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Pharmacoepigenetics in gastrointestinal tumors: *MGMT* methylation and beyond

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1. ABSTRACT

Epigenetic mechanisms are involved in gastrointestinal (GI) cancer pathogenesis. Insights into the molecular basis of GI carcinogenesis led to the identification of different epigenetic pathways and signatures that may play a role as therapeutic targets in metastatic colorectal cancer (mCRC) and non-colorectal GI tumors. Among these alterations, *O6-methylguanine DNA methyltransferase* (*MGMT*) gene promoter methylation is the most investigated biomarker and seems to be an early and frequent event, at least in CRC. Loss of expression of MGMT as a result of gene promoter methylation has been associated with interesting activity of alkylating agents in mCRC. However, the optimal methods for the definition of the *MGMT* status and additional predictive factors beyond *MGMT* in GI malignancies are lacking. Here we review the current role of *MGMT* methylation and other epigenetic alterations as potential treatment targets in GI tumors.

2. INTRODUCTION

In the last decade, we have witnessed significant advances in the management of several advanced gastrointestinal (GI) malignancies. New therapeutic agents (targeted drugs or cytotoxic compounds) have been proved effective against both metastatic colorectal cancer (mCRC) (1) and non-colorectal GI tumors such as pancreatic, gastric and liver cancers (2-4). However, prognosis of patients with advanced GI tumors remains dismal and the development of alternative treatment approaches is needed.

Epigenetics has generated great interest as a valuable companion to cancer genetics in the unravelling of tumor initiation and progression in recent years (5). Even more intriguingly, epigenetic alterations have been proved effective in predicting disease course in specific solid malignancies (*e.g.* glioblastoma), thus adding additional prognostic information to conventional pathologic and clinical features in the clinics. As regards treatment response, epigenetics is being explored as a predictive tool for activity and efficacy of both cytotoxics and biologic agents and in the case of central-nervous system malignancies it has already entered the clinical practice. In this scenario, epigenetics thus offers a different perspective to investigate and develop anti-tumor drugs with the ability to overcome resistance to conventional treatments. In fact, DNA methylation, histone post-translational modifications and microRNAs cooperate with somatic gene alterations in the multistep carcinogenetic process in most cancer types, contributing to tumor growth, metastatization and drug resistance (5). Two main epigenetics-based approaches have been explored in GI tumors after the failure of available therapies: the use of demethylating agents in order to interfere with gene expression and the administration of specific drugs in selected patient subgroups according to key gene silencing by DNA methylation.

In this review, we will describe the current evidence supporting the role of *O6-methylguanine DNA methyltransferase* (*MGMT*) gene promoter methylation as a promising treatment biomarker and we will review the other epigenetic pathways currently under evaluation as putative therapeutic targets in GI malignancies.

Table 1. Phase II clinical trials with alkylating agents in mCRC

Reference	Drug	Schedule	No. (MGMT+)	% with >3 previous lines (range of lines)	Methods	RR (MGMT+) (%)	DCR (MGMT+) (%)	mPFS (MGMT+)	mOS (MGMT+)
Amatu [23]	DTIC	250 mg/sqm d1-4 q21	68 (26)	54% (2-7)	PCR	3 (8)	15 (44)	1.7 (NR)	NR
Hochhauser [24]	TMZ	150 mg/sqm/daily d1-7 q14	37 (37)	5% (2-4)	PCR	3 (3)	44 (44)	NR	NR
Pietrantonio [25]	TMZ	150 mg/sqm/daily d1-5 q28	32 (32)	37% (2-5)	PCR	12 (12)	31 (31)	1.8 (1.8)	8.4 (8.4)
Pietrantonio [27]	TMZ	75 mg/sqm/daily d1-21 q28	21 (21)*	Median: 3 (2-5)	PCR	24 (24)	30 (30)	2.2 (2.2)	NR

Abbreviations: mCRC: metastatic colorectal cancer; DTIC: dacarbazine; TMZ: temozolomide; d: day; q: repeated every (days); No.: number (of patients); *MGMT*+: *MGMT* methylated; PCR: (methylation-specific) polymerase chain reaction; RR: response rate; DCR: disease control rate; mPFS: median progression-free survival (months); mOS: median overall survival (months); NR: not reported. *preliminary results

3. *MGMT* METHYLATION IN MCRC

3.1. Current evidence supporting a role for *MGMT* methylation as therapeutic target in mCRC

Epigenetic mechanisms of gene silencing are often deregulated in CRC and essentially contribute to colorectal carcinogenesis. In particular, DNA methylation is one of the most studied genetic aberrations in CRC and 20% of all CRCs display a cytosine guanine (CpG) dinucleotides island methylation phenotype (CIMP) (6): CIMP is characterized by multiple promoter hypermethylation of tumor-related genes and is associated with distinct clinico-pathologic features and genetic signatures in early stage CRC (7). MGMT is a DNA repair protein that removes O6-methylguanine adducts in DNA by transferring the methyl group to a cysteine residue in the active site of the protein, thus restoring guanine in DNA and allowing cell survival (8).

Loss of MGMT expression is associated with diminished DNA-repair activity and may therefore play a significant role in disease progression. MGMT deficiency (which is primarily due to promoter hypermethylation) has been reported in approximately 40% of CRC cases (9). Of note, it is thought to be an early event in CRC pathogenesis (10), succeeding APC mutation but preceding *KRAS* mutation (11): *MGMT* promoter hypermethylation is closely associated with the G: C>A: T mutation in *KRAS* (12,13). MGMT expression has been recently correlated with shorter overall survival (OS) in CRC and retained its prognostic value independently of treatment and other histopathologic variables (14).

Alkylating agents, like temozolomide and dacarbazine, are currently employed mainly in the treatment of high-grade glioma and metastatic melanoma. Temozolomide is the oral prodrug of dacarbazine, which is able to induce genotoxic damage in cancer and normal cells. Alkylating agents induce DNA damage by the creation of O6-methylguanine adducts, which may induce G1/S cell-cycle arrest and p53-dependent apoptosis (15). Cancer cells may repair DNA damages induced by alkylating agents through the MGMT enzymatic activity (16). In temozolomide-