Temozolomide in Advanced Neuroendocrine Neoplasms: Pharmacological and Clinical Aspects

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Key Words

Neuroendocrine neoplasms · Pancreatic neuroendocrine neoplasms · Neuroendocrine carcinomas · Ki-67 labelling index · Progression-free survival

Abstract

Alkylating agents, such as streptozocin and dacarbazine, have been reported as active in neuroendocrine neoplasms (NENs). Temozolomide (TMZ) is an oral, potentially less toxic derivative of dacarbazine, which has shown activity both as a single agent and in combination with other drugs. Nevertheless, its role in NENs has not been well defined. Several retrospective and prospective phase I–II studies have been published describing its use in a variety of NENs. In a retrospective series, the combination of capecitabine and TMZ was reported to be associated with a particularly high tumour response in pancreatic NENs as a first-line treatment. Although in NENs, determination of the O⁶-methylguanine-DNA methyltransferase (MGMT) status has been suggested

as a predictive biomarker of response, its role still remains investigational, awaiting validation along with the establishment of the optimal detection method. Metronomic schedules have been reported to potentially overcome MGMT-related drug resistance. Toxicity is manageable if well monitored. We reviewed the literature regarding pharmacological and clinical aspects of TMZ, focusing on specific settings of NENs, different schedules, toxicity and safety profiles, and potential predictive biomarkers of response.

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Introduction

Neuroendocrine neoplasms (NENs) constitute a relatively rare and heterogeneous group of tumours, arising from cells of the diffuse neuroendocrine system most

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commonly encountered in the gastrointestinal (GI) tract and the lung [1]. Neuroendocrine cells are characterized by amine and neuropeptide hormone production that, when biologically active, can lead to distinct clinical syndromes. Despite the diversity in tissue origin, the majority of tissues are well differentiated and have common features, including their growth pattern, expression of neuroendocrine markers, and response to treatment [2].

Since their initial description, the nomenclature and classification of NENs has evolved, hindering the collection of epidemiological information and comparison between published studies [3]. In 2010, a revised version of the WHO classification of gastroenteropancreatic NENs introduced specific term changes, including 'neuroendocrine' to indicate neoplastic cells expressing neural markers such as synaptophysin, and 'neuroendocrine neoplasm', which encompasses both well-differentiated and poorly differentiated neoplasms of neuroendocrine cells [4]. NENs were then separated further by their proliferative activity, based on the Ki-67 labelling index (LI) and/or mitotic index that defined grading (G), and differentiation [into well-differentiated neuroendocrine tumours (NETs; G1: Ki-67 LI <3%; G2: Ki-67 LI 3–20%) and poorly differentiated neuroendocrine carcinomas (NECs; G3: Ki-67 LI >20%)].

Traditionally, cytotoxic chemotherapy, primarily with alkylating agents, has been used in patients with locally advanced or metastatic NENs, but it has been associated with variable efficacy depending on inherent tumoural characteristics [5–7]. Following the implementation of specific histopathological classification criteria, it became apparent that the primary site of origin, tumour differentiation, and the extent of the disease can affect prognosis and guide treatment. Although the major part of the information regarding the response of gastroenteropancreatic NENs to various treatments is derived from retrospective studies, randomized prospective studies have recently demonstrated a clinical benefit with molecular therapies targeting angiogenesis and the mTOR pathway, such as with sunitinib [8] and everolimus [9], in patients with advanced progressing pancreatic NENs (pNENs). Although targeted therapies yielded prolonged progression-free survival (PFS) in patients with pNENs, they are rarely associated with objective radiographic tumour shrinkage by Response Evaluation Criteria in Solid Tumors (RECIST) [10].

Although the role of cytotoxic chemotherapy in NENs has not been as well defined, its use is recommended in certain settings by guidelines for NEN management [11–14]. In poorly differentiated NENs, a combination of plat-

inum and etoposide is usually proposed as a gold standard – although based on old and relatively limited evidence [7]. It is more difficult to find a well-defined role for conventional chemotherapy in well-differentiated/moderately differentiated NENs, where several agents, mainly 5-fluorouracil (5-FU), capecitabine (CAP), streptozocin (STZ), dacarbazine (DTIC), and oxaliplatin, have been studied [5]. Temozolomide (TMZ), a less toxic and oral derivative of intravenous DTIC, is an alkylating agent more commonly investigated in the context of low-/intermediate-grade NENs, but it has also been evaluated in high-grade NENs [6, 15, 16]. Brain and endocrine glands have different embryologic origin, as they are derived from the ectodermal and endodermal layers, respectively, but they share biologic pathways including resistance to available chemotherapeutic drugs. This is evident by the finding that low levels of the DNA repair enzyme O⁶-methylguanine-DNA methyltransferase (MGMT) are associated with sensitivity to TMZ in both NENs and glioblastomas [17].

The present review focuses on the current knowledge of TMZ in NENs, including its pharmacological and clinical aspects, possible predictive biomarkers, published and ongoing studies, and clinical experience.

Pharmacology

Pharmacokinetics

TMZ is a small molecule that is rapidly absorbed after oral administration with a bioavailability of approximately 100%. The peak plasma concentration is obtained at a median time of maximal concentration of 1 h. Food reduces TMZ absorption by 9%, but this is not a clinically significant effect [18]. Because TMZ is highly lipophilic, it has a large mean volume of distribution of 0.4 l/kg and is able to cross the blood-brain barrier. Moreover, TMZ is promptly metabolized and eliminated with a mean half-life of about 1.8 h [18]. Within 8 h, it is almost entirely cleared from plasma, exhibiting linear kinetics over the therapeutic dosing range of 75–250 mg/m²/day [19, 20].

