World Journal of Clinical Cases

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.12998/wjcc.v2.i12.835

World J Clin Cases 2014 December 16; 2(12): 835-839
ISSN 2307-8960 (online)

Role of MGMT as biomarker in colorectal cancer

Alessandro Inno, Giuseppe Fanetti, Maria Di Bartolomeo, Stefania Gori, Claudia Maggi, Massimo Cirillo, Roberto Iacovelli, Federico Nichetti, Antonia Martinetti, Filippo de Braud, Ilaria Bossi, Filippo Pietrantonio

Alessandro Inno, Stefania Gori, Massimo Cirillo, Medical Oncology Department, Ospedale Sacro Cuore Don Calabria, Negrar, 37100 Verona, Italy
Giuseppe Fanetti, Radiotherapy Unit, European Institute of Oncology, 20100 Milan, Italy
Maria Di Bartolomeo, Claudia Maggi, Roberto Iacovelli, Federico Nichetti, Antonia Martinetti, Filippo de Braud, Ilaria Bossi, Filippo Pietrantonio, Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, 20100 Milan, Italy
Author contributions: All authors gave substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; final approval of the version to be published.
Correspondence to: Filippo Pietrantonio, MD, Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian, 20100 Milan, Italy. filippo.pietrantonio@istitutotumori.mi.it
Telephone: +39-2-23903807 Fax: +39-2-23902149
Received: July 23, 2014 Revised: September 1, 2014
Accepted: October 14, 2014
Published online: December 16, 2014

Abstract

O^6-methylguanine DNA methyltransferase (MGMT) gene promoter methylation plays an important role in colorectal carcinogenesis, occurring in about 30%-40% of metastatic colorectal cancer. Its prognostic role has not been defined yet, but loss of expression of *MGMT*, which is secondary to gene promoter methylation, results in an interesting high response to alkylating agents such as dacarbazine and temozolomide. In a phase 2 study on heavily pre-treated patients with *MGMT* methylated metastatic colorectal cancer, temozolomide achieved about 30% of disease control rate. Activating mutations of *RAS* or *BRAF* genes as well as mismatch repair deficiency may represent mechanisms of resistance to alkylating agents, but a dose-dense schedule of temozolomide may potentially restore sensitivity in *RAS*-mutant patients. Further development of temozolomide in *MGMT* methylated colorectal cancer includes investigation of synergic combinations with other agents such as fluoropyrimidines and research for additional biomarkers, in order to better define the role of temozolomide in the treatment of individual patients.

Key words: Colorectal cancer; O^6-methylguanine DNA methyltransferase; Temozolomide; Dacarbazine; Biomarker

Core tip: O^6-methylguanine DNA methyltransferase (MGMT) methylation is involved in colorectal carcinogenesis and represents a predictive biomarker for alkylating agents in metastatic colorectal cancer. In fact, patients with chemorefractory metastatic colorectal cancer with *MGMT* methylation derived promising response from treatment with dacarbazine or temozolomide, and ongoing research is investigating the efficacy of temozolomide in combination with other chemotherapy drugs for *MGMT*-methylated colorectal cancer. Future challenges include the combination with biologic drugs and the research for additional biomarkers.

Inno A, Fanetti G, Di Bartolomeo M, Gori S, Maggi C, Cirillo M, Iacovelli R, Nichetti F, Martinetti A, de Braud F, Bossi I, Pietrantonio F. Role of MGMT as biomarker in colorectal cancer. *World J Clin Cases* 2014; 2(12): 835-839 Available from: URL: http://www.wjgnet.com/2307-8960/full/v2/i12/835.htm DOI: http://dx.doi.org/10.12998/wjcc.v2.i12.835

ROLE OF MGMT IN THE DEVELOPMENT OF COLORECTAL CANCER

Colorectal carcinogenesis is a complex, multistep and still not completely understood process including both genetic and epigenetic alterations. DNA damage certainly plays a central role in cancer development and progression, especially when the DNA repair machinery is not

efficient.

