Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0278.v1

Article

Safety and activity of metronomic temozolomide in second-line treatment of advanced neuroendocrine neoplasms.

Salvatore Tafuto[1*], Claudia von Arx[1,2*], Monica Capozzi[1], Fabiana Tatangelo[3], Manuela Mura[2], Roberta Modica[4], Maria Luisa Barretta[5], Antonella Di Sarno[6], Maria Lina Tornesello[7], Annamaria Colao[4] and Alessandro Ottaiano[8]

[1] Department of Abdominal Oncology, Istituto Nazionale Tumori, IRCCS - Fondazione "G. Pascale", Naples, Italy.
[2] Department of Surgery and Cancer, Imperial College London, London, UK
[3] Department of Pathology, Istituto Nazionale Tumori, IRCCS - Fondazione "G. Pascale", Naples, Italy.
[4] Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy.
[5] UOC of Radiology, Istituto Nazionale Tumori, IRCCS - Fondazione "G. Pascale", Naples, Italy.
[6] UOC of Oncology, A.O. dei Colli, Monaldi Unit, Naples, Italy.
[7] Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Naples, Italy
[8] SSD Innovative Therapies for Abdominal Metastases - Department of Abdominal Oncology, Istituto Nazionale Tumori, IRCCS – Fondazione "G. Pascale", Naples, Italy.
On behalf of ENETs (European NeuroEndocrine Tumors Society) Center of Excellence of Naples, Italy
* Correspondence: Monica Capozzi, PharmD; m.capozzi@istitutotumotri.na.it; Tel: +390815903680

Abstract: Background. The front-line treatment of advanced NeuroEndocrine Neoplasms (NENs) depends on clinical and pathological factors but there are no standard second-line therapies. **Methods.** Metastatic NENs patients were treated at the ENETS (European NeuroEndocrine Tumor Society) center of excellence of Naples (Italy), from 2014 to 2017 with second-line metronomic temozolomide, 75 mg/m2 *per os* "one week on/one week off". Toxicity was graded with NCI-CTC criteria v4.0; objective responses with RECIST v1.1 and performance status (PS) according to ECOG. **Results.** Twenty-six consecutive patients were treated. Median age was 65.5 years. The predominant primary organs were pancreas and lung. Grading was G2 in 11 patients, G3 in 15. Eleven patients presented with PS 1 and 15 with PS 2. The median time-on-temozolomide therapy was 12.2 months (95% CI: 11.4-19.6). No G3/G4 toxicities were registered. Complete response was obtained in 1 patient, partial response in 4, stable disease in 19 (disease control rate: 92.3%), and progressive disease in 2. The median overall survival from temozolomide start was 28.3 months. PS improved in 73% of patients. **Conclusions.** Metronomic temozolomide is a safe and active treatment for G2 and G3 NENs. Prospective and larger trials are needed to confirm these results.

Keywords: Neuroendocrine neoplasms, chemotherapy, temozolomide, metronomic treatment, second-line.

1. Introduction

Neuroendocrine neoplasms (NEN) are a group of tumors arising from the neuroendocrine cell compartment present in different tissues. Their incidence is increasing probably because of improvements in diagnosis and pathological identification [1]. The therapy depends on origin, grading, and presence and localization of metastases. When the tumor is localized, surgery is the gold standard treatment [2]; unfortunately, metastases particularly to the liver may occur at clinical presentation (20%) or after (38%) the initial diagnosis [3]. Hepatic surgery in resectable metastases leads to 4-year survival rate of about 70%. However, first-line systemic treatments are recommended in metastatic unresectable NENs by the most important guidelines (European Neuroendocrine Tumor Society, North American Neuroendocrine Tumor Society) in order to control hormone-

© 2019 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0278.v1

dependent symptoms when present and/or to improve survival [4, 5]. However, still to date, there is a lack of evidence for standard second line treatments in progressive NENs, this phase of disease typically requires multidisciplinary approaches that may reside on external radiotherapy, organ-directed treatments (chemoembolization, embolization, etc.), biologics and peptide receptor radionuclide therapy [4, 5]. A detailed description of these therapeutic options is beyond the scopes of the present study.