TMZ and its metabolites are excreted predominantly by the renal tubules, while cytochrome P450 enzymes play only a minor role. Pharmacokinetic studies have also shown that a creatinine clearance <40 ml/min and hepatic impairment have a minimal effect on TMZ clearance [21]. TMZ does not accumulate in plasma with repeated and increasing dosing [18] and, unlike other alkylating agents that cross-link the DNA, causes less cumulative toxicity such as myelotoxicity [19, 20].

Pharmacodynamics

Mechanism of Action

TMZ belongs to a novel class of alkylating agents which contain an imidazole ring and is structurally and functionally similar to DTIC. It is a prodrug, highly stable at the acid pH values of the stomach, but in the presence of slightly alkaline pH values, such as in blood and tissues, it undergoes spontaneous hydrolysis to the active compound 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC), which is further degraded to reactive methyl diazonium ions. The therapeutic benefit with TMZ depends on its ability to alkylate/methylate DNA, which most often occurs at the N⁷ and O⁶ positions of guanine residues, causing base pair mismatch (fig. 1). This methylation damages the DNA, and when mismatch repair (MMR) enzymes attempt to remove the O⁶-methylguanine adduct, they produce single- and double-strand breaks in the DNA, triggering cell death by apoptosis [18].

Resistance

Three main DNA repair mechanisms are involved in TMZ resistance [22]: MGMT, the MMR system, and the poly(ADP)-ribose polymerase (PARP) pathway, which is responsible for base excision repair (fig. 2). Primary resistance to TMZ is directly related to high MGMT expression [23], while the secondary mechanism is related to the MMR system in MGMT-lacking cells [24]. The third mechanism depends on the PARP pathway, which is involved in removing mainly N⁷-methylguanine and O³-methyladenine adducts. The base excision repair pathway seems to have a marginal role compared to the MGMT and MMR pathways, since the removal of N⁷- and O³-methyl adducts does not result in DNA double-strand breaks [18, 25].

Interactions with Other Drugs

Since TMZ is weakly bound to human plasma proteins and its metabolism is minimally affected by cytochrome P450, an interaction with other drugs is quite uncommon. A population analysis indicated that administration of valproic acid (VPA) decreases the clearance of TMZ by about 5%, which corresponds to the rate of TMZ metabolized by the liver. Interestingly, a combination of VPA and TMZ in vitro seems to enhance its anti-tumour effect in TMZ-resistant glioblastoma multiforme (GBM) cells, which correlates with a VPA-mediated, reduced MGMT expression [26].

MGMT and TMZ

MGMT is a protein encoded by a gene located on chromosome band 10q26, which is crucial for genome stabil-

Fig. 1. Mechanism of action of TMZ.

ity [27]. It repairs the naturally occurring mutagenic DNA lesion O⁶-alkylguanine back to guanine and prevents mismatch and errors during DNA replication and transcription. Accordingly, loss of the *MGMT* gene increased the carcinogenic risk in mice after exposure to alkylating agents [28]. Alkylation DNA damage triggers the MMR system, leading to cell apoptosis. Although alkylating drugs preferentially modify the guanine base at the N⁷ position, O⁶-alkylguanine seems to be the main carcinogenic lesion in the DNA. MGMT is not a true enzyme, since it removes the alkyl group from the lesion in a stoichiometric reaction and the active enzyme is not regenerated after it has been alkylated ('suicide enzyme') [29] (fig. 1).

MGMT protein expression and MGMT methylation rates seem to be different in several GI-NENs and pNENs. MGMT deficiency as assessed by immunohistochemistry (IHC) ranges from 24 to 51% in pNENs [17, 30], while no case of MGMT deficiency has so far been recorded in GI-NENs [17]. In a retrospective study of 110 patients with NET treated with TMZ-based chemotherapy, 21 patients had tissue evaluable for MGMT IHC; partial responses (PR) were observed in 4 of the 5 patients with deficient MGMT (all pancreatic) but in none of the 16 patients showing intact MGMT [17]. On the other hand, Strosberg and co-workers found no significant correlation between MGMT IHC and tumour response in 59 patients with pNETs treated with TMZ combined with CAP (Strosberg, ENETS 2015, H16 poster). By contrast, MGMT methylation appears to be more common in other GI-NENs than in pNENs (25 vs. 0%, $p = 0.03$) [31].

Fig. 2. Mechanisms of resistance to TMZ.

However, in a retrospective cohort study in which 15/93 (16.1%) of the tumours exhibited methylated MGMT, no significant difference was reported between NENs originating from the foregut and those from the midgut [32]. So far, no study comparing MGMT expression as assessed by IHC with MGMT methylation in other GI-NENs or pNENs has been reported.

Furthermore, MGMT methylation as assessed by methylation-specific polymerase chain reaction (MSP) is a very rare finding in poorly differentiated GI-NECs and does not seem to compromise the effect of TMZ. In a retrospective study of 25 patients with NECs receiving second-line TMZ-based therapy, MGMT methylation was found only in 1 out of 20 patients. This patient was also negative for MGMT expression as assessed by IHC and was responsive to TMZ. The overall objective response was 71%, showing that TMZ may be successfully used in NECs regardless of the MGMT methylation status [16]. No data on IHC in GI-NECs are currently available.

MGMT Detection Methods

The two main techniques for MGMT status detection include MGMT protein expression assessment by IHC and molecular analysis of *MGMT* gene promoter methylation by polymerase chain reaction (PCR). IHC is a sim-

ple, low-cost, and practical method commonly used in diagnostic histopathology that identifies protein expression patterns in heterogeneous areas within a tumour; MGMT staining in endothelial cells adjacent to or within the tumour can provide an internal positive control [17, 33]. One study found no significant difference in the response rate (RR) to TMZ according to MGMT protein expression [34], while another study reported a trend toward improved PFS and overall survival (OS) in patients with MGMT protein deficiency [17]. Observer variability and the lack of a clear association with patient survival have called into question the value of MGMT protein expression assessment by IHC and impeded its use as a clinical biomarker for the prediction of response to alkylating therapy [35].

