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

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.

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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'-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'-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]. 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[5].

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)[6]. 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[7]. Loss of MGMT expression is more frequent in CRC with microsatellite instability, suggesting that methylated MGMT select cellular clones with MMR deficient status[8].

However, MGMT loss also plays a role in microsatellite-stable CRC through a mechanism of chromosomal instability[9]. 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>T mutations, whereas it was present only in 32% of tumors with non-G>T KRAS mutations and in 35% of tumors with wild-type KRAS[10,11]. 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[12]. Shima et al[13] 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[14].

**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[15]. In a phase I 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[16]. 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[17]. 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[18]. 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[19]. 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.
Amatu et al.\textsuperscript{[21]} evaluated the activity of dacarbazine in 68 heavily pretreated mCRC patients. The response rate was only 3\% with 2 partial responses, and 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 Hochauer et al.\textsuperscript{[22]} with TMZ given at 150 mg/m\textsuperscript{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\textsuperscript{[23]}. All the patients received TMZ at the standard dose of 150 mg/m\textsuperscript{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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[26]}. Patients with chemo-refractory disease were treated with TMZ at a dose of 75 mg/m\textsuperscript{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

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

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

\textsuperscript{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: 0.40-1.0), P = 0.0982; \textsuperscript{2}The study enrolled 86 patients with MGMT-m metastatic CRC (M crc), esophageal, head and neck and non small cell lung cancer; this table reports data of patients with mCRC only; \textsuperscript{3}Only MGMT-m patients were enrolled in the study; \textsuperscript{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.
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 R-14 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/m² per day refracted in a BID dosing on days 10-14 and capecitabine 750 mg/m² BID on days 1-14 of a 28-d-cycle[2]. 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[17,20]. 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, R-AS 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[20] 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.

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P- Reviewer: Persano L, Sulkowski S
S- Editor: Tian YL
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