Temozolomide (TMZ) is an orally active alkylating agent analogue of the dacarbazine. In monotherapy and at the standard doses of 150-200 mg/m2 once daily for 5 every 28 days, TMZ showed to be active in pre-treated patients affected by NENs with response rates (RR) of 14% in patient with G1/G2 NENs [6] and a disease control rate (DCR) of 38% in G3 NEC [7]. In association with other drugs, namely capecitabine, everolimus, bevacizumab and octreotide, and thalidomide the RR ranges between 17-70 % [8-16]. The large part of these studies are small (<25 patients) and/or retrospective because of the low incidence of the disease. The most frequent reported all-grade toxicities of TMZ single-agent or combined with other drugs are anemia, leucopenia, thrombocytopenia, hand-foot syndrome and gastrointestinal. However, a discontinuation rate of TMZ up to 55% is reported [16], and in association with everolimus, the treatment with TMZ has been precautionary administrated for a maximum of 6 months in order to reduce toxicity [13].

The use a metronomic schedule of TMZ represents a possible way to reduce toxicity. Metronomic TMZ (mTMZ) consists on lower daily doses with greater frequency of administration. The main biological effects reside on anti-angiogenic activity [17-19] and immune-modulation leading to improvement of dendritic cells function [20] and selective depletion of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs), which are potent immunosuppressive cells within the tumor microenvironment [21-24].

There are no studies in literature evaluating the activity and safety of mTMZ in advanced pre-treated NENs. In this study, we evaluated the efficacy of mTMZ in a consecutive series of 26 NENs patients treated at the ENETS (European NeuroEndocrine Tumor Society) center of Naples.

2. Experimental Section

2.1. Patients and treatment

This was a retrospective study approved by the Scientific Directorate (among criteria: reliable and verifiable source of data, consecutiveness of the cases to reduce biases, adequate follow-up, monocentric radiologic evaluations) of the National Cancer Institute of Naples and conducted at the ENETS Center of Excellence in Naples (Italy). The ENETS center of Naples internal database collects continuously data about NENs' patients from three different institutions; it was utilized to identify consecutive cases of patients with advanced G2-G3 NENs, progressed after a first-line systemic therapy and treated with second-line mTMZ therapy between 2014 and 2017. Following the procedures of our Institute, retrospective studies are submitted only to the approval of Scientific Directorate and do not require ethical approval.

Information about patients and disease characteristics (age, gender, PS, comorbidities, stage), histology (primary tumor site and size, Ki 67 status), previous treatments (surgery and/or systemic treatments) were collected and used as descriptive outcomes. The treatment schedule consisted on oral administration of "one week on/one week off" TMZ at 75 mg/m2 until unacceptable toxicity or progression. The drug was taken on an empty stomach (1 hour before or 2 hours after eating), with a full glass of water. Written informed consent was obtained before prescribing and starting therapy.

2.2. Efficacy and safety evaluation

For efficacy evaluation, tumor response was defined according to RECIST 1.1 (Response Evaluation Criteria in Solid Tumors). Tumor assessment was performed every three months through Computed Tomography (CT) scans. The objective response rate (ORR) was the sum of complete (CR)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0278.v1

+ partial responses (PR). The disease control rate (DCR) was the sum of CRs +PRs + stable diseases (SD). The clinical benefit was documented by the treating physicians in the patient records and it was evaluated as improvement of ECOG (Eastern Cooperative Oncology Group) PS (Performance Status). Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI–CTCAE version 4).

2.3. Descriptive analyses and statistical methods

Data are predominantly descriptive. Progression free survival (PFS) was calculated as the time elapsed from the date of mTMZ initiation to the date of disease progression or death for any cause (whichever occurred first). Patients who were alive with no disease progression were censored at the date of last visit. Overall Survival (OS) was defined as the time from the start of mTMZ administration to the date of death for any cause. Patients who were alive were censored at the date of data analysis. The median PFS (mPFS) and the median OS (mOS) curves were depicted using the Kaplan-Meier method. Exploratory subgroup analyses were done by Log-rank test.