Since IHC detection of MGMT protein expression lacks standardization and reproducibility, determination of gene silencing by PCR has been explored as an alternative mechanism to assess MGMT status. Retrospective translational studies of *MGMT* promoter methylation in GBM patients have consistently shown that patients with *MGMT* gene silencing benefit from TMZ treatment [36]. A recent prospective randomized study employing MSP indicated a statistically significantly higher PFS of TMZ-retreated GBM patients with methylated MGMT pro-

moter [37]. Until recently there has been a paucity of information regarding *MGMT* gene silencing by promoter hypermethylation in NENs. A new retrospective study reported data on 53 NEN patients treated with alkylating agents such as TMZ, STZ, and DTIC [38]. Tumour tissue was analysed by MSP and pyrosequencing for *MGMT* promoter methylation and by IHC for *MGMT* protein expression. Both PFS and OS following the first therapy with alkylating agents (TMZ, DTIC, and STZ) were prolonged in patients with *MGMT* protein loss. This study provides important information on the role of MSP and pyrosequencing, highlighting the need for prospective, randomized studies to address these associations. Another retrospective analysis, presented by Strosberg et al. [39] at the 2015 ENETS meeting, showed no correlation between *MGMT* IHC and tumour response in 59 out of 144 pNET patients treated with TMZ and CAP.

TMZ in pNENs

Compared to other NENs, pNENs show higher RR to cytotoxic chemotherapy, particularly to STZ – an agent approved 30 years ago, either as monotherapy [40] or in combination with doxorubicin – and/or 5-FU [41]. Although early prospective studies using poorly defined histopathological classification criteria and means of assessing response to treatment demonstrated high RR of pNENs to STZ and DTIC/doxorubicin/5-FU-based chemotherapy, recent important retrospective studies using RECIST criteria have revealed an RR of approximately 40% [42, 43].

Another active drug that has demonstrated activity in pNENs is DTIC. A phase II Eastern Cooperative Oncology Group (ECOG) study investigated the effect of 850 mg/m² DTIC intravenously in 50 patients with advanced pNENs and showed an overall RR of 33% [44]. However, this regimen was associated with severe toxicity, with 2 deaths and cases of grade IV toxicity, mainly haematologic.

More recently, TMZ was shown to exhibit comparable overall RR in the treatment of NENs, albeit with less toxicity [15, 34]. Based on the good RR and tolerability, TMZ has gained a primary role in pNEN treatment, both alone and in combination with other cytotoxic or targeted agents that may have a synergistic effect. Based on the high vascularity of NENs, one of the first prospective TMZ studies investigated the combination of TMZ (at a median dose of 150 mg/m² on days 1–7 and 15–21) and thalidomide (at a median dose of 100 mg daily) in 29 pa-

tients with a variety of NENs, including 11 pNENs. An RR of 45% among pNENs using RECIST criteria and a median duration of response in the entire population of 13.5 months was obtained [45].

Another antiangiogenic-based combination is that of TMZ and bevacizumab (BVZ) reported in one phase II and one retrospective study [46, 47]. In the phase II study, 34 patients (including 15 with pNENs) were treated with TMZ (at 150 mg/m² orally per day on days 1–7 and days 15–21) together with BVZ (at a dose of 5 mg/kg intravenously on days 1 and 15 of each 28-day cycle). In the subset of patients with pNENs, the radiological RR was 33% (5 PR of 15%), PFS was 14.3 months, and OS was 41.7 months [47]. In a retrospective study, a metronomic schedule of 100 mg TMZ daily, combined with BVZ at 7.5 mg/kg every 21 days along with octreotide LAR at 30 mg monthly, was administered to 15 patients with NENs (7 with pNENs) [46]. The overall RR according to RECIST criteria was 64% and with respect to pNENs included 1 complete response (CR), 4 PR, 1 stable disease (SD), and 1 progressive disease (PD). However, no assessment of the *MGMT* status was available in both of these studies. Although the number of patients treated was small, the metronomic schedule had fewer side effects and higher RR, probably due to the continuous low-dose TMZ that may saturate *MGMT* and enhance TMZ sensitivity [48]. In addition, metronomic TMZ administration seems to inhibit angiogenesis and vasculogenesis by continuously exposing the more slowly proliferating endothelial cells of the tumour to the damaging action of the cytotoxic therapy [49].

Single-agent TMZ therapy, at a dose of 200 mg/m² on days 1–5 every 28 days until disease progression or intolerable toxicity development, has also been explored in 7 pretreated pNEN cases with disease progression, achieving a median survival of 7.0 months in the second-line setting [50]. Another interesting combination has recently been published and included the administration of the mTOR inhibitor everolimus with TMZ. Forty-three patients with pNEN were treated with TMZ at a dose of 150 mg/m² per day on days 1–7 and days 15–21 in combination with everolimus at 10 mg daily in each 28-day cycle for 6 months. Among 40 evaluable patients, 16 (40%) experienced a PR; PFS was 15.4 months, although OS was not reached at the time of analysis [51].

