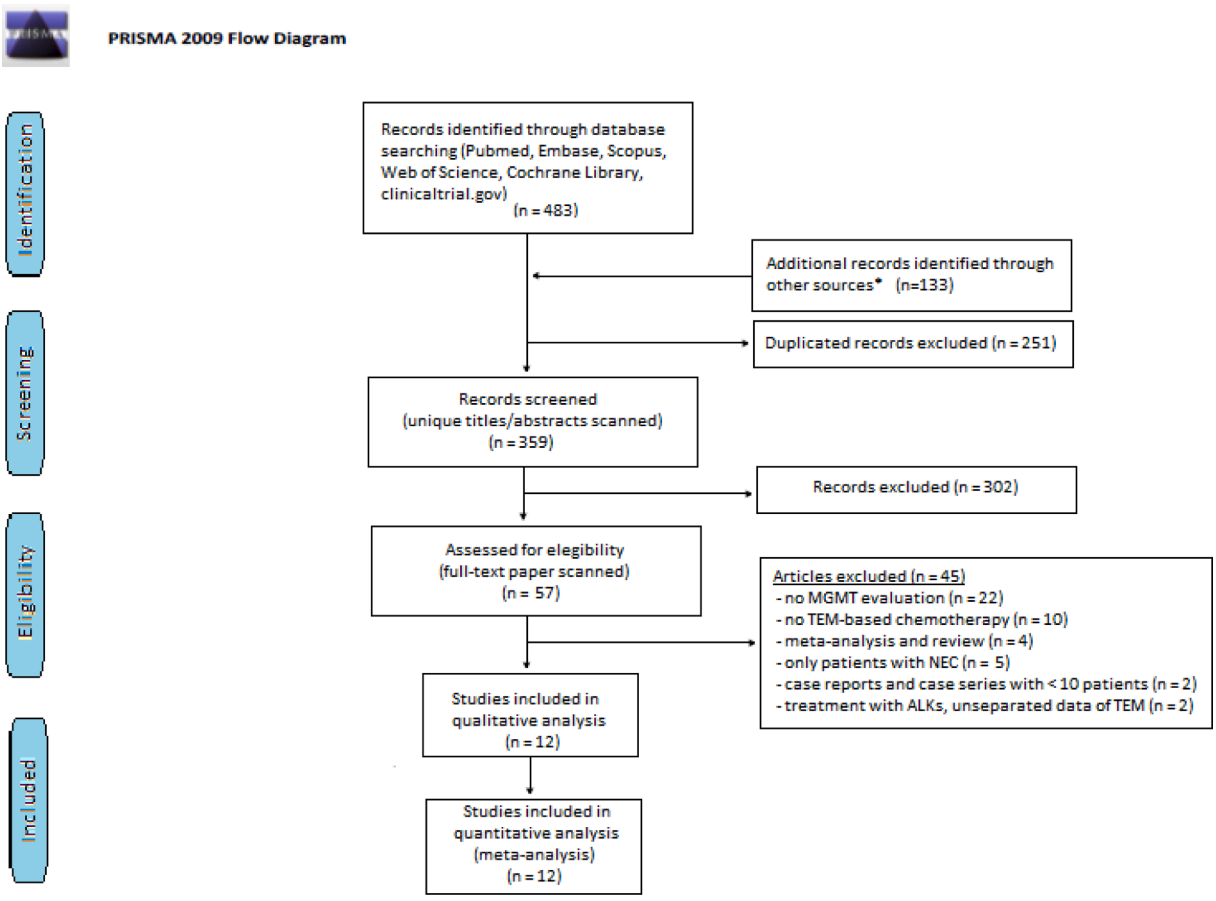

Fig. 1. Flow diagram representing the systematic review process performed according to PRISMA statement.

heterogeneity, was also calculated [30]. The analyses were conducted with R package meta. A p-value less than 0.05 was considered statistically significant in all analyses.

The ORR was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST criteria) [31]. The PFS was defined as the time from start the treatment to radiological progression or death for any cause and TTP was defined as the time from start the treatment to radiological progression. The OS was defined as the time from start of TEM-based treatment to death from any cause.

Quality assessment and risk of publication bias

The selected articles were assessed according to "The Newcastle-Ottawa Scale (NOS)", which includes three subscales (selection,

Table 1
Summary of the selected studies.

Authors	Date of publication	Study design	Centers involved	Time frame	Country	Total of patients per study N	Pts treated with TEM-based therapy N (%)	Pts with valid MGMT test N (%)	Pts treated with TEM and valid MGMT test N (%)	Ref.
Viudez et al	2021	retrospective	multicenter	2016–2020	Spain	76®	34 (45)	73 (96)	34 (45)	34
Wang et al	2020	retrospective	multicenter	2011–2017	China	151	151 (100)	43 (29)	43 (29)	33
Spada et al	2020	retrospective	multicenter	2008–2020	Italy	170	170 (100)	49 (29)	49 (29)	35
Campana et al	2018	retrospective	multicenter	2008–2016	Italy-France	95	95 (100)	95 (100)	95 (100)	36
Owen et al	2017	retrospective	na	2009–2013	US	38	38 (100)	20 (53)	20 (53)	37
Girot et al	2017	retrospective	multicenter	2010–2016	France	22	22 (100)	22 (100)	22 (100)	38
Cives et al	2016	retrospective	monocenter	2005–2014	US	143	143 (100)	52 (36)	52 (36)	39
Cros et al	2016	retrospective	monocenter	2005–2012	France	43	43 (100)	43 (100)	43 (100)	40
Walter et al	2015	retrospective	monocenter	1982–2012	France	107*	15 (14)	89 (83)	15 (14)	41
Schmitt et al	2014	retrospective	monocenter	na	Switzerland	141§	10 (7)	9 (6)	9 (6)	42
Kulke et al	2009	retrospective	multicenter	na	US	101	101 (100)	96 (95)	96 (95)	43
Ekeblad et al	2007	retrospective	monocenter	1999–2006	Sweden	36	36 (100)	23 (64)	23 (64)	44

Pts: patients. na: not available. TEM: temozolomide. Ref: reference. ®Of the 76 patients treated in this study, 34 patients received TEM-CAP and 42 patients received everolimus. * Of the 107 patients included in this study only 15 patients were treated with TEM-based chemotherapy.
§ Of the 141 patients with operated NETs only 10 cases developed metastases and were treated with TEM-based chemotherapy.