3. Results

3.1. Patients' and disease characteristics

From 2014 to 2017, twenty-six consecutive patients with advanced G2-G3 NENs in progression after a first line chemotherapy were treated with second-line mTMZ. Characteristics of patients and their disease are reported in **Table 1**.

Table 1. Characteristics of patients and disease.

Characteristics	No.
Age, years	
Median	65
Range	32-88
Gender	
Male	13
Female	13
Grading	
G1	0
G2	11
G3	15
KI-67 level	
3 - 20	11
20 - 55	10
>55	5
Performance Status	
0	0
1	11
2	15
Site of primary tumor	
Pancreas	5
Lung	5
Stomach	3

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0278.v1

Miscellanea	
Head and Neck	2
Small bowel	3
Rectum	1
Gallbladder	1
Cutaneous	1
Unknown Primary Origin	5
No. of involved metastatic sites	
1	13
2	8
≥ 3	5
Previous treatments	
Platinum-based treatments	12
Chemotherapy non-platinum based	2
Somatostatin analogues	8
Clinical trials drugs	4

The median age was 65.5 years (range: 32-88 years) and the genders were equally represented (13 patients were male and 13 patients were female). Fifteen patients (58%) had an ECOG PS of 2 before starting the second line treatment, while 11 patients (42%) presented with a PS equal to 1. No patient had a PS of 0. Grading is a fundamental characteristic to drive therapeutic choices, G2 NENs were 42% and G3 58%. Among the G3 NENs, 10 out of 15 (67%) had a Ki67 between 20% and 55%. The predominant primary sites were pancreas and lung, whereas the predominant site of metastasis was the liver followed by loco-regional nodes and bone. In half of the patients, metastases were present in a single site, and the liver was the only involved site in the 81% of patients. In contrast, 8 patients (31%) had two different sites of metastasis, and in 5 patients (19%) the sites of metastasis were equal or more than three.

The majority of patients (54%) was previously treated with chemotherapy, whereas 31% received SomatoStatin Analogues (SSAs) as first line treatment, and 15% received other treatments including immunotherapy or targeted/biologic therapies. Of the 14 patients who received first line chemotherapy, 12 received platinum-based treatments and two non-platinum chemotherapy regimens. In addition, among the chemo-treated patients, 10 (71%) had a G3 NEN but 4 (29%) had a G2 NEN. In the latter patients, the choice to administer chemotherapy was based on the primary site of the NENs and/or on the Ki67: two atypical carcinoids with a Ki67 ≥15%, one intracranial neuroendocrine tumor and one NEN of unknown primary origin with a Ki67 of 18%. Of the 8 patients who received SSAs, half received octreotide and half lanreotide.

3.2. Efficacy and Safety

At last follow-up (median follow-up from mTMZ start: 29 months), 16 (62%) patients were alive, and 8 (31%) were still on treatment with mTMZ. All the twenty-six patients were evaluable for response. The objective response rate (ORR) to second line mTMZ was 19%, with one complete response (CR) and four partial responses (PR). An additional 73% of patients achieved stable disease (SD) as best response (**Table 2; Figure 1a**).

Table 2. Efficacy estimates of second-line metronomic TMZ.

	No. (%)
Response to therapy	
Complete Response	1 (3.8)
Partial Response	4 (15.4)
Stable Disease	19 (73.1)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0278.v1

Progressive Disease	2 (7.6)
Median PFS (18 events)	9 months
Median OS (10 events)	28.3 months