In a retrospective study, 30 patients with metastatic, mostly well-differentiated or moderately differentiated pNENs received first-line therapy with CAP (at 750 mg/m² twice daily on days 1–14) and TMZ (at 200 mg/m² once daily on days 10–14 every 28 days). Twenty-one pa-

tients (70%) achieved an overall response (0 CR, 21 PR, 8 SD, and 1 PD), while their PFS was 18 months and the survival rate at 2 years was 92% [15]. A synergistic relationship between CAP and TMZ has been postulated. A preliminary study has suggested that metastatic pNENs express low levels of MGMT [17], potentially explaining the high RR of TMZ in pNENs. Additionally, the DNA damage induced by CAP through incorporation of 5-fluorodeoxyuridine triphosphate into the DNA and reduction of thymidine pools by inhibition of thymidylate synthase via 5-fluorodeoxyuridine monophosphate may reduce the repair activity of MGMT, thereby potentiating the effects of TMZ on the DNA strand [15, 52–54]. A second retrospective study evaluated 18 pretreated patients with a broad range of metastatic NENs (GI and pancreatic) who received 600 mg/m² of CAP orally twice daily on days 1–14 (max. 1,000 mg orally twice daily) and TMZ at 150–200 mg/m² divided into 2 doses daily on days 10–14 of a 28-day cycle [53]. Although it is difficult to extrapolate precise data on pNENs, the total RR was 64% (1 CR, 10 PR, 4 SD, and 3 PD), PFS was 14.0 months, and OS from the diagnosis of liver metastases was 83 months. The MGMT status was not evaluated, but the rationale of this scheme was based on in vitro data suggesting that twice-daily TMZ dosing may be superior to once-daily dosing because the first dose depletes MGMT levels, thus allowing the second dose to methylate guanines with a decreased repair capacity from MGMT [55]. A third retrospective study evaluated 7 patients with metastatic pNENs who were treated with CAP at a flat dose of 1,000 mg orally twice daily on days 1–14 and TMZ at 200 mg/m² in 2 divided doses daily on days 10–14 of a 28-day cycle [56]. Three patients achieved a PR, and 2 reached SD. The total RR was 43%, a clinical benefit was noted in 71% of the cases, and the median duration of response was 8 months. In the later study, MGMT analysis was not carried out.

Based on these encouraging results, the interest in the effect that TMZ may exert on pNENs is evolving. In a prospective study, 28 patients (11 with pNENs) received CAP at 1,500 mg/m²/day divided into 2 daily doses (max. 2,500 mg/day) on days 1–14 and TMZ at 150–200 mg/m² divided into 2 daily doses on days 10–14, with 2 weeks off in a 28-day cycle. Four patients with pNENs (36%) showed a PR, 6 (55%) SD, and 1 (9%) PD; PFS was longer than 18.2 months [57]. Another retrospective study tested the same regimen in 29 patients, 14 of whom had well-differentiated pNENs. The overall RR was 14%, and 59% of the patients showed SD. Of note, all patients with PR had MGMT gene inactivation and TS polymorphism, sug-

gesting that it could serve as a predictive biomarker [58]. A third retrospective study tested a slightly higher chemotherapy dose including CAP at 2,000 mg/m²/day divided into 2 daily doses on days 1–14 and TMZ at 200 mg/m² on days 10–14 of each 28-day cycle. Of the 29 patients included, 14 had pNENs. Although RR were not reported, PFS and OS of the pNEN patients was 4.9 and 18.8 months, respectively [59]. Another interesting prospective study included 35 patients with advanced NENs who received 7.8 GBq of ¹⁷⁷Lu-octreotate every 8 weeks, with 14 days of CAP at 1,500 mg/m² for 4 cycles and 200 mg/m² of TMZ in the last 5 days of each CAP cycle. Although no specific RR was reported for pNENs, the overall responses in GI-NENs were CR in 16%, PR in 41%, SD in 37%, and PD in 6% of the patients [60]. More recently, a multicentre, retrospective study on NECs has expanded currently existing treatment options for such tumours beyond platinum-based chemotherapy [61], as patients with a Ki-67 LI <55% were more responsive to TMZ than to platinum-based chemotherapy [62].

The majority of these studies have limitations including a relatively small number of patients treated, heterogeneity of the tumour characteristics, and differences in the regimens applied and responses observed. Furthermore, while most of the studies to date have evaluated TMZ in combination with other agents, the relative efficacy of these combinations compared to TMZ alone has not been formally evaluated. The combination most widely studied has been the combination of CAP and TMZ (table 1). Whether this combination is in fact superior to TMZ alone is uncertain. This question is currently being addressed in an ongoing clinical trial (NCT01824875) entitled TMZ with or without CAP in Treating Patients with Advanced pNENs.

TMZ in Thoracic NENs

So far, around 150 patients with thoracic (lung and thymic) NENs have been reported in retrospective and prospective studies of TMZ-based chemotherapy [16, 17, 34, 46, 47, 63–68] (table 2). TMZ as a single agent was tested in 20 patients, 13 with bronchial and 7 with thymic NENs, as a part of a retrospective analysis of 36 pretreated patients with advanced NENs [34]. In this series, TMZ was mostly given when all other treatment options had failed, obtaining a 67% overall rate of clinical benefit (PR and SD), with lung NENs achieving 31 PR and 31% SD, whereas thymic NENs showed 71% SD. The overall time to progression was 7 months, with no difference between