Table 2
Summary of studies with MGMT expression, evaluation methods (IHC, PSQ or MSP) and their possible correlation.

Author	Pts with MGMT test	IHC						PSQ						MSP				Correlation among techniques
	N	n	Kit	Cut Off	Low	High	NI	n	Kit	Cut Off	Hyper-CH3	Un-CH3	NI	n	Hyper-CH3	Un-CH3	NI	
Viudez	73	73	Mouse anti-MGMT monoclonal antibody (abcam)	Deficiency = 0%	46 (63%)	27 (37%)	0	–	–	–	–	–		–	–	–	–	na
Wang	43	43	Anti-MGMT monoclonal antibody (Fuzhou Maxim Biotech)	Three cut-off applied: deficiency =0%; low < 10%; low = negative/ weak°	11 (26%) 15 (35%) 19 (44%)	32 (74%) 28 (65%) 24 (56%)	0 0 0	–	–	–	–	–	–	–	–	–	–	na
Spada	49	–	–	–	–	–	–	49	MGMT plus®, (Diatech Pharmacogenetic)	Meth ≥ 5%	31 (63%)	18 (37%)	0	–	–	–	–	na
Campana	95	–	–	–	–	–	–	53	PyroMark Q24 CpG MGMT Kit (Qiagen)	Meth > 8%	15 (28%)	38 (72%)	0	42	12 (29%)	30 (71%)	0	na
Owen	20	20	MS-470B (Thermofisher)	low < 10%	12 (60%)	8 (40%)	0	–			–	–	–	–	–	–	–	na
Girot	22	22	MT3.1 (Merck Millipore)	low < 10%	13 (59%)	9 (41%)	0	–	–	–	–	–	–	22*	3 (14%)	17 (77%)	2 (9%)	NO correlation found
Cives	65	65	MS-470-P1 (Thermo Scientific)	Three cut-off applied: deficiency = 0%; low < 10%; Allred score < 4*	- 15 (23%) 20 (31%) 19 (29%)	- 37 (57%) 32 (49%) 33 (51%)	13 (20%)	–	–	–	–	–	–	–	–	–	–	na
Cros	43	43	MT3.1 (Thermo Scientific)	Low: nuclear staining intensity (0–3) multiplied by the percentage of stained cells (0–100%) < 50	30 (70%)	13 (30%)	0	29	PyroMark Q24 MGMT kit	Meth ≥ 7%	15 (52%)	14 (48%)	0	–	–	–	–	High methylation associated with low MGMT IHC (<=100) (p = 0.08)
Walter	107	107	MT23.2 (Invitrogen)	Negative < 10%	29 (27%)	60 (56%)	18 (17%)	107	PyroMark Q96 MGMT kit	Meth ≥8%	24 (22%)	75 (70%)	8 (8%)	107	12 (11%)	87 (81%)	8 (8%)	Concordance rate 89% between PSQ and IHC and 84% between MSP and IHC
Schmitt	10	10	MT 3.1 (Gen Tech)	Low ≤ 5%	4 (40%)	5 (50%)	1 (10%)	–	–	–	–	–	–	10**	3 (30%)	7 (70%)	0	NO correlation found
Kulke	97	97	MT 3.1 (Lab Vision)	Deficency = 0%	19 (20%)	77 (79%)	1 (1%)	–	–	–	–	–	–	–	–	–	–	na
Ekeblad	23	23	MAB16200 (Chemicon)	Low < 10%	10 (43%)	13 (57%)	0	–	–	–	–	–	–	–	–	–	–	na

Pts = patients. N or n = number. IHC = immunohistochemistry; PSQ = pyrosequensing; MSP or MS-PCR = Methylation-specific polymerase chain reaction. NI = test not interpretable. * The immunostaining signal was expressed as the sum of scores representing the proportion and staining intensity of negative and positive tumor cell nuclei (Allred et al., 1993) **Primer extension-based quantitative PCR. ° Analyzed based on the immunoreactivity scored into 4 categories according to the intensity of the staining: 0, 1+, 2+, and 3+. The percentage of positive tumor cells was also scored semiquantitatively on a 6-tiered scale: 0 (0%), 1 (≤1%), 2 (1–10%), 3 (11–33%), 4 (34–66%), and 5 (>66%). The sum of both intensity and percentage scores defined the final score, which was classified as negative (score 0), weak (score 1–3), moderate (scores 4–6), or strong (scores 7–8).

comparability, and exposure), to assess the quality of non-randomized studies in meta-analyses [32]. For comparability, where the NOS requires to verify whether the study controls for confounders, we decided to assign points only to studies controlling for covariates with respect to the associations of our interest, regardless of the focus of the study. Studies with a score of 7–9 stars were considered to have a low risk of bias, studies with a score of 4–6 were considered to have a medium risk of bias, and studies with a score of ≤3 were considered to have a high risk of bias. Risk of bias assessment was conducted independently by two authors (PTA and GP) and disagreement was settled by consensus.

Publication bias was estimated using Begg's Funnel plot, in which the mean result of each study was plotted against its standard error; the corresponding Egger's test was performed to test for small study effects.

Results

Characteristics of the included studies

A flow chart representing the systematic review process performed according to PRISMA statement is shown in Fig. 1. Our systematic research retrieved 616 documents and after removing 251 duplicates 359 records were screened for inclusion. In the screening phase 302 records were excluded and 57 articles were selected to assess eligibility. According to the selection criteria, we identified twelve eligible articles [33–44].

As reported in Table 1, the twelve selected studies [33–44] were retrospective. Five were monocentric [39–42,44], six multicentric [33–36,38,43] and one unspecified [37]. Eight studies were from Europe, three from US and one from Asia. The time frame reported in ten studies ranged from 1982 to 2020.

Among a total of 1123 patients included in the twelve studies, 858 (76%) received TEM-based chemotherapy and of these 513 (60%) underwent an assessment of MGMT status. Some of these patients were either not treated with TEM or assessed MGMT: 134 patients underwent MGMT evaluation but had not received TEM-based chemotherapy and 345 patients who received TEM-based chemotherapy, had not performed MGMT status (Supplementary Fig. 1).

Demographic and clinical features of patients

We collected the demographic and clinical characteristics of the patients for each study when available (Supplementary Table 3). Seven studies [33,35–37,41,43,44] included patients with pancreatic (pan)-NET and extra-pancreatic (EP)-NET origin while five studies [34,38–40,42] included only patients with pan-NETs. The patients with EP-NETs had gastrointestinal, bronchial, thymic, and other origins. Three studies [37,43,44] included patients with pheochromocytoma or paraganglioma in ≤5% of cases.