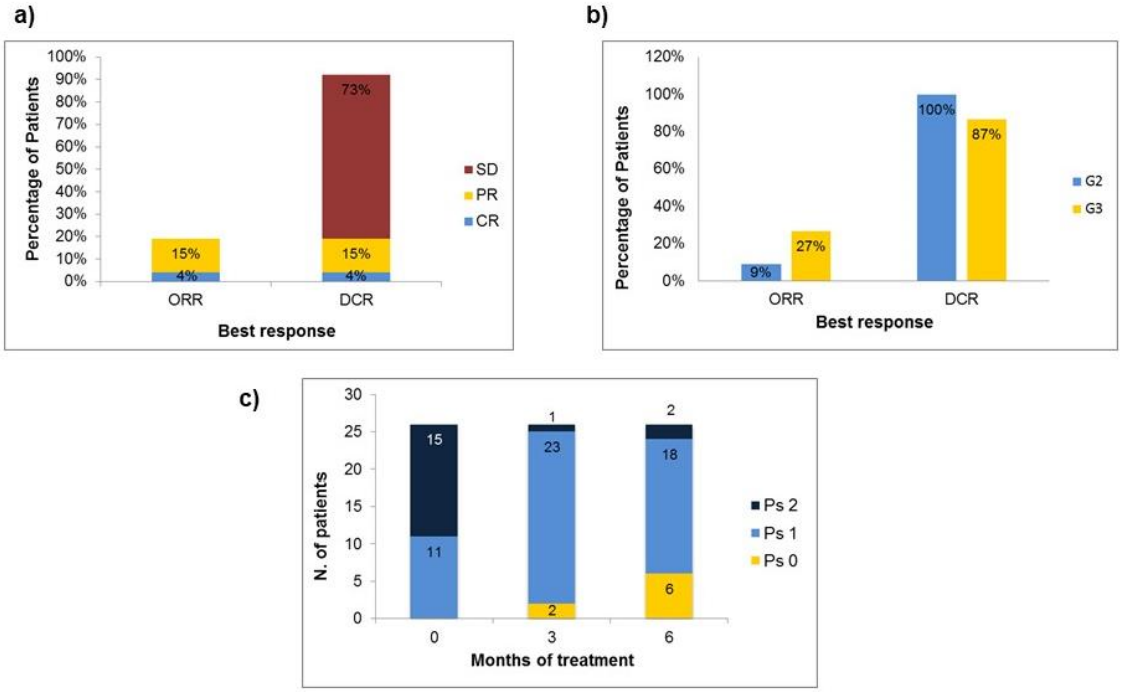
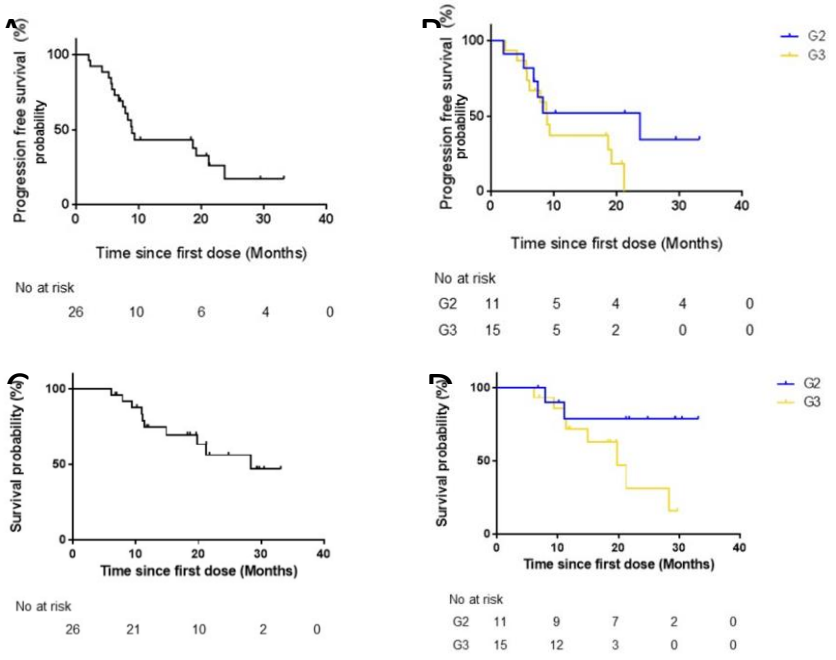


Figure 1. Response rates with metronomic temozolomide in all patients (a) and according to grading (G2 vs G3) of the tumor (b). Improvement of Performance Status over 3 and 6 months of treatment (c). ORR=Overall Response Rate; DCR= Disease Control Rate; SD=Stable Disease; PR=Partial Response; CR=Complete Response; PS= Performance Status.