Table 1. TMZ regimens and responses in pNENs assessed by RECIST criteria

Study	Type of study	Regimen	Number of patients with pNENs/ number of total NEN patients	Patients pretreated with chemotherapy, %	CR/PR, %	SD, %	TTP or PFS, months
Kulke et al. [45], 2006	phase II	TMZ 150 mg/m ² /d, d1–7 and d15–21 thalidomide 50–400 mg/d, d1–30, q4wk	11/29	45	4/21	68	NA
Ekeblad et al. [34], 2007	retrospective	TMZ monotherapy 200 mg/m ² , d1–5, q4wk	12/36	94	0/14	54	TTP: 7
Strosberg et al. [15], 2011	retrospective	CAP 750 mg/m ² ×2/d, d1–14 TMZ 200 mg/m ² /d, d10–14, q4wk	30/30	0	0/70	27	PFS: 18
Welin et al. [16], 2011	retrospective	TMZ 150–200 mg/m ² /d, d1–5 CAP 750–1,000 mg ×2, d1–14 BVZ 5 mg/kg, d14, d28	10/25	100	4/29	38	PFS: 6
Koumarianou et al. [46], 2012	retrospective	TMZ 100 mg/d, d1–21 BVZ 7.5 mg/kg d1, q3wk	7/14	80	7/57	21	TTP: 36
Chan et al. [47], 2012	phase II	TMZ 150 mg/m ² /d, d1–14 BVZ 5 mg/kg d1 and d15, q4wk	15/34	21	0/15	65	PFS: 14
Claringbold et al. [60], 2012	phase I–II	CAP 1,500 mg/m ² /d, d1–14 TMZ 200 mg/m ² /d, d10–14, q4wk ¹⁷⁷ Lu-octreotate 7.8 GBq d5, q8wk (4 cycles)	17 GEP/35	18	15/38	38	PFS: 31
Chan et al. [51], 2013	phase I/II	TMZ 150 mg/m ² /d, d1–7 and d15–21 everolimus 5–10 mg/d, q4wk	40/40	23	0/40	53	PFS: 15
Fine et al. [53], 2013	retrospective	CAP 600 mg ×2/d, d1–14 TMZ 150–200 mg/m ² :2/d, d10–14, q4wk	10/18	61	5/55	22	PFS: 14
Saif et al. [56], 2013	retrospective	CAP 1,000 mg ×2/d, d1–14 TMZ 200 mg/m ² :2/d, d10–14, q4wk	7/7	57	0/42	28	PFS: 12
Peixoto et al. [59], 2014	retrospective	CAP 1,500 mg/m ² /d, d1–14 TMZ 200 mg/m ² /d, d10–14, q4wk	14/29	48	NA	NA	PFS: 5

TTP = Time to progression; GEP = gastroenteropancreatic; d = day; ×2 = twice; :2 = divided into 2 doses; q3/4/8wk = every 3/4/8 weeks; NA = data not available.

thoracic NENs and pNENs. MGMT status as evaluated by IHC did not correlate with the primary tumour site, and no significant difference in overall RR was found between tumours with high and those with low MGMT protein expression. In another study, 25 patients with NECs progressive on first-line, mainly platinum-based chemotherapy were treated with a TMZ-based regimen. Among these patients were 3 with atypical lung carcinoids with a Ki-67 LI >20% [16]. Although specific results for lung NENs were not reported, the 16 patients with Ki-67 LI <60% and positive somatostatin receptor scintigraphy were more responsive than the others. In a further study that used metronomic TMZ in 15 patients, 1 patient with a lung NEN showed PD [46]. In the phase II trial that included 34 patients with NENs of different tissue origin treated with TMZ and BVZ, 12% of the patients had lung

NENs, but specific results regarding the RR and the survival times of this subgroup were not reported [47]. Over the last 3 years, six small studies have been published describing results of TMZ-based therapy in homogeneous thoracic NEN populations: two on bronchial, two on small cell, and two on thymic NENs [63–68] (table 2). The bronchial NEN studies included 45 patients treated with TMZ monotherapy and showed a 66–71% clinical benefit rate and a 5- to 10-month PFS [63, 66]. In the thymic group, 13 patients treated with TMZ and CAP showed a 90–100% clinical benefit rate [64, 65]. In the small cell lung cancer group, 89 patients were treated with either a 5-day or a 21-day TMZ regimen, achieving a 12–23% overall RR [67, 68].

Although the relatively small size of the above studies limits the interpretation of the results and specific data

Table 2. TMZ regimens and responses in thoracic NENs assessed by RECIST criteria

Study	Type of study	Regimen	Number of patients with thoracic NENs/ number of total NEN patients	Pretreated patients with chemotherapy, %	CR+PR, %	SD, %	TTP or PFS, months
Ekeblad et al. [34], 2007	retrospective	TMZ 200 mg/m ² /d, d1–5, q4wk	20/36 (7 thymic, 13 bronchial)	NA	0/31	25	TTP: 7
Welin et al. [16], 2011	retrospective	TMZ 150–200 mg/m ² /d, d1–5 CAP 750–1,000 mg ×2, d1–14 BVZ 5 mg/kg, d14, d28	3/25 (bronchial)	100	32	40	PFS: 6
Koumariou et al. [46], 2012	retrospective	TMZ 100 mg/d, d1–21 BVZ 7.5 mg/kg, d1, q3wk	1/15 (bronchial)	80	0	0	TTP: 9
Chan et al. [47], 2012	prospective	–	4/34 (bronchial)	NA	NA	NA	NA
Crona et al. [65], 2013	retrospective	TMZ 150–200 mg/m ² /d, d1–5, q4wk (±sorafenib 1 patient, ±CAP/BVZ 1 patient)	10/28 (thymic)	NA	0/20	70	TTP: 20
Crona et al. [63], 2013	retrospective	TMZ 150–200 mg/m ² /d, d1–5, q4wk	31/31 (bronchial)	35	0/14	52	PFS: 5
Saranga-Perry et al. [64], 2013	retrospective	TMZ 170–190 mg/m ² /d, d10–14 CAP 600–750 mg/d ×2, d1–14	3/3 (thymic)	100	67	33	NA
Chong et al. [66], 2014	retrospective	TMZ 150–200 mg/m ² /d, d1–5, q4wk	14/300 (bronchial)	NA	14	57	PFS: 10
Pietanza et al. [67], 2012	phase II	TMZ 75 mg/m ² /d, d1–21, q4wk	64 (SCLC)	100	1.5/19	9	TTP: 1.6
Zauderer et al. [68], 2014	phase II	TMZ 200 mg/m ² /d, d1–5, q4wk	25/25 (SCLC)	100	0/12	NA	NA