Most of the studies used the WHO 2010 classification for GEP origin [45] and the WHO 2004 classification for bronchial origin [46]; three recent studies [33-35] used the WHO 2019 classification [2] and only for two older studies [43,44] it was not specified. Given this heterogeneity, we reported the value of Ki67 index. Overall, in our analysis pancreatic origin are dominant and the majority had an intermediate Ki67 index (range: 3–20%).

Temozolomide-based chemotherapy

Among 858 patients receiving a TEM-based chemotherapy: 577 (67%) received TEM plus capecitabine (CAPTEM), 52 (6%) TEM plus bevacizumab [43], 44 (5%) TEM plus thalidomide [43] and 185 (22%) TEM as single agent (Supplementary Table 4).

Two-hundred eighty-three (33%) patients received TEM-based chemotherapy as first line, 541 (63%) as further line and for 34 (4%) was not specified. Eight studies [33–39,42] reported the type of prior therapy, that was chemotherapy in seven studies [33–37,39,42]. Some of these patients had received a prior alkylating agent (e.g., streptozotocin) as reported in Supplementary Table 4.

MGMT expression

The status of MGMT was assessed for each study using various methods as reported in Table 2. Immunohistochemistry was used in ten studies [33,34,37–44], pyrosequencing (PSQ) was performed in four studies [35,36,40,41] and methylation-specific-PCR (MSP) or primer extension-based quantitative PCR (MS-PCR) was done in four studies [36,38,41,42]. Overall, only one method (IHC or PSQ) was used in seven studies [33–35,37,39,43,44], two methods (IHC and PSQ or MSP) in four studies [36,38,40,42], and three methods (IHC and PSQ and MSP) in only one study [41].

We observed that the low expression of MGMT assessed by IHC in the different ten studies ranged from 20% to 70%. Instead, the hyper-methylation (hyper-CH3) of the MGMT promoter assessed by PSQ ranged from 22% to 63% and assessed by MSP or MS-PCR ranged from 11% to 30%.

In four studies was reported the concordance among techniques with contradictory results. Girot et al [38] and Schmitt et al [42] could not find correlation between ICH and MSP or MS-PCR. Walter et al [41] found 89% of concordance rate between PSQ and IHC and 84% between MSP and IHC. Also Cros et al [40] reported that high methylation was associated with low MGMT IHC (cut off ≤ 100) ($p = 0.08$).

However high grade of variability was present within the techniques applied in the studies. The MGMT protein expression evaluated by IHC were performed with different antibodies, dilutions and antigen retrieval systems. Even the assessment of the expression level of MGMT was performed using different criteria. Two studies [34,43] defined MGMT deficiency as complete absence of staining in all cancer cells, four studies [37,38,41,44] adopted a cut off value of 10% of positive tumor cell to define MGMT deficiency whereas Schmitt [42] reported a threshold of 5%, Cros [40] evaluated the nuclear staining intensity and the percentage of stained cells, and other two studies [33,39] grouped the cases based on multiple assessment scoring systems. Similarly, PSQ was performed with different kit and thresholds (5% [35], 7% [40] and 8% [36,41]) for hyper-methylation assessment.

Quality assessment of the studies

All studies were evaluated with the Newcastle-Ottawa Scale for cohort studies. Five studies scored 5, six studies scored 6, and only one study scored 7 (Supplementary Table 7). Eleven out of twelve studies should be considered with an intermediate risk of bias while only one study should be considered with low risk of bias. The main reasons for the intermediate score in the eleven studies were related to the absence of a direct comparison between the two groups (MGMT deficient versus MGMT proficient) in all studies and the absence of follow-up data reporting in the majority of them.

We also obtained asymmetric funnel plots for ORR (Supplementary Fig. 6), PFS/TTP and OS (Supplementary Fig. 7). This suggests the possibility of publication bias or a systematic difference between higher and lower precision studies (typically "small study effects").

Meta-analysis

MGMT status and overall response rate

Eleven articles [33,35–44] provided ORR data for this analysis (Supplementary Table 5). It was available in 814 (95%) of the 858 patients treated with TEM-based chemotherapy because in 34 patients the radiological tumor assessment was not done. Correlation between ORR and MGMT status was obtained for 385 patients who had valid MGMT test and sufficient follow-up. In particular, the correlation of ORR with MGMT evaluated by IHC was in 227 (59%) cases, with the methylation method (PSQ or MSP) in 187 (49%) cases and with both methods in 29 (8%) cases (Supplementary Fig. 1). Regarding methylation, ORR was available in 63 (34%) patients with PSQ and 124 (64%) patients with MSP or MS-PCR, respectively.