O^6-methylguanine DNA methyltransferase (MGMT) is a DNA repair enzyme codified by the *MGMT* gene at locus 10q26[1]. MGMT removes alkyl groups from the O^6-position of the guanine acting itself as an acceptor, and such reaction leads to an irreversible inactivation of the enzyme[2]. *MGMT* transcription is regulated by epigenetic mechanisms. Methylation of the CpG dinucleotides in the promoter region of *MGMT* results in gene silencing, MGMT loss of expression and inability to remove alkyl groups from methylated guanine, with a consequent alteration of the normal DNA structure[3].

While protecting normal cell from carcinogens, MGMT activity also protects tumor cells from lethal effects of chemotherapy with alkylating agents such as dacarbazine (DTIC) or temozolomide (TMZ), widely used for the treatment of melanoma and glioblastoma. In glioblastoma, in fact, *MGMT* methylation has been identified as a relevant prognostic factor and as an independent predictive factor of benefit from TMZ[4,5]. For melanoma, the predictive and prognostic role of *MGMT* methylation status is controversial, but patients with MGMT-methylated melanoma treated with DTIC seem to be at higher risk of treatment-related adverse events[6].

MGMT promoter methylation is a frequent and relevant event in colorectal cancerogenesis, with a low expression of MGMT secondary to gene silencing observed in 27 to 40% of metastatic chemorefractory colorectal cancer (mCRC)[7]. However, MGMT loss has been also demonstrated in normal colorectal tissue, suggesting that *MGMT* silencing is only one of several steps needed for the accumulation of DNA damage and cell transformation. MGMT loss has been defined as a "field defect", since it is neither necessary nor sufficient to cancer progression - *i.e.*, in a multistep process, it represents only one of the earlier passages leading to carcinogenesis. In fact, a second level of defense against DNA damage is represented by the mismatch repair (MMR) system which leads cell to apoptosis in presence of serious genomic alterations[8]. Loss of MGMT expression is more frequent in CRC with microsatellite instability, suggesting that methylated *MGMT* select cellular clones with MMR deficient status[9].

However, MGMT loss also plays a role in microsatellite-stable CRC through a mechanism of chromosomal instability[10]. During DNA transcription, methylated guanine is wrongly recognized as adenine causing C:G to A:T transition mutations. MGMT may prevent guanine to adenine transition in ras-family genes, while the loss of MGMT activity may increase the likelihood of *RAS* and *TP53* mutations. In fact, *MGMT* methylation was found in 71% of mCRC with *KRAS* G>A mutations, whereas it was present only in 32% of tumors with non-G>A KRAS mutations and in 35% of tumors with wild-type *KRAS*[11,12]. In *KRAS*-mutant tumors, *MGMT* promoter methylation occurs before *KRAS* mutations and it represents an early event in the adenoma-carcinoma sequence. Therefore, there should be a high concordance of *MGMT* methylation status between primary tumor and distant metastases.

Despite its defined position in the pathogenesis of CRC, the prognostic role of *MGMT* is still controversial. Few studies directed to investigate the prognostic role of *MGMT* methylation have been published with different results. In a series of 116 patients, a reduction of recurrence rate after adjuvant therapy has been reported in *MGMT* methylated patients[13]. Shima *et al*[14] analyzed a group of 855 patients with CRC showing no prognostic value of MGMT loss or gene promoter hypermethylation. No benefit from 5-fluorouracil (5-FU)- or oxaliplatin-based regimens was reported in presence of *MGMT* promoter methylation or *MGMT* loss[15].

ACTIVITY OF ALKYLATING AGENTS IN MGMT-METHYLATED COLORECTAL CANCER

DTIC and its oral derivative TMZ exert their cytotoxic effects through methylation of DNA at the N3 position of adenine and at the N7 and O6 positions of guanine. Although N7-methyl-guanine and N3-methyl-adenine represent the majority of adducts, cytotoxicity of DTIC and TMZ seems to be mainly due to DNA methylation at the O6-position of guanine, which leads to DNA double strand breaks and subsequent inhibition of DNA replication and apoptosis. As the cytotoxic effect of TMZ is mediated primarily through methylation of O6-guanine, the predominant mechanism of tumor resistance to DTIC and TMZ is *MGMT* expression.