The overall DCR was 92%. The ORR and DCR in patients with G2 NENs were 9% and 100%, respectively, while for those with G3 NENs the ORR was 27% and the DCR was 87% (**Figure 1b**). A clinical improvement of the basal PS was reported in 73% of patients (**Figure 1c**). The mPFS was 9 months and longer for patients with G2 NENs (mPFS: 23.6 months) compared to patients with G3 NENs (mPFS: 8.9 months), although not significant (P=0.16) (**Figure 2**).



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0278.v1

Figure 2. Kaplan-Meier curves of progression-free survival and overall survival in all patients (A and C) and according to grading (B and D).

The mOS was 28.3 months in the entire population. The mOS was 19.8 months in patients with G3 NENs and not reached in patient with G2 NENs (p=0.60) (**Figure 2**). No G3/G4 toxicities were registered (**Table 3**); no dose reductions were reported. The two most common adverse events were anemia and asthenia (Table 3). The median time-on-TMZ therapy was 12.2 months (95% CI: 11.4-19.6). No patient discontinued treatment for the occurrence of severe adverse events.

Table 3. Summary of adverse events.

Toxicity	G1		G2		G3/G4	
	No	%	No	%	No	%
Anaemia	11	42.3	13	50.0	0	0.0
Asthenia	9	34.6	12	46.1	0	0.0
Neuropathy	8	30.7	10	38.4	0	0.0
Neutropenia	8	30.7	8	30.7	0	0.0
Nausea	7	26.9	8	30.7	0	0.0
Hyperbilirubinemia	7	26.9	6	23.1	0	0.0
Alkaline phosphatase	3	11.5	0	0.0	0	0.0
Hyperglycaemia	4	15.3	6	23.1	0	0.0
Thrombocytopenia	0	0.0	6	23.1	0	0.0

4. Discussion

Currently, the optimal schedule for TMZ have still not been established. Different schedules have been used in recent trials both in monotherapy and in association with other drugs [6-16]. Although a significant activity has been constantly revealed with these schedules, the median time on TMZ was negatively influenced by G3/G4 toxicity [6, 13, 16] with a reported discontinuation rate up to 55%. This highlights the need to minimize toxicity.

Albeit retrospective and exploratory, we report the first "hypothesis generating" study with mTMZ 75mg/m2 "one week on-one week off" scheme in NENs. We suggest that this schedule might represent a feasible second-line treatment in patients with advanced G2 and G3 NENs. In fact, the treatment was associated with an ORR of 19% and an overall DCR of 92%; no G3/G4 adverse events and no interruption of treatment for toxicity were registered. In addition, treatment with mTMZ determined a clinical benefit through improvement of PS.

The clinical advantages of a low-dose administration of TMZ have been explored over the last 20 years and are mainly based on i) a lower toxicity profile eventually associated ii) to a better quality of life [25-28]. Furthermore, beyond these clinical advantages, mTMZ, but not the conventional scheme, is able to trigger anti-angiogenetic and immune-mediated pathways [17-23]. NENs are hypervascularised tumors and overexpress a plethora of proangiogenic molecules and related receptors [29-31]. Therefore, given their high dependence from angiogenic pathways, the metronomic schedule, through its predominant anti-angiogenic action, could represent a stronger candidate for NENs treatment. In fact, it has been shown that the metronomic regimen, unlike the conventional full-dose treatment, is associated to a better negative control of the angiogenic pathways preventing endothelial cells survival and re-growth [31-33], and reducing tumor microvessel density [19]. Additionally, metronomic therapy exerts its anti-angiogenetic activity through the increase of the inhibitor thrombospodin-1 (THBS-1) and the inhibition of the hypoxia-inducible factor 1 (HIF-1) [34]. This latter effect could be particularly interesting for combination with mTOR inhibitors (i.e.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0278.v1

everolimus). Inhibition of mTORC1 (mTOR Complex 1) causes the loss of a negative feedback loop that activates HIF-1 [35]; therefore, the association of an mTOR inhibitor with mTMZ might preserve the activity of this loop. In addition to these anti-angiogenetic effects, mTMZ has immune-stimulatory effects through reduction of circulating regulatory T cells (Tregs) and modulation of dendritic cells. Thus, mTMZ deserves to be considered for future trials in combination with immunotherapy or other biologics. For instance, cabozantinib, a multi-tyrosin-kinase inhibitor, is currently emerging for the treatment of carcinoid and pancreatic NENs [36] and it has been shown to be effective with TMZ in glioblastoma [37]. This could be one of the promising future therapeutic associations in NENs.