TTP = Time to progression; d = day; ×2 = twice; q3/4wk = every 3/4 weeks; NA = data not available; SCLC = small cell lung cancer.

regarding lung NENs are not available, non-pancreatic primaries seemed to be less responsive to the TMZ regimen than pNENs. Additionally, TMZ-based chemotherapy seems to be somewhat more active in patients with bronchial and thymic NENs as compared to small cell lung cancer. However, in order to substantiate its efficacy, a specific prospective study of lung and thymic NENs with TMZ as a single agent or in combination is warranted; in such a study, an ancillary biological analysis should be included to validate the potential predictive role of MGMT expression.

TMZ in NENs from Other Primary Sites

Malignant Chromaffin Cell Tumours

Chromaffin cell tumours (phaeochromocytomas and paragangliomas) were initially considered to be relatively benign, but recent studies have documented that approximately 30% (mainly paragangliomas) may follow a truly malignant course, whereas 25–30% may develop in

the context of familial syndromes [69]. Several indices have been used to predict their malignant potential, including succinate dehydrogenase B (SDHB) mutations, which carry the highest risk. Treatment options for these patients are relatively limited, although radiopharmaceuticals with either ¹³¹I-metaiodobenzylguanidine or ⁹⁰Y- or ¹⁷⁷Lu-radiolabelled octreotide have elicited some meaningful responses [70]. Chemotherapy using a cyclophosphamide-DTIC-vincristine regimen is currently recommended for the treatment of malignant phaeochromocytoma and paraganglioma (MPP) [69]. Due to relatively low RR to these modalities, TMZ was used for the first time in a phase II study in 3 patients with MPP, employing the combination of TMZ (at 150 mg/m² for 7 days followed by a 7-day rest) and thalidomide (administered at doses of 50–400 mg without discontinuation for 28 days) [45]. Since then, only sporadic case reports using TMZ either alone or in combination with other agents have appeared, demonstrating a potential benefit [71, 72]. A recent French retrospective multicentre study investigated the response to TMZ monotherapy (at a

mean dose intensity of 172 mg/m²/day for 5 days every 28 days) in 15 consecutive patients with documented progressive MPP for whom information regarding *SDHB* mutation and *MGMT* promoter methylation and *MGMT* IHC was available [10 patients (67%) carried an *SDHB* mutation] [13]. Median PFS was 13.3 months after a median follow-up of 35 months. Five patients developed a PR (33%), 7 SD (47%), and 3 PD (20%). PR were observed only in patients with *SDHB* mutations. *MGMT* IHC was negative in the tumour samples from 4 patients who responded to treatment, whereas *SDHB* germline mutation was associated with hypermethylation of the *MGMT* promoter and low expression of *MGMT*. This study demonstrated that TMZ is an effective anti-tumour agent in patients with *SDHB*-related MPP; its effectiveness is partially explained by the silencing of *MGMT* expression as a consequence of *MGMT* promoter hypermethylation in *SDHB*-mutated tumours. These data suggest that genotyping may guide the response to treatment with TMZ in MPP and provide a rationale for tailor-made treatment.

Non-Pancreatic GI-NENs

Non-pancreatic primary GI-NENs are heterogeneous and include tumours of midgut (small intestine and proximal colon), foregut (stomach and duodenum), and hindgut origin. Multiple studies indicate dramatically lower responses of GI-NENs to TMZ-based chemotherapy compared to pNENs [45, 47]. One exception is a prospective study of CAP and TMZ (CAPTEM) in 28 patients, which reported an RR of 41% among 12 'carcinoid tumour' patients [57]. However, the specific primary sites of these patients were unclear, and further data are necessary to evaluate whether the CAPTEM combination is active in specific subtypes of GI-NENs.

Treatment Schedules and Toxicity

TMZ has been administered to patients with solid tumours for nearly 15 years in different schedules and dosages. In the published literature on NENs, TMZ has been administered in different combinations, dosages, and schedules to approximately 350 patients over time (table 3). There is retrospective and prospective evidence of activity, but due to the low number of patients included in the individual studies and the variety of regimens used, it is difficult to identify an optimal combination and schedule (table 3).

Single Agent

The recommended schedule for TMZ as a single agent comes from early clinical trials with TMZ conducted by the Cancer Research Campaign (now Cancer Research UK) and consists of an oral administration of 200 mg/m² daily for 5 consecutive days repeated every 4 weeks [73]. The 5-day schedule was believed to enhance *MGMT* depletion and hence to sensitize *MGMT*-intact cells to the effects of the methylating agent [73]. The overall toxicity observed in the first NEN study with this schedule was as follows: 14% grade III thrombocytopenia, 9% grade III and IV fatigue, and 3% grade IV neutropenia [34]. The most intensive schedule studied in clinical trials comprised TMZ administration in a 4-hour schedule to patients with metastatic melanoma, but the efficacy was not sufficiently enhanced and the toxicity was increased [73].