The results of our meta-analysis for MGMT status and ORR are shown

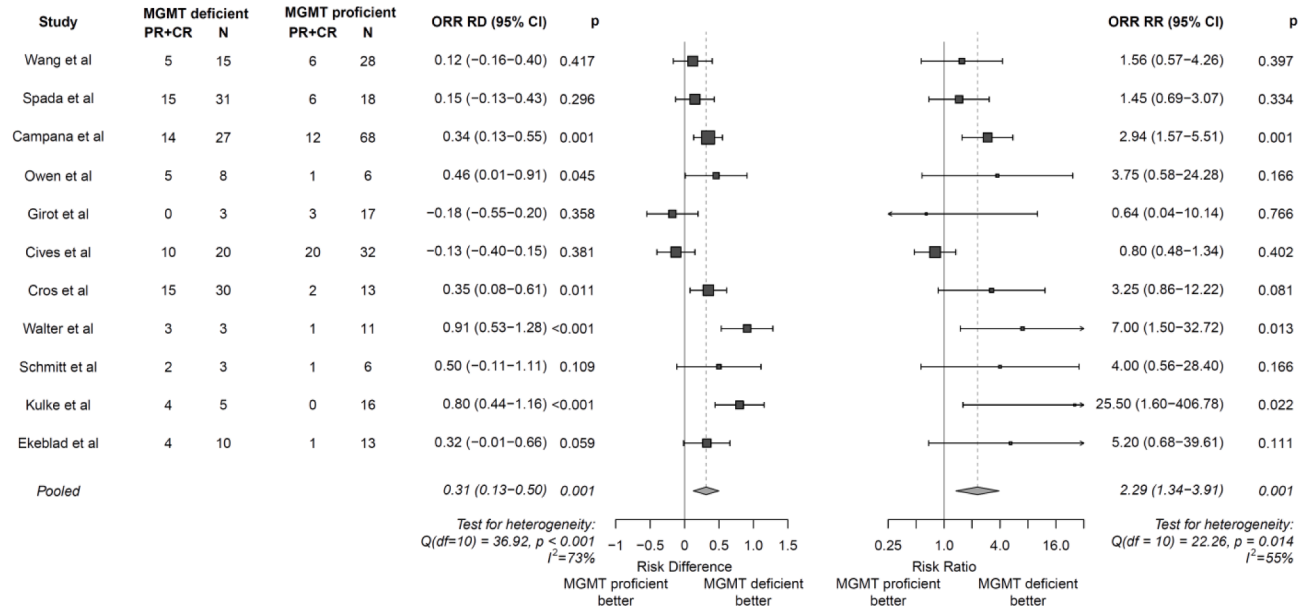

Fig. 2. Forest plots representing the meta-analysis of risk differences and risk ratios for the ORR in MGMT deficient vs MGMT proficient.

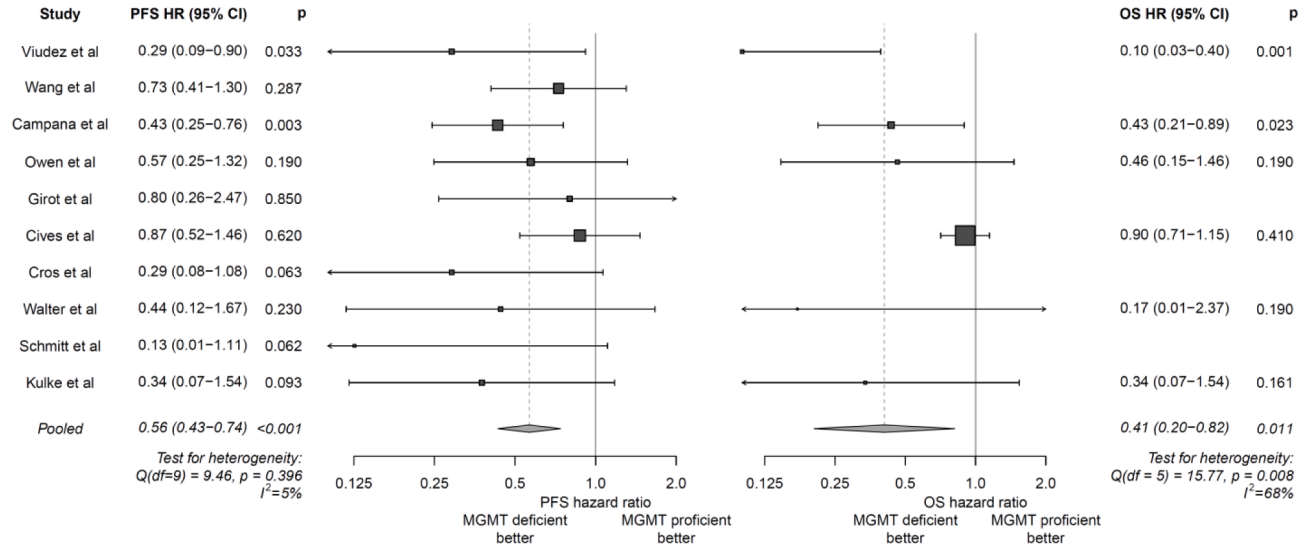

Fig. 3. Forest plots representing the meta-analysis of progression-free survival and overall survival hazard ratios for MGMT deficient vs MGMT proficient.

in Fig. 2. The pooled data analysis showed a risk difference (RD) of 0.31 (95% CI: 0.13–0.50; $p < 0.001$; I^2: 73%) and a risk ratio (RR) of 2.29 (95% CI: 1.34–3.91; $p < 0.001$; I^2: 55%) in patients with MGMT deficient status (defined as low protein expression and/or promoter hyper-methylated) compared to patients with MGMT proficient status (defined as high protein expression and/or promoter un-methylated). According to different methods of detecting the MGMT status, we performed a subgroup analysis. Considering the immunohistochemical method (Supplementary Fig. 2), our meta-analysis indicated higher ORR in patients with low MGMT protein expression compared to patients with high MGMT protein expression (RR 2.41; 95% CI: 1.11–5.21; $p = 0.025$; I^2: 54%). Similarly, for the methylation methods (PSQ or MSP/MS-PCR) (Supplementary Fig. 3), the ORR was significantly greater in patients with hyper-methylated promoter than in patients with un-methylated promoter (RR 2.45; 95% CI: 1.40–4.30; $p = 0.002$; I^2: 22%). There were similar results between the subgroups.

MGMT status and progression free-survival or time to progression

In ten [33,34,36–43] of the twelve studies this correlation was reported (Supplementary Table 6). Our pooled analysis (Fig. 3) indicated that patients with NETs with MGMT deficient status had significantly longer PFS compared to patients with MGMT proficient status (HR 0.56; 95% CI: 0.43–0.74; $p < 0.001$, I^2: 5%). In the subgroup analysis according to the IHC method we obtained a longer PFS in patients with low protein expression compared to patients with high protein expression (HR = 0.63, 95% CI: 0.47–0.83; $p = 0.001$, I^2: 0%) (Supplementary Fig. 4). Likewise, the meta-analysis for the studies with the methylation method (PSQ and MSP/MS-PCR) indicated that PFS is longer in patients with hyper-methylated MGMT promoter compared to patients with un-methylated MGMT promoter (HR = 0.43, 95% CI: 0.28–0.67; $p < 0.001$, I^2: 0%) (Supplementary Fig. 5).

MGMT status and overall survival

The median OS was available in six [34,36,37,39,41,43] of the twelve studies (Supplementary Table 6). Analysis of the pooled data

showed a longer OS (HR 0.41; 95% CI: 0.20–0.62; p = 0.011, I^2: 68%) in patients with NET with MGMT deficiency compared to patients with MGMT proficiency (Fig. 3). We observed that this advantage was evident in the methylation group (HR 0.41; 95% CI: 0.20–0.82; p = 0.011, I^2: 0%) and not in the protein expression group (HR 0.40; 95% CI: 0.15–1.07; p = 0.068, I^2: 75%) (Supplementary Fig. 4 and Supplementary Fig. 5).