Notably, the evaluation of O6-methylguanine DNA methyltransferase (MGMT), which repairs the methylation at the O6-position of guanine induced by alkylating agents thus reducing their cytotoxic effects, could have a predictive role in patients treated with mTMZ [38-41]. However, it did not show to be significant in our series (data not shown). Our group is going to accumulate more data about this issue. In fact, expression and activity of MGMT could be useful biomarkers to further optimize mTMZ-based therapy.

In conclusion, our study is a proof of concept that an intermittent schedule of mTMZ at 75 mg/m^2 can be a suitable treatment in advanced, G2-3 NENs. Despite its exploratory and retrospective nature, the efficacy of mTMZ in monotherapy here reported is similar to that shown in other retrospective trials with conventional schedules of TMZ monotherapy; conversely, the toxicity profile is clearly better. These results make the metronomic schedule a strong candidate also for combination treatments.

On the basis of these results and to further investigate the role of mTMZ in second-line treatment of G3 NENs, a study is currently ongoing at the ENETS center of Naples. This larger and prospective clinical trial named TENEC trial (TEmozolomide in NeuroEndocrine Carcinoma), is supported by ITANET (ITalian Association for NEuroendocrine Tumors) and aims to confirm the efficacy and toxicity results of mTMZ as well as its modulating effects on host' immune system.

Supplementary Materials: None.

Author Contributions: The authors contributed as follows: conceptualization, A.O., C.V.A., S.T., A.C.; methodology, A.O. and M.C.; validation, C.V.A. M.C., M.M., F.T., S.T.; formal analysis, A.O., M.C. and C.V.A.; investigation, A.C., S.T., C.V.A., M.C., R.M., M.M., F.T., M.L.B., M.L.T., A.D.S.; data curation, C.V.A. and A.C.; writing and original draft preparation, A.O., M.C., S.T., C.V.A.

Funding: This research was funded by Lega Italiana per la Lotta contro i Tumori (LILT), Naples section.

Acknowledgments: We thank Dr. Alessandra Trocino, Librarian at the Library of Istituto Nazionale Tumori Fondazione 'G Pascale', Naples, Italy, for her excellent bibliographic service and assistance.

References

1. Dasari A, Shen C, Halperin D, Zhao B, 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; 3:1335-1342.
2. National Comprehensive Cancer Network. Neuroendocrine and Adrenal Tumor (Version 2.2018. https://www.nccn.org/professionals/physician_gls/PDF/neuroendocrine.pdf
3. Hallet J, Law CH, Cukier M, Saskin R, Liu N, Singh S. Exploring the rising incidence of neuroendocrine tumors: a population-based analysis of epidemiology, metastatic presentation, and outcomes. Cancer. 2015; 121:589-97.
4. Pavel M, O'Toole D, Costa F, Capdevila J, Gross D, Kianmanesh R et al. ENETS Consensus Guidelines Update for the Management of Distant Metastatic Disease of Intestinal, Pancreatic, Bronchial Neuroendocrine Neoplasms (NEN) and NEN of Unknown Primary Site. Neuroendocrinology 2016; 103:172-185.
5. Strosberg JR, Halfdanarson TR, Bellizzi A, Chan JA, Dillon JS, Heaney AP et al. The North American Neuroendocrine Tumor Society Consensus Guidelines for Surveillance and Medical Management of Midgut Neuroendocrine Tumors Pancreas. 2017; 46:707-714.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0278.v1