TMZ showed *in vitro* activity against several human malignancies, including colorectal cancer[16]. In a phase 1 study on solid tumors comparing a novel schedule for TMZ given twice a day for more than 5 consecutive days with the standard schedule of a daily administration for 5 consecutive days, the drug activity was quite disappointing with only 1 out of 12 mCRC patients responding to treatment[17]. However, patients enrolled in the trial were not molecularly selected according to the *MGMT* methylation status.

In a pilot study, 66 patients with refractory metastatic cancer were treated according to the molecular tumor profiling, and TMZ was effective in 2 cases of advanced CRC exhibiting loss of *MGMT* expression[18]. Consistently with these data, Schacham-Schmueli and colleagues described 2 patients with mCRC and a low expression of MGMT who were treated with TMZ achieving an impressive clinical response[19]. To explore the hypothesis that in mCRC the activity of TMZ is confined to tumors with low levels of *MGMT*, a phase II trial combined the alkylating drug with lomeguatrib, a nontoxic low-molecular weight pseudosubstrate which inactivates MGMT[20]. Unfortunately, the study was terminated early for futility, after 19 instead of the 30 patient initially planned had been recruited. The main reason why no objective response was observed, as the same authors stated, may be related to the low doses of TMZ and lomeguatrib used in the trial.

Table 1 Phase 2 clinical trials with alkylating agents in metastatic chemorefractory colorectal cancer

Ref.	Schedule	*n* (*MGMT*-m)	RR (*MGMT*-m)	DCR (*MGMT*-m)	PFS mo (*MGMT*-m)	OS mo (*MGMT*-m)
Amatu *et al*[21]	DTIC 250 mg/m^2 per day, d 1-4 q21d	68 (26)	3% (8%)	12% (44%)	1.7 (NR)[1]	NR
Hochauser *et al*[22]	TMZ 150 mg/m^2 per day 7 d on/7 d off	37[2] (37)	3% (3%)	44% (445)	NR	NR
Pietrantonio *et al*[23]	TMZ 150 mg/m^2 per day d 1-5, q28d	32[3] (32)	12% (12%)	31% (31%)	1.8 (1.8)	8.4 (8.4)
Pietrantonio *et al*[26]	TMZ 75 mg/m^2 per day, d 1-21 q28d	21[4] (21)	24% (24%)	30% (30%)	2.2 (2.2)	NR

[1]Median PFS for *MGMT*-m patients was not reported, but hazard ratio for progression between methylated and non-methylated patients was 0.66 (95%CI: 0.40-1.10), P = 0.0982; [2]The study enrolled 86 patients with MGMT-m metastatic CRC (Mcrc), esophageal, head and neck and non small cell lung cancer; this table reports data of patients with mCRC only; [3]Only MGMT-m patients were enrolled in the study; [4]Preliminary results. DTIC: Dacarbazine; TMZ: Temozolomide; RR: Response rate; DCR: Disease control rate; PFS: Progression-free survival; OS: Overall survival; MGMT-m: MGMT-methylated; NR: Not reported.

Amatu *et al*[21] evaluated the activity of dacarbazine in 68 heavily pretreated mCRC patients. The response rate was only 3% with 2 partial responses, but a preplanned analysis based on *MGMT* methylation status in the individual tumors showed that objective responses only occurred in patients with *MGMT* methylated cancer. In the *MGMT*-methylated group, a significantly higher disease control rate (44% *vs* 6%, P = 0.012) and a trend toward longer progression-free survival (PFS) were also observed (Table 1). These results provided the proof-of-concept that dacarbazine, and consequently its derivative TMZ, are effective only in patients with mCRC harboring methylation in the *MGMT* gene promoter.

Patients with advanced aerodigestive tract and colorectal cancers and methylation of *MGMT* gene promoter were treated by Hochhauser *et al*[22] with TMZ given at 150 mg/m^2 per day on a 7-day-on/7-day-off schedule. A low response rate (3%) was reported in patients with mCRC (Table 1), suggesting that *MGMT* methylation may be not the only factor determining response to TMZ. Particularly, cell death induced by TMZ also depends on the integrity of MMR pathways. In this study, all the patients with objective response were MMR-proficient, and most MMR-deficient patients experienced a disease progression, but data were limited to drive definitive conclusions about the correlation between MMR status and response to TMZ.