Discussion

Our systematic review and meta-analysis strengthened the hypothesis that MGMT could have a role as a predictor of response/efficacy to TEM-based chemotherapy in NETs, that is a long-debated issue in the NET field. Particularly it showed that (1) patients with MGMT-deficient NETs treated with TEM-based chemotherapy had a higher ORR compared with MGMT-proficient NETs and this difference was observed for both IHC and methylation. A significantly longer PFS (2) was also observed for MGMT-deficient compared with MGMT-proficient and this difference was more remarkable with methylation than IHC; (3) finally, a significantly better OS was observed for MGMT-deficient compared with MGMT-proficient NET patients even though this advantage was statistically significant only for methylation.

Single studies with contradictory results led to investigating some variables. Among the others we discuss three of them as follows. Primarily, clinical and biological heterogeneity of NETs. Differences in methylation of MGMT have been previously reported between the different primary sites. It is estimated that MGMT methylation in panNENs is about 50% [38–40,42] whereas it is approximately 0–15% in lung and gastrointestinal NENs [41,43]. So, different MGMT status of the primary sites may be cause different sensitivity to TEM-based treatment. Additionally, potential differences in MGMT status between the primary site and metastatic lesions within the same patient could contribute to this discordance. Indeed one study showed higher rates of MGMT activity in metastases than in the primary lesion [48], however another small study suggested that MGMT alterations were comparable in primary NETs and their metastatic sites [49].

Secondly, the different methods used to assess the MGMT status. The evaluation of MGMT status remains a critical point because of the lack of standardize procedures. Indeed, different methods may be adopted to forecast the deficiency/proficiency of MGMT DNA repair mechanism. MGMT deficiency can be assessed at epigenomic level, evaluating the promoter methylation status or at protein level, assessing the immunohistochemical staining. The concordance between these nonequivalent evaluations is still a debated issue as different studies reported contradictory results [38,40,42]. Of note, the two-level evaluations can produce different results as the gene expression can be regulated not only by methylation silencing but other mechanisms such as long noncoding RNAs (lncRNAs) [50], transcriptional regulation via p53 [51] and NF-Kb/STAT3 dependent mechanism [52] may affect the protein translation. Moreover, the intra-tumor heterogeneity adds complexity to this scenario [53]. Traditionally, MGMT status has been assessed by methylation-specific PCR (MS-PCR), a qualitative PCR evaluating CpG islands in the promoter region, that has shown to have a predictive role in clinical trials [54]. From this starting point have been developed other methods such as quantitative methylation-specific PCR techniques, that allow a quantification of the methylation level of few CpG dinucleotides. These techniques require a step of bisulfite conversion, are easy to perform, have a dichotomic positive/negative result or a methylation quantitative value. However, these approaches have some limitations as they are biased by the tumor cell content, they interrogate few CpG sites in a context of heterogeneity in the methylation levels of CpG sites [55] and they lack of standardized methylated cutoff value for considering MGMT methylation positive. On the other side, different efforts have been made to evaluate the predictive value of MGMT protein expression by IHC [56]. Immunohistochemical staining is faster, less expensive, and easy to implement even if less reproducible and more affected to subjective interpretation. There are different cut-offs for considering MGMT positive/negative cases and there is a lack of uniformly defined criteria for IHC interpretation. Among different studies MGMT IHC staining results are reported as dichotomic positive/negative evaluations [43] or low, intermediate or high expression [37] or the proportion of stained cell, based on nuclear staining (grade 0–3) [40]. A step forward has been reached with the introduction of PSQ assays, that allows the quantification of the methylation level of single CpG dinucleotides in the promoter region– first exon of MGMT. This method is laborious and requires a dedicated instrument, the pyro-sequencer, but is robust and reproducible. Moreover, there are commercially available kit for MGMT evaluation starting from FFPE tissue, validated for in vitro diagnostic use [57], with defined cut-off for the positive/negative evaluation. Therefore to date, there has been growing evidences that support the use of PSQ as the method of choice for MGMT evaluation in routine clinical practice [58,59]. Nevertheless, the best predictive value may be reached combining the protein expression and methylation status, even evaluated with Methyl-BEAMing, a digital PCR with high sensitivity [60]. However, the proteic-epigenomic concordant results are associated to modulation of chemotherapeutic response [61,62].

Thirdly, the type of drug associated with TEM. Among these, the most commonly used is CAP. *In-vitro* studies showed that 5-FU depletes tumor levels of MGMT [63]. So capecitabine, which is converted to 5-FU, depletes tumor MGMT levels thereby enhancing the alkylating effect of TEM. Synergistic activity was observed to be schedule dependent, requiring TEM to be given after continuous exposure to CAP [64]. In our systematic review, two studies that exclusively used the TEM-CAP regimen found conflicting results [37,39]. At ASCO 2018, Kunz et al [16] presented the results of a randomized phase II study that compared TEM with TEM-CAP in patients with advanced panNETs (E2211 trial) obtaining a significant improvement in PFS (median PFS of 14.4 versus 22.7 months; HR 0.58) and a non-significant improvement in ORR (27.8% versus 33.3%; p = 0.47). However, the pending results included the MGMT assessed by IHC and by promoter methylation method, which we look forward to seeing soon.

Our results are in line with two recently published meta-analyses that have addressed the issue of the predictive value of MGMT in NETs treated with alkylating agents. Gao et al [65] reported that MGMT deficiency rate detected with both IHC and promoter methylation with PSQ can effectively predict the response to alkylating agents. Similarly, Zhirong et al [66] suggested that MGMT status can be used as a biological indicator of response to alkylating agents.

Our analysis shows several limitations and therefore its results should be taken with caution. (1) The selection criteria were very flexible, for example some studies had a limited number of NECs, paragangliomas, pheochromocytomas and others unspecified. (2) All the included studies were retrospective and almost all did not have our topic as their primary endpoint. In fact, using the NOS scale we found that most of the included studies have an intermediate risk of bias (score 4–6). (3) The methods of detecting of MGMT in the various studies were various. (4) Funnel plots showed asymmetry, suggesting the presence of publication bias. On the basis of these limitations, we would like to highlight the risk of bias in the event that the results of our analysis will be taken into account for clinical decisions.