6. Ekeblad S, Sundin A, Janson ET, Welin S,Granberg D, Kindmark H et al. Temozolomide as monotherapy is effective in treatment of advanced malignant neuroendocrine tumors. Clin Cancer Res 2007; 13: 2986–2991.
7. Olsen IH, Sørensen JB, Federspiel B, Kjaer A,Hansen CP, Knigge U et al. Temozolomide as second or third line treatment of patients with neuroendocrine carcinomas. Scientific World Journal 2012; 2012: 170496.
8. Strosberg JR, Fine RL, Choi J, Nasir A, Coppola D, Chen DT et al. First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. Cancer 2011; 117: 268-275.
9. Welin S, Sorbye H, Sebjornsen S, Knappskog S, Busch C, Öberg K. Clinical effect of temozolomide-based chemotherapy in poorly differentiated endocrine carcinoma after progression on first-line chemotherapy. Cancer 2011;117: 4617-4622.
10. Saif MW, Kaley K, Brennan M, Garcon MC, Rodriguez G, Rodriguez T. A retrospective study of capecitabine/temozolomide (CAPTEM) regimen in the treatment of metastatic pancreatic neuroendocrine tumors (pNETs) after failing previous therapy. JOP 2013;14:498-501.
11. 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;71:663-670.
12. Saranga-Perry V, Morse B, Centeno B, Kvols L, Strosberg J. Treatment of metastatic neuroendocrine tumors of the thymus with capecitabine and temozolomide: a case series. Neuroendocrinology 2013;97:318-321.
13. Chan JA, Blaszkowsky L, Stuart K, Zhu AX, Allen J, Wadlow R et al. A prospective, phase 1/2 study of everolimus and temozolomide in patients with advanced pancreatic neuroendocrine tumor. Cancer 2013;119:3212–3218.
14. Koumarianou A, Antoniou S, Kanakis G, Economopoulos N, Rontogianni D, Ntavatzikos A et al. Combination treatment with metronomic temozolomide, bevacizumab and long-acting octreotide for malignant neuroendocrine tumors. Endocr Relat Cancer 2012;19:L1-L4.
15. Chan JA, Stuart K, Earle CC, Clark JW, Bhargava P, Miksad R et al. Prospective study of bevacizumab plus temozolomide in patients with advanced neuroendocrine tumors. J Clin Oncol 2012;30:2963-2968.
16. Kulke MH, Stuart K, Enzinger PC, Ryan DP, Clark JW, Muzikansky A, Vincitore M et al. Phase II study of temozolomide and thalidomide in patients with metastatic neuroendocrine tumors. J Clin Oncol 2006;24:401-406.
17. Kurzen H, Schmitt S, Näher H, Möhler T. Inhibition of angiogenesis by non-toxic doses of temozolomide. Anticancer Drugs. 2003;14:515-522.
18. Sun C, Yu Y, Wang L, Wu B, Xia L, Feng F, Ling Z et al. Additive antiangiogenesis effect of ginsenoside Rg3 with low-dose metronomic temozolomide on rat glioma cells both in vivo and in vitro. J Exp Clin Cancer Res. 2016;35:32.
19. Woo JY, Yang SH, Lee YS, Lee SY, Kim J, Hong YK. Continuous Low-Dose Temozolomide Chemotherapy and Microvessel Density in Recurrent Glioblastoma. J Korean Neurosurg Soc. 2015;58:426-431.
20. Kaneno R, Shurin GV, Tourkova IL, Shurin MR. Chemomodulation of human dendritic cell function by antineoplastic agents in low noncytotoxic concentrations. J Transl Med 2009;7:58.
21. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, Solary E et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother 2007;56:641-648.
22. Banissi C, Ghiringhelli F, Chen L, Carpentier AF. Treg depletion with a lowdose metronomic temozolomide regimen in a rat glioma model. Cancer Immunol Immunother 2009;58:1627-1634.
23. Zhao J, Cao Y, Lei Z, Yang Z, Zhang B, Huang B. Selective depletion of CD4+CD25+Foxp3+ regulatory T cells by low-dose cyclophosphamide is explained by reduced intracellular ATP levels. Cancer Res 2010;70:4850-4858.
24. Kan S, Hazama S, Maeda K, Inoue Y, Homma S, Koido S et al. Suppressive Effects of Cyclophosphamide and Gemcitabine on Regulatory T-Cell Induction. In Vitro. Anticancer Res 2012;32:5363-5369.
25. Fidler IJ, Ellis LM. Chemotherapeutic drugs—more really is not better. Nat Med 2000;6:500-502.
26. Gatenby RA, Silva AS, Gillies RJ, Frieden BR. Adaptive Therapy. Cancer Res 2009;69:4894-4903.
27. Scharovsky OG, Mainetti LE, Rozados VR. Metronomic chemotherapy: changing the paradigm that more is better. Curr Oncol 2009;16:7-15.
28. Pasquier E, Kavallaris M, André N. Metronomic chemotherapy: new rationale for new directions. Nat Rev Clin Oncol 2010;7:455-465.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 July 2019 doi:10.20944/preprints201907.0278.v1