A phase 2 study run by our research group at the National Cancer Institute of Milan enrolled 32 patients with *MGMT*-methylated mCRC who progressed after all the approved standard therapies including fluoropyrimidines, oxaliplatin, irinotecan and, if *KRAS* wild-type, also cetuximab or panitumumab[23]. All the patients received TMZ at the standard dose of 150 mg/m^2 per day for 5 consecutive days every 28 d until disease progression or unacceptable toxicity. This was the first trial of TMZ given to mCRC patients selected for *MGMT* methylation status. The study met its primary end-point of promising activity, with 4 (12%) partial responses and 6 (19%) disease stabilizations; median PFS and overall survival (OS) were 1.8 and 8.4 mo, respectively (Table 1). TMZ was well tolerated, with severe thromocytopenia occurring in only 1 patient and no other grade 3-4 toxicities observed. Dose reduction was necessary in 3 patients, and no patients underwent early discontinuation due to adverse events. The study also explored potential predictive biomarkers, showing that none of the patients with mCRC harboring a mutation in the mitogen-activated protein kinases (MAPK), either *RAS* or *BRAF*, achieved a response. Conversely, four of nine patients with *RAS* and *BRAF* wild-type tumors had an objective response (0% *vs* 44%, P = 0.004). These results confirmed what was already shown for glioblastoma, namely that MAPK signaling may enhance MGMT activity and drive cellular resistance to TMZ[24]. Interestingly, none of the patients included in this trial were MMR-deficient, and this might explain the clinically meaningful response rate observed in this study.

Other trials are currently ongoing, with the aim to shed more light on the role of TMZ as single agent and investigate predictive biomarkers in patients with *MGMT* methylated CRC (Eudract n. 2012-003338-17; 2012-002766-13).

FUTURE CHALLENGES

It was previously shown that a dose-dense TMZ regimen results in prolonged depletion of MGMT in blood mononuclear cells and possibly in the tumor[25]. This schedule of administration may have enhanced activity due to higher cumulative dose and might be able to restore treatment sensitivity in *RAS* mutant tumors. Preliminary results from a mono-institutional, phase Ⅱ, open label, single arm study with dose-dense TMZ were recently presented by our group[26]. Patients with chemorefractory disease were treated with TMZ at a dose of 75 mg/m^2 given daily for 21 consecutive days of a 4-week cycle, up to 6 cycles or until disease progression or unacceptable toxicity. Seventeen out of 21 patients were evaluable for RECIST response. Tumor response, which was the primary endpoint of the study, was 24%. Interestingly, all patients with tumor response had a mutation of either *KRAS* (3 patients) or *BRAF* (1 patient). These preliminary data confirmed the encouraging activity of

TMZ in molecularly selected patients with *MGMT* methylation-positive, chemorefractory mCRC (Table 1). The good safety profile of dose-dense TMZ, as well as the response rate obtained in the *RAS* mutant population, are promising and warrant further prospective randomized confirmation.

There is a strong rationale for combining TMZ with fluoropyrimidines, based on preclinical data in slow-growing carcinoid cell line. It was found that TMZ and 5-FU have synergistic activity in a schedule-dependent manner in which 5-FU exposure preceded TMZ by 5–7 d with maximal synergism at 9 d. When the two agents were delivered concomitantly or if TMZ preceded 5-FU, *in vitro* cytotoxicity was additive but not synergistic. From these translational studies, researchers from the Columbia university formulated the CAPTEM regimen using TMZ for 5 d at 150-200 mg/m^2 per day refracted in a BID dosing on days 10-14 and capecitabine 750 mg/m^2 BID on days 1-14 of a 28-d-cycle[27]. The TMZ was given twice-a-day instead of the standard daily dosing because the first dose binds MGMT, thus allowing the second dose to methylate guanines in presence of a decreased repair activity of MGMT[17,28]. Given the potential synergy of CAPTEM combination in mCRC, we planned a randomized phase Ⅱ study of second-line CAPTEM *vs* FOLFIRI after failure of prior first-line oxaliplatin-based treatment in patients with advanced, *MGMT* methylated, *RAS* mutated CRC (Eudract n 2014-002417-36). The primary objective of the study is to demonstrate the superiority of CAPTEM over standard FOLFIRI in terms of PFS; secondary endpoints are response rate, safety, quality of life, OS, with an exploratory biomarkers sub-study.