In conclusion, our analysis suggested that MGMT status may be predictive of TEM activity and efficacy. However, due to the high heterogeneity of the evaluated studies the risk of biases should be considered. Particularly it should be considered that studies with different primary sites, tumor grade, prior lines of therapies were selected. So, currently there is no absolute evidence for mandatory testing MGMT in clinical practice to decide on TEM-based chemotherapy in patients with NETs. However, the results of our analysis should be taken as a solid hypothesis to be investigated in well-designed homogeneous prospective clinical trials that will specifically address this topic. At the time of writing this paper, seven ongoing clinical trials are investigating the MGMT as predictive biomarker in NETs treated with TEM-based

Table 3
Ongoing clinical trials evaluating the status of MGMT as a predictive factor to TEM-based therapy.

NCT.gov identifier	Phase	Status	Population	Drug regimen	MGMT method	MGMT as endpoint
NCT03217097 (MGMT-NET)	II	R	Neuroendocrine Tumors	Alkylating-based chemotherapy (STZ, DTIC, TEM)	PSQ & IHC	1°
NCT01525082	II	U	Metastatic or Unresectable Pancreatic Neuroendocrine Tumors	TEM, Capecitabine and Bevacizumab	IHC & MS-PCR	2°
NCT02231762 (SONNET)	II	C	Progressive GEP-NET	TEM and Lanreotide Autogel	IHC & MS-PCR	2°
NCT01824875 (E2211)	II	ANR	Advanced Pancreatic Neuroendocrine Tumors	TEM with or without Capecitabine	IHC & promoter methylation	2°
NCT02698410 (ATLANT)	II	C	Thoracic Neuroendocrine Tumors	TEM and Lanreotide Autogel	IHC & MS-PCR	2°
NCT01465659	I/II	ANR	Advanced Pancreatic Neuroendocrine Tumors	Pazopanib and TEM	IHC	3°
NCT02358356 (CONTROL NETS)	II	R	Neuroendocrine Tumors	CAPTEM and Radionuclide Therapy Octreotate Lutetium-177	IHC	IV

R = Recruiting, U = Unknown, C = completed, ANR = Active, not recruiting, IV = investigational. IHC = immunohistochemistry, PSQ = pyrosequencing, MSP or MS-PCR = Methylation-specific PCR. TEM = temozolomide. STZ = streptozotocin. DTIC = dacarbazine. CAPTEM = capecitabine plus temozolomide.

chemotherapy as their primary, secondary or exploratory endpoint, as shown Table 3.

Funding

No funding was received for this manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: FN reports Honoraria: Novartis, Ipsen, Merck, Sanofi-Aventis, Advanced Accelerator Applications. Consulting or Advisory Role: Novartis/Ipsen, Advanced Accelerator Applications, Pfizer, Ipsen, Merck Serono, MSD Oncology, Wren Laboratories Europe.

GC Research Funding: Novartis (Institutional), Merck Serono (Inst), Ipsen (Inst), MSD (Inst) has received honoraria from Pfizer, Novartis, Lilly, Roche; fees for expert testimony and medical education from Pfizer; and has participated in advisory boards for Pfizer, Roche, Lilly, Novartis, Seattle Genetics, Celltrion. All the declarations are outside the submitted work.

All the other authors have no potential conflicts of interest to disclosure.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ctrv.2021.102261.

References

[1] Dasari A, Shen C, Halperin D, Zhao Bo, Zhou S, Xu Y, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. JAMA Oncol 2017;3(10):1335. https://doi.org/10.1001/jamaoncol.2017.0589.
[2] Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. Histopathology 2020;76(2):182–8. https://doi.org/10.1111/his.v76.210.1111/his.13975.
[3] Modlin IM, Pavel M, Kidd M, Gustafsson BI. Review article: Somatostatin analogues in the treatment of gastroenteropancreatic neuroendocrine (carcinoid) tumours. Aliment Pharmacol Ther 2010. https://doi.org/10.1111/j.1365-2036.2009.04174.x.
[4] Pearson RK. Everolimus for advanced pancreatic neuroendocrine tumors. 10.1056/NEJMoa1009290. N Engl J Med 2011;2011:138–9.
[5] Raymond E, Dahan L, Raoul J-L, Bang Y-J, Borbath I, Lombard-Bohas C, et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med 2011;364(6):501–13. https://doi.org/10.1056/NEJMoa1003825.
[6] Kong G, Hicks RJ. Peptide Receptor Radiotherapy: Current Approaches and Future Directions. Curr Treat Options Oncol 2019;20(10). https://doi.org/10.1007/s11864-019-0677-7.