29. Scoazec JY. Angiogenesis in neuroendocrine tumors: therapeutic applications. Neuroendocrinology 2013;97:45-56.
30. Besig S, Voland P, Baur DM, Perren A, Prinz C. Vascular endothelial growth factors, angiogenesis, and survival in human ileal enterochromaffin cell carcinoids. Neuroendocrinology 2009;90:402-415.
31. Zhang J, Jia Z, Li Q, Wang L, Rashid A, Zhu Z et al. Elevated expression of vascular endothelial growth factor correlates with increased angiogenesis and decreased progression-free survival among patients with low-grade neuroendocrine tumors. Cancer 2007);109:1478-1486.
32. Zhou Q, Guo P, Wang X, Nuthalapati S, Gallo JM. Preclinical pharmacokinetic and pharmacodynamic evaluation of metronomic and conventional temozolomide dosing regimens. J Pharmacol Exp Ther 2007;321:265-275.
33. Lambrescu I, Fica S, Martins D, Spada F, Cella C, Bertani E et al. Metronomic and metronomic-like therapies in neuroendocrine tumors – rationale and clinical perspectives. Cancer Trea Rev 2017;55:46-56.
34. André N, Carré M, Pasquier E. Metronomics: towards personalized chemotherapy? Nat Rev Clin Oncol 2014;11:413-431.
35. Figlin RA, Kaufmann I, Brechbiel J. Targeting PI3K and mTORC2 in metastatic renal cell carcinoma: new strategies for overcoming resistance to VEGFR and mTORC1 inhibitors. Int J Cancer. 2013;133:788-796.
36. Chan JA, Faris JE, Murphy JE, Blaszkowsky LS, Kwak EL, McCleary NJ et al. Phase II trial of cabozantinib in patients with carcinoid and pancreatic neuroendocrine tumors (pNET). Journal of Clinical Oncology 2017;35:4_suppl, 228-228.
37. Schiff D, Desjardins A,Cloughesy T, Mikkelsen T, Glantz M, Chamberlain MC et al. Phase 1 Dose Escalation Trial of the Safety and Pharmacokinetics of Cabozantinib Concurrent With Temozolomide and Radiotherapy or Temozolomide After Radiotherapy in Newly Diagnosed Patients With High-Grade Gliomas . Cancer 2016;122:582-587.
38. 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; 100:35-44.
39. Walter T, van Brakel B, Vercherat C, Hervieu V, Forestier J, Chayvialle JA. O6-Methylguanine-DNA methyltransferase status in neuroendocrine tumors: prognostic relevance and association with response to alkylating agents. British Journal of Cancer 2015; 112:523-531.
40. Kulke MH, Hornick JL, Frauenhoffer C, Hooshmand S, Ryan DP, Enzinger PC et al. O6-methylguanine DNA methyltransferase deficiency and response to temozolomide-based therapy in patients with neuroendocrine tumors. Clinical Cancer Research 2009;15:338-345.
41. Raj N, Klimstra DS, Horvat N, Zhang L, Chou JF, Capanu M et al. O6-Methylguanine DNA Methyltransferase Status Does Not Predict Response or Resistance to Alkylating Agents in Well-Differentiated Pancreatic Neuroendocrine Tumors.Pancreas. 2017;46:758-763.