In conclusion, there is growing evidence that *MGMT* methylation status may serve as a predictive biomarker of response to TMZ in mCRC. TMZ is a promising agent for the treatment of *MGMT*-methylated mCRC, and the future research should establish the best schedule of TMZ and should also investigate how to integrate TMZ with the current available therapeutic options for mCRC, whether as single agent in chemorefractory patients or in combination with other active drugs in first or second line. The combination of TMZ with fluoropyrimidines is based on a strong rationale and is currently being investigated, but also the combination of TMZ with irinotecan[29] has been proven to be feasible and it may deserve evaluation in mCRC. TMZ-containing chemotherapy may also provide an interesting backbone for the addition of biologic agents. However, the identification of additional biomarkers is a priority for future research, in order to select individual patients who may benefit the most from alkylating agents.

REFERENCES

1 **Natarajan AT**, Vermeulen S, Darroudi F, Valentine MB, Brent TP, Mitra S, Tano K. Chromosomal localization of human O6-methylguanine-DNA methyltransferase (MGMT) gene by in situ hybridization. *Mutagenesis* 1992; **7**: 83-85 [PMID: 1635460 DOI: 10.1093/mutage/7.1.83]

2 **Pegg AE**. Mammalian O6-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic and therapeutic agents. *Cancer Res* 1990; **50**: 6119-6129 [PMID: 2205376]

3 **Gerson SL**. MGMT: its role in cancer aetiology and cancer therapeutics. *Nat Rev Cancer* 2004; **4**: 296-307 [PMID: 15057289 DOI: 10.1038/nrc1319]

4 **Hegi ME**, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; **352**: 997-1003 [PMID: 15758010 DOI: 10.1056/NEJMoa043331]

5 **Reifenberger G**, Hentschel B, Felsberg J, Schackert G, Simon M, Schnell O, Westphal M, Wick W, Pietsch T, Loeffler M, Weller M. Predictive impact of MGMT promoter methylation in glioblastoma of the elderly. *Int J Cancer* 2012; **131**: 1342-1350 [PMID: 22139906 DOI: 10.1002/ijc.27385]

6 **Hassel JC**, Sucker A, Edler L, Kurzen H, Moll I, Stresemann C, Spieth K, Mauch C, Rass K, Dummer R, Schadendorf D. MGMT gene promoter methylation correlates with tolerance of temozolomide treatment in melanoma but not with clinical outcome. *Br J Cancer* 2010; **103**: 820-826 [PMID: 20736948 DOI: 10.1038/sj.bjc.6605796]

7 **Esteller M**, Herman JG. Generating mutations but providing chemosensitivity: the role of O6-methylguanine DNA methyltransferase in human cancer. *Oncogene* 2004; **23**: 1-8 [PMID: 14712205 DOI: 10.1038/sj.onc.1207316]

8 **Allan JM**, Travis LB. Mechanisms of therapy-related carcinogenesis. *Nat Rev Cancer* 2005; **5**: 943-955 [PMID: 16294218 DOI: 10.1038/nrc1749]

9 **Svrcek M**, Buhard O, Colas C, Coulet F, Dumont S, Massaoudi I, Lamri A, Hamelin R, Cosnes J, Oliveira C, Seruca R, Gaub MP, Legrain M, Collura A, Lascols O, Tiret E, Fléjou JF, Duval A. Methylation tolerance due to an O6-methylguanine DNA methyltransferase (MGMT) field defect in the colonic mucosa: an initiating step in the development of mismatch repair-deficient colorectal cancers. *Gut* 2010; **59**: 1516-1526 [PMID: 20947886 DOI: 10.1136/gut.2009.194787]