Combinations

The extent to which combination therapy may increase toxicity is difficult to assess, given the different dose schedules of TMZ used in various studies [45–47, 51, 57, 60, 67, 74, 75] (table 1). One study included 29 NEN patients treated with a dose-dense schedule of TMZ at 150 mg/m²/day on days 1–7 every other week and thalidomide at 200 mg/day. Although the treatment was effective, the study had 55% of the patients discontinuing treatment due to high toxicity, mainly grade III–IV lymphopenia. Three patients developed opportunistic infections, indicating a severe immunocompromising effect of the regimen [45]. Based on the fact that opportunistic infections occurred during TMZ-induced lymphopenia and that these patients had not received anti-infection prophylaxis, a recommendation to administer prophylactic sulfamethoxazole/trimethoprim was established for further studies with the same regimen. Additional clinical variables such as age, concurrent steroid treatment, poor nutritional status, and compromised performance status are also likely to increase the risk of infections. In addition, prophylactic antifungal and antiviral (mainly antiherpetic) drugs might be needed to control the infection risk or viral reactivation [76]. In a second prospective study combining TMZ (at 150 mg/m² orally per day on days 1–7 and days 15–21) with BVZ (at a dose of 5 mg/kg/day intravenously on days 1 and 15 of each 28-day cycle) the most commonly observed toxicities were grade III or IV lymphopenia (53%) and thrombocytopenia (18%). Prophylactic antibiotics were included in this study, and only 1 patient developed an opportunistic infection [47]. A third study of TMZ in combination with everolimus also used the dose-intensive schedule of TMZ at 150 mg/m² for 7 days every other week

Table 3. Schedules and dosages of TMZ in NENs

Type of study	TMZ schedule	Study	Patients	Drugs	Schedule	Toxicity
Retrospective	conventional	Ekeblad et al. [34], 2007	36	TMZ	TMZ 200 mg/m ² /d, d1–5, q4wk	2 patients, GIII–IV: fatigue
		Strosberg et al. [15], 2011	30	TMZ + CAP	CAP 1,500 mg/m ² /d, d1–14, TMZ 200 mg/m ² /d, d10–14, q4wk	4 patients, GIII–IV: anaemia, thrombocytopenia
		Welin et al. [16], 2011	25	TMZ ± CAP ± BVZ	CAP 1,500–2,000 mg/m ² /d, d1–14 TMZ 150–200 mg/m ² /d, d10–14 BVZ 5 mg/kg, d14 and d28, q4wk	1 patient, GIII thrombocytopenia and leucopenia
		Crona et al. [63], 2013	31	TMZ	TMZ 150–200 mg/m ² /d, d1–5, q4wk	3 patients, GIII thrombocytopenia; 1 patient, GIII leucopenia
		Saif et al. [56], 2013	7	TMZ + CAP	CAP 2,000 mg/m ² /d, d1–14 TMZ 200 mg/m ² /d, d10–14, q4wk	2 patients, GIII: 1 thrombocytopenia and 1 fatigue
		Fine et al. [53], 2013	18	TMZ + CAP	CAP 2,000 mg/m ² /d, d1–14 TMZ 150–200 mg/m ² /d, d10–14, q4wk	2 patients, GIII thrombocytopenia
Prospective	conventional	Kesavan et al. [86], 2014	65	TMZ + CAP + ¹⁷⁷ Lu-octreotate	CAP 1,500 mg/m ² /d, d1–14 TMZ 200 mg/m ² /d, d10–14, q4wk ¹⁷⁷ Lu-octreotate 7.8 GBq	5 patients, GIII: 1 nausea, 2 neutropenia, 2 angina
	dose-dense	Kulke et al. [45], 2006	29	TMZ + thalidomide	TMZ 150 mg/m ² /d, d1–7 and d14–20 thalidomide 50–400 mg/d, q4wk	GIII–IV: 69% lymphopenia, 10% opportunistic infections
		Chan et al. [47], 2012	34	TMZ + BVZ	TMZ 150 mg/m ² /d, d1–7 and d15–21 BVZ 5 mg/kg, d14 and d28, q4wk	GIII–IV: 53% lymphopenia, 18% thrombocytopenia
		Chan et al. [51], 2013	43	TMZ + everolimus	TMZ 150 mg/m ² /d, d1–7 and d15–21 everolimus 5–10 mg/d, q4wk	GIII–IV: 44% lymphopenia, 16% thrombocytopenia
	metronomic	Koumarianou et al. [46], 2012	15	TMZ + BVZ + octreotide	TMZ 100 mg/d BVZ 7.5 mg/kg, q3wk octreotide LAR 30 mg, q4wk	GIII: 1 patient, vomiting

d = Day; q3/4wk = every 3/4 weeks.

[51]. Due to concerns regarding toxicity, TMZ was administered for a maximum of 6 months. This study showed that the combination of TMZ and everolimus was both active and well tolerated.

A study on GBM employing 75 mg/m² TMZ for 6 weeks every 8 weeks, analysed for lymphocyte subsets, showed protracted lymphopenia induced in 60% of the patients and affecting the CD4⁺ compartment preferen-

tially [77]. TMZ given on a less dose-intense schedule at a dose of 200 mg/m² for 5 consecutive days may be better tolerated, although prospective data are limited. For instance, in the CAPTEM study by Strosberg et al. [15] employing CAP at 750 mg/m² twice daily on days 1–14 and TMZ at 200 mg/m² once daily on days 10–14 every 28 days, despite the lack of antimicrobial prophylaxis, only 3 patients developed clinically non-significant infections (2 herpes zoster and 1 herpes labialis); toxicities included 2 grade IV haematologic cases (anaemia and thrombocytopenia) and 2 cases of grade III transaminasaemia. However, because this study was retrospective in nature, it is difficult to compare the relative toxicities to data from prospective trials. In a second CAPTEM study employing 600 mg/m² of CAP orally twice daily on days 1–14 (max. 1,000 mg orally twice daily) and TMZ at 150–200 mg/m² divided into 2 doses daily on days 10–14 of a 28-day cycle, the toxicity was mild and included 1 grade III toxicity (thrombocytopenia) [53]. Another combination study employing 7.8 GBq of ¹⁷⁷Lu-octreotate every 8 weeks, with 14 days of CAP at 1,500 mg/m² for 4 cycles and 200 mg/m² of TMZ on days 10–14, resulted in 1 case of grade III nausea (3%) and 2 cases of grade III neutropenia (6%), whereas 2 patients developed angina (6%) [60].