[7] Strosberg J, Goldman J, Costa F, Pavel M. The Role of Chemotherapy in Well-Differentiated Gastroenteropancreatic Neuroendocrine Tumors. Frontiers of hormone research 2015. https://doi.org/10.1159/000403785.
[8] Kouvaraki MA, Ajani JA, Hoff P, Wolff R, Evans DB, Lozano R, et al. Fluorouracil, doxorubicin, and streptozocin in the treatment of patients with locally advanced and metastatic pancreatic endocrine carcinomas. J Clin Oncol 2004;22(23):4762–71. https://doi.org/10.1200/JCO.2004.04.024.
[9] Delaunoit T, Ducreux M, Baudin E, Boige V, Lasser P, Rougier P, et al. Treatment of pancreatic endocrine tumours with adriamycine-streptozotocin association: evaluation of efficacy and prognostic factors of response and survival. Eur J Cancer 2001;37:S313. https://doi.org/10.1016/S0959-8049(01)81649-X.
[10] Bajetta E, et al. 5-fluorouracil, dacarbazine, and epirubicin in the treatment of patients with neuroendocrine tumors. Cancer 1998. https://doi.org/10.1002/(SICI)1097-0142(19980715)83:2<372::AID-CNCR23>3.0.CO;2-P.
[11] Koumarianou A, Kaltsas G, Kulke MH, Oberg K, Strosberg JR, Spada F, et al. Temozolomide in Advanced Neuroendocrine Neoplasms: Pharmacological and Clinical Aspects. Neuroendocrinology 2015;101(4):274–88. https://doi.org/10.1159/000430816.
[12] Tafuto S, von Arx C, Capozzi M, Tatangelo F, Mura M, Modica R, et al. Safety and Activity of Metronomic Temozolomide in Second-Line Treatment of Advanced Neuroendocrine Neoplasms. J Clin Med 2019;8(8):1224. https://doi.org/10.3390/jcm8081224.
[13] Ferrarotto R, Testa L, Riechelmann RP, Sahade M, Siqueira LT, Costa FP, et al. Combination of capecitabine and oxaliplatin is an effective treatment option for advanced neuroendocrine tumors. Rare Tumors 2013;5(3):121–5. https://doi.org/10.4081/rt.2013.e35.
[14] Brixi-Benmansour H, Jouve J-L, Mitry E, Bonnetain F, Landi B, Hentic O, et al. Phase II study of first-line FOLFIRI for progressive metastatic well-differentiated pancreatic endocrine carcinoma. Dig Liver Dis 2011;43(11):912–6. https://doi.org/10.1016/j.dld.2011.07.001.
[15] Kulke MH, Wu B, Ryan DP, Enzinger PC, Zhu AX, Clark JW, et al. A phase II trial of irinotecan and cisplatin in patients with metastatic neuroendocrine tumors. Dig Dis Sci 2006;51(6):1033–8. https://doi.org/10.1007/s10620-006-8001-3.
[16] Kunz PL, et al. A randomized study of temozolomide or temozolomide and capecitabine in patients with advanced pancreatic neuroendocrine tumors: A trial of the ECOG-ACRIN Cancer Research Group (E2211). J Clin Oncol 2018;36:4004.
[17] Kaina B, Christmann M, Naumann S, Roos WP. MGMT: Key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. DNA Repair (Amst) 2007;6(8):1079–99. https://doi.org/10.1016/j.dnarep.2007.03.008.
[18] Pfeifer G. Defining driver DNA methylation changes in human cancer. Int J Mol Sci 2018;19(4):1166. https://doi.org/10.3390/ijms19041166.
[19] Shiraishi A. Increased susceptibility to chemotherapeutic alkylating agents of mice deficient in DNA repair methyltransferase. Carcinogenesis 2000;21(10):1879–83. https://doi.org/10.1093/carcin/21.10.1879.
[20] Bugni JM, Meira LB, Samson LD. Alkylation-induced colon tumorigenesis in mice deficient in the Mgmt and Msh6 proteins. Oncogene 2009;28(5):734–41. https://doi.org/10.1038/onc.2008.426.
[21] Kitange GJ, Carlson BL, Schroeder MA, Grogan PT, Lamont JD, Decker PA, et al. Induction of MGMT expression is associated with temozolomide resistance in glioblastoma xenografts. Neuro Oncol 2009;11(3):281–91. https://doi.org/10.1215/15228517-2008-090.
[22] Weller M, Tabatabai G, Kästner B, Felsberg J, Steinbach JP, Wick A, et al. MGMT promoter methylation is a strong prognostic biomarker for benefit from dose-intensified temozolomide rechallenge in progressive Glioblastoma: The DIRECTOR Trial. Clin Cancer Res 2015;21(9):2057–64. https://doi.org/10.1158/1078-0432.CCR-14-2737.
[23] Hegi ME, Diserens A-C, Gorlia T, Hamou M-F, de Tribolet N, Weller M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352(10):997–1003. https://doi.org/10.1056/NEJMoa043331.
[24] Schraml Peter, Von Teichman Adriana, Mihic-Probst Daniela, Simcock Mathew, Ochsenbein Adrian, Dummer Reinhard, et al. Predictive value of the MGMT

promoter methylation status in metastatic melanoma patients receiving first-line temozolomide plus bevacizumab in the trial SAKK 50/07. Oncol Rep 2012;28(2):654–8. https://doi.org/10.3892/or.2012.1826.
[25] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol 2009;62(10):e1–34. https://doi.org/10.1016/j.jclinepi.2009.06.006.
[26] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 2009;62(10):1006–12. https://doi.org/10.1016/j.jclinepi.2009.06.005.
[27] Guyot P, Ades AE, Ouwens MJNM, Welton NJ. Enhanced secondary analysis of survival data: reconstructing the data from published Kaplan-Meier survival curves. BMC Med Res Methodol 2012;12:9.
[28] Cortés J, González JA, Campbell MJ, Cobo E. A hazard ratio was estimated by a ratio of median survival times, but with considerable uncertainty. J Clin Epidemiol 2014;67:1172–7.
[29] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
[30] Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.
[31] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J 2009;45(2):228–47. https://doi.org/10.1016/j.ejca.2008.10.026.
[32] Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses.
[33] Wang W, Zhang Yu, Peng Y, Jin K-Z, Li Y-L, Liang Y, et al. A Ki-67 Index to Predict Treatment Response to the Capecitabine Temozolomide (CAPTEM) Regimen in Neuroendocrine Neoplasms: A Retrospective Multicenter Study. Neuroendocrinology 2020. https://doi.org/10.1159/000510159.
[34] Viúdez A, Crespo G, Gómez Dorronsoro ML, Arozarena I, Marín-Méndez JJ, Custodio A, et al. Usefulness of an immunohistochemical score in advanced pancreatic neuroendocrine tumors treated with CAPTEM or everolimus. Pancreatology 2021;21(1):215–23.
[35] Spada F, Maisonneuve P, Fumagalli C, Marconcini R, Gelsomino F, Antonuzzo L, et al. Temozolomide alone or in combination with capecitabine in patients with advanced neuroendocrine neoplasms: an Italian multicenter real-world analysis. Endocrine 2021;72(1):268–78. https://doi.org/10.1007/s12020-020-02421-2.
[36] Campana D, Walter T, Pusceddu S, Gelsomino F, Graillot E, Prinzi N, et al. Correlation between MGMT promoter methylation and response to temozolomide-based therapy in neuroendocrine neoplasms: an observational retrospective multicenter study. Endocrine 2018;60(3):490–8. https://doi.org/10.1007/s12020-017-1474-3.
[37] Owen DH, Alexander AJ, Konda B, Wei L, Hemminger JA, Schmidt CR, et al. Combination therapy with capecitabine and temozolomide in patients with low and high grade neuroendocrine tumors, with an exploratory analysis of O6-methylguanine DNA methyltransferase as a biomarker for response. Oncotarget 2017;8(61):104046–56. https://doi.org/10.18632/oncotarget.v8i6110.18632/oncotarget.22001.
[38] Girot P, et al. 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 2017. https://doi.org/10.1097/MEG.0000000000000874.
[39] Cives M, Ghayouri M, Morse B, Brelsford M, Black M, Rizzo A, et al. Analysis of potential response predictors to capecitabine/temozolomide in metastatic pancreatic neuroendocrine tumors. Endocr Relat Cancer 2016;23(9):759–67. https://doi.org/10.1530/ERC-16-0147.
[40] Cros J, et al. MGMT expression predicts response to temozolomide in pancreatic neuroendocrine tumors. Endocr Relat Cancer 2016;23:625–33.
[41] Walter T, van Brakel B, Vercherat C, Hervieu V, Forestier J, Chayvialle J-A, et al. O6-Methylguanine-DNA methyltransferase status in neuroendocrine tumours: Prognostic relevance and association with response to alkylating agents. Br J Cancer 2015;112(3):523–31.
[42] Schmitt AM, Pavel M, Rudolph T, Dawson H, Blank A, Komminoth P, et al. Prognostic and predictive roles of MGMT protein expression and promoter methylation in sporadic pancreatic neuroendocrine neoplasms. Neuroendocrinology 2014;100(1):35–44.
[43] Kulke MH, et al. O 6 -methylguanine DNA methyltransferase deficiency and response to temozolomide-based therapy in Patients with Neuroendocrine Tumors. Clin Cancer Res 2009;15:338–45.
[44] Ekeblad S, et al. Temozolomide as monotherapy is effective in treatment of advanced malignant neuroendocrine tumors. Clin Cancer Res 2007;13:2986–91.
[45] Bosman F, Carneiro F, Hruban R, Theise N. WHO Classification of Tumours of the Digestive System. Forth Edition. Lyon: International Agency for research on canser; 2010. http://dx.doi.10.1017/CBO9781107415324.004.
[46] Beasley MB, Brambilla E, Travis WD. The 2004 World Health Organization classification of lung tumors. Semin Roentgenol 2005;40:90–7.
[48] Christmann M, Verbeek B, Roos WP, Kaina B. O6-Methylguanine-DNA methyltransferase (MGMT) in normal tissues and tumors: Enzyme activity, promoter methylation and immunohistochemistry. Biochimica et Biophysica Acta - Reviews on Cancer 2011;1816(2):179–90. https://doi.org/10.1016/j.bbcan.2011.06.002.
[49] House MG, et al. Aberrant Hypermethylation Tumor Suppressor Genes in Pancreatic Endocrine Neoplasms. Ann Surg 2003. https://doi.org/10.1097/01.sla.0000086659.49569.9e.
[50] Wu P, et al. Lnc-TALC promotes O6-methylguanine-DNA methyltransferase expression via regulating the c-Met pathway by competitively binding with miR-20b-3p. Nat Commun 2019;10:2045.
[51] Bocangel D, Sengupta S, Mitra S, Bhakat KK. p53-Mediated down-regulation of the human DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) via interaction with Sp1 transcription factor. Anticancer Res 2009;29:3741–50.
[52] Lavon I, et al. Novel Mechanism whereby Nuclear Factor κB Mediates DNA Damage Repair through Regulation of O 6 -Methylguanine-DNA-Methyltransferase. Cancer Res 2007;67:8952–9.
[53] Wenger A, et al. Intratumor DNA methylation heterogeneity in glioblastoma: implications for DNA methylation-based classification. Neuro Oncol 2019;21:616–27.
[54] Hegi ME, et al. Clinical Trial Substantiates the Predictive Value of O-6-Methylguanine-DNA Methyltransferase Promoter Methylation in Glioblastoma Patients Treated with Temozolomide. Clin Cancer Res 2004;10:1871–4.
[55] Chai R-C, et al. A novel analytical model of MGMT methylation pyrosequencing offers improved predictive performance in patients with gliomas. Mod Pathol 2019;32:4–15.
[56] Pandith AA, et al. Concordant association validates MGMT methylation and protein expression as favorable prognostic factors in glioma patients on alkylating chemotherapy (Temozolomide). Sci Rep 2018;8:6704.
[57] Beltrán-García J, Osca-Verdegal R, Mena-Mollá S, García-Giménez JL. Epigenetic IVD Tests for Personalized Precision Medicine in Cancer. Front Genet 2019;10.
[58] Johannessen Lene E, Brandal Petter, Myklebust TorÅge, Heim Sverre, Micci Francesca, Panagopoulos Ioannis. MGMT Gene Promoter Methylation Status – Assessment of Two Pyrosequencing Kits and Three Methylation-specific PCR Methods for their Predictive Capacity in Glioblastomas. *Cancer Genomics -*. Proteomics 2018;15(6):437–46.
[59] Lemelin A, et al. O6-methylguanine-DNA methyltransferase (MGMT) status in neuroendocrine tumors: a randomized phase II study (MGMT-NET). Dig Liver Dis 2019;51:595–9.
[60] Barault L, et al. Digital PCR quantification of MGMT methylation refines prediction of clinical benefit from alkylating agents in glioblastoma and metastatic colorectal cancer. Ann Oncol 2015;26:1994–9.
[61] Lalezari S, et al. Combined analysis of O6-methylguanine-DNA methyltransferase protein expression and promoter methylation provides optimized prognostication of glioblastoma outcome. Neuro Oncol 2013;15:370–81.
[62] Sartore-Bianchi A, et al. Digital PCR assessment of MGMT promoter methylation coupled with reduced protein expression optimises prediction of response to alkylating agents in metastatic colorectal cancer patients. Eur J Cancer 2017;71:43–50.
[63] Murakami J, Lee Y-J, Kokeguchi S, Tsujigiwa H, Asaumi J-I, Nagatsuka H, et al. Depletion of O6-methylguanine-DNA methyltransferase by O 6-benzylguanine enhances 5-FU cytotoxicity in colon and oral cancer cell lines. Oncol Rep 2007. https://doi.org/10.3892/or10.3892/or.17.6.1461.
[64] Fine RL, Gulati AP, Krantz BA, Moss RA, Schreibman S, Tsushima DA, et al. Capecitabine and temozolomide (CAPTEM) for metastatic, well-differentiated neuroendocrine cancers: The Pancreas Center at Columbia University experience. Cancer Chemother Pharmacol 2013;71(3):663–70. https://doi.org/10.1007/s00280-012-2055-z.
[65] Gao HL, et al. O-6-Methylguanine-DNA Methyltransferase and Response to Alkylation Agents in Neuroendocrine Tumor: A Meta-Analysis. J Pancreas 2017.
[66] Qi Z, Tan H. Association between MGMT status and response to alkylating agents in patients with neuroendocrine neoplasms: A systematic review and meta-analysis. Biosci Rep 2020;40(3). https://doi.org/10.1042/BSR20194127.