10 **Shen L**, Kondo Y, Rosner GL, Xiao L, Hernandez NS, Vilaythong J, Houlihan PS, Krouse RS, Prasad AR, Einspahr JG, Buckmeier J, Alberts DS, Hamilton SR, Issa JP. MGMT promoter methylation and field defect in sporadic colorectal cancer. *J Natl Cancer Inst* 2005; **97**: 1330-1338 [PMID: 16174854 DOI: 10.1093/jnci/dji275]

11 **Esteller M**, Risques RA, Toyota M, Capella G, Moreno V, Peinado MA, Baylin SB, Herman JG. Promoter hypermethylation of the DNA repair gene O(6)-methylguanine-DNA methyltransferase is associated with the presence of G: C to A: T transition mutations in p53 in human colorectal tumorigenesis. *Cancer Res* 2001; **61**: 4689-4692 [PMID: 11406538]

12 **Esteller M**, Toyota M, Sanchez-Cespedes M, Capella G, Peinado MA, Watkins DN, Issa JP, Sidransky D, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-ras in colorectal tumorigenesis. *Cancer Res* 2000; **60**: 2368-2371 [PMID: 10811111]

13 **Nagasaka T**, Sharp GB, Notohara K, Kambara T, Sasamoto H, Isozaki H, MacPhee DG, Jass JR, Tanaka N, Matsubara N. Hypermethylation of O6-methylguanine-DNA methyltransferase promoter may predict nonrecurrence after chemotherapy in colorectal cancer cases. *Clin Cancer Res* 2003; **9**: 5306-5312 [PMID: 14614014]

14 **Shima K**, Morikawa T, Baba Y, Nosho K, Suzuki M, Yamauchi M, Hayashi M, Giovannucci E, Fuchs CS, Ogino S. MGMT promoter methylation, loss of expression and prognosis in 855 colorectal cancers. *Cancer Causes Control* 2011; **22**: 301-309 [PMID: 21140203 DOI: 10.1007/s10552-010-9698-z]

15 **Kim JC**, Choi JS, Roh SA, Cho DH, Kim TW, Kim YS. Promoter methylation of specific genes is associated with the phenotype and progression of colorectal adenocarcinomas. *Ann Surg Oncol* 2010; **17**: 1767-1776 [PMID: 20077021 DOI:

10.1245/s10434-009-0901-y]
16 **Raymond E**, Izbicka E, Soda H, Gerson SL, Dugan M, Von Hoff DD. Activity of temozolomide against human tumor colony-forming units. *Clin Cancer Res* 1997; **3**: 1769-1774 [PMID: 9815562]
17 **Spiro TP**, Liu L, Majka S, Haaga J, Willson JK, Gerson SL. Temozolomide: the effect of once- and twice-a-day dosing on tumor tissue levels of the DNA repair protein O(6)-alkylguanine-DNA-alkyltransferase. *Clin Cancer Res* 2001; **7**: 2309-2317 [PMID: 11489806]
18 **Von Hoff DD**, Stephenson JJ, Rosen P, Loesch DM, Borad MJ, Anthony S, Jameson G, Brown S, Cantafio N, Richards DA, Fitch TR, Wasserman E, Fernandez C, Green S, Sutherland W, Bittner M, Alarcon A, Mallery D, Penny R. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. *J Clin Oncol* 2010; **28**: 4877-4883 [PMID: 20921468 DOI: 10.1200/JCO.2009.26.5983]
19 **Shacham-Shmueli E**, Beny A, Geva R, Blachar A, Figer A, Aderka D. Response to temozolomide in patients with metastatic colorectal cancer with loss of MGMT expression: a new approach in the era of personalized medicine? *J Clin Oncol* 2011; **29**: e262-e265 [PMID: 21220612 DOI: 10.1200/JCO.2010.32.0242]
20 **Khan OA**, Ranson M, Michael M, Olver I, Levitt NC, Mortimer P, Watson AJ, Margison GP, Midgley R, Middleton MR. A phase II trial of lomeguatrib and temozolomide in metastatic colorectal cancer. *Br J Cancer* 2008; **98**: 1614-1618 [PMID: 18475294 DOI: 10.1038/sj.bjc.6604366]
21 **Amatu A**, Sartore-Bianchi A, Moutinho C, Belotti A, Bencardino K, Chirico G, Cassingena A, Rusconi F, Esposito A, Nichelatti M, Esteller M, Siena S. Promoter CpG island hypermethylation of the DNA repair enzyme MGMT predicts clinical response to dacarbazine in a phase II study for metastatic colorectal cancer. *Clin Cancer Res* 2013; **19**: 2265-2272 [PMID: 23422094 DOI: 10.1158/1078-0432.CCR-12-3518]
22 **Hochhauser D**, Glynne-Jones R, Potter V, Grávalos C, Doyle TJ, Pathiraja K, Zhang Q, Zhang L, Sausville EA. A phase II study of temozolomide in patients with advanced aerodigestive tract and colorectal cancers and methylation of the O6-methylguanine-DNA methyltransferase promoter. *Mol Cancer Ther* 2013; **12**: 809-818 [PMID: 23443801 DOI: 10.1158/1535-7163.MCT-12-0710]
23 **Pietrantonio F**, Perrone F, de Braud F, Castano A, Maggi C, Bossi I, Gevorgyan A, Biondani P, Pacifici M, Busico A, Gariboldi M, Festinese F, Tamborini E, Di Bartolomeo M. Activity of temozolomide in patients with advanced chemorefractory colorectal cancer and MGMT promoter methylation. *Ann Oncol* 2014; **25**: 404-408 [PMID: 24379162 DOI: 10.1093/annonc/mdt547]
24 **Sato A**, Sunayama J, Matsuda K, Seino S, Suzuki K, Watanabe E, Tachibana K, Tomiyama A, Kayama T, Kitanaka C. MEK-ERK signaling dictates DNA-repair gene MGMT expression and temozolomide resistance of stem-like glioblastoma cells via the MDM2-p53 axis. *Stem Cells* 2011; **29**: 1942-1951 [PMID: 21957016 DOI: 10.1002/stem.753]
25 **Tolcher AW**, Gerson SL, Denis L, Geyer C, Hammond LA, Patnaik A, Goetz AD, Schwartz G, Edwards T, Reyderman L, Statkevich P, Cutler DL, Rowinsky EK. Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. *Br J Cancer* 2003; **88**: 1004-1011 [PMID: 12671695]
26 **Pietrantonio F**, de Braud F, Maggi C, Milione M, Iacovelli R, Castano A, Perrone F, Bossi I, Ricchini F, Pusceddu S, Leone G, Dominoni F, Saibene G, Di Bartolomeo M. Dose-dense temozolomide (TMZ) in patients with advanced chemorefractory colorectal cancer (CRC) and MGMT promother methylation. *Ann Oncol* 2014; **25**: 14-104 [DOI: 10.1093/annonc/mdu165]
27 **Fine RL**, Gulati AP, Krantz BA, Moss RA, Schreibman S, Tsushima DA, Mowatt KB, Dinnen RD, Mao Y, Stevens PD, Schrope B, Allendorf J, Lee JA, Sherman WH, Chabot JA. Capecitabine and temozolomide (CAPTEM) for metastatic, well-differentiated neuroendocrine cancers: The Pancreas Center at Columbia University experience. *Cancer Chemother Pharmacol* 2013; **71**: 663-670 [PMID: 23370660 DOI: 10.1007/s00280-012-2055-z]
28 **Balmaceda C**, Peereboom D, Pannullo S, Cheung YK, Fisher PG, Alavi J, Sisti M, Chen J, Fine RL. Multi-institutional phase II study of temozolomide administered twice daily in the treatment of recurrent high-grade gliomas. *Cancer* 2008; **112**: 1139-1146 [PMID: 18246536 DOI: 10.1002/cncr.23167]
29 **Reynés G**, Balañá C, Gallego O, Iglesias L, Pérez P, García JL. A phase I study of irinotecan in combination with metronomic temozolomide in patients with recurrent glioblastoma. *Anticancer Drugs* 2014; **25**: 717-722 [PMID: 24322542 DOI: 10.1097/CAD.0000000000000059]

P- Reviewer: Persano L, Sulkowski S **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Wu HL

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com