Other less common non-haematologic toxicities reported with the use of TMZ in NENs include GI side effects such as nausea, vomiting, diarrhoea, elevated aspartate transaminase/alanine transaminase and total bilirubin, as well as mucocutaneous toxicity such as skin rash and pruritus [45–47, 51, 57, 60, 67, 74, 75]. Additionally, some non-haematologic toxicities have been ascribed to the use of other agents used in combination with TMZ (e.g. hypertension and proteinuria related to the use of BVZ, hand and foot syndrome related to the use of CAP, and hyperglycaemia and hypercholesterolaemia related to the use of everolimus) [45–47, 51, 57, 60, 67, 74, 75]. Such toxicities have to be accounted for in patients with NENs.

Schedules

So far, various schedules with TMZ have been used. In GBM, a general consensus regarding the standard schedule has not been reached, even though the 5/28-day schedule seems the most commonly used. Other types of schedules, named ‘dose-dense’, ‘prolonged’, and ‘metronomic’, have been used, mainly outside clinical trials [78]. The dose-dense schedule is also called ‘week on/week off’ and consists of TMZ at 150 mg/m²/day over 7 days followed by 7 days of rest, repeated every 2 weeks (7/15-day

schedule). The prolonged schedule is a 21/28-day schedule, repeated on day 29, using 75–100 mg/m²/day over 21 days. The dose-dense and prolonged schedules have been compared in patients with advanced solid tumours. For the dose-dense schedule, the maximum tolerated dose was 150 mg/m²/day. For the prolonged schedule, the maximum tolerated dose ranged from 85 mg/m² daily in heavily pretreated patients to 100 mg/m² daily in minimally pretreated patients. The authors concluded that the prolonged TMZ schedule depletes MGMT levels and may potentiate TMZ activity [79]. In another study, patients treated with TMZ (100 mg/m²/day for 21 consecutive days every 28 days) for indications other than NEN experienced profound and prolonged lymphopenia with blood counts recovering to normal levels only after 12 weeks [80].

The metronomic schedule uses TMZ at 50 mg/m²/day without interruption and is associated with minor toxicity [81]. In a phase I study, TMZ was administered for 6–7 weeks at a starting dose of 50 mg/m² daily, with a planned escalation up to 100 mg/m², in patients with GBM. Although the patients received an increased dose compared to the 5-day schedule, pharmacokinetic data showed activity with no plasma accumulation of TMZ and no further increase in toxicity [19]. In another metronomic study employing 100-mg TMZ capsules daily combined with BVZ at 7.5 mg/kg every 21 days along with octreotide LAR at 30 mg monthly in 15 patients with NENs [46], haematologic toxicities were 2 cases of grade I neutropenia and 2 cases of grade II thrombocytopenia.

Duration of Therapy

For those patients who develop long-term responses to treatment, a question arises on how long to continue TMZ therapy. As TMZ is a DNA-damaging agent, development of secondary malignancies is a potential cause for concern. This concern has been sustained by the finding of a number of TMZ-treated GBM patients developing myelodysplastic syndrome, leukaemia, or aplastic anaemia [82–85]. While no such toxicities have been reported in NEN patients, this may be due to the relative lack of prospective data and long-term follow-up in NEN patients treated with TMZ. There are no recommendations regarding the maximum duration of therapy, but a 1- to 39-month duration of treatment has been reported [15, 34, 45, 47, 53, 59].

Ongoing Clinical Trials of TMZ in NENs

- 1 A phase II study from Stanford University, USA (NCT01525082): CAP, Temozolomide and Bevacizumab for Metastatic or Unresectable Pancreatic Neuroendocrine Tumors
- 2 A phase I/II study from Northwestern University, USA (NCT01465659): Temozolomide and Pazopanib Hydrochloride in Treating Patients with Advanced Pancreatic Neuroendocrine Tumors That Cannot Be Removed by Surgery
- 3 A randomized phase II study from the ECOG and National Institutes of Health, USA (NCT01824875): Temozolomide with or without Capecitabine in Treating Patients with Advanced Pancreatic Neuroendocrine Tumors
- 4 A phase II study from Haukeland University Hospital, Norway, and collaborators from the Nordic countries (NCT02248012): Everolimus and Temozolomide in Advanced Gastroenteropancreatic Neuroendocrine Carcinoma (G3)

Conclusions

TMZ has shown activity as a single agent or in combination with other drugs in approximately 450 patients with advanced NENs. However, the majority of the data is derived from observational and small, single-institution, prospective phase II studies using a variety of differ-

ent TMZ dose schedules. The combination of CAPTEM is a commonly employed regimen, and has been associated with objective responses in either chemotherapy-naïve and/or heavily pretreated patients mainly with pNENs, although NENs from other GI sites and non-GI primaries may also be responsive. Currently, there are no data comparing the efficacy of this regimen to other TMZ-based combinations or to TMZ alone, nor are there prospective data comparing this regimen to STZ-based therapies. Although determination of the MGMT status has been suggested as a predictive biomarker of response, its role remains still investigational, awaiting validation along with the establishment of the optimal detection method.

In conclusion, although TMZ appears to be a promising agent for the management of advanced NENs, there are several open questions regarding the timing of therapy, optimal schedule, duration of treatment, and long-term toxicity that should be studied in prospective trials. Large multicentre, randomized, prospective studies are needed to substantiate its use and possible role in the adjuvant and neoadjuvant setting in advanced well-differentiated and poorly differentiated NENs.

Disclosure Statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. This study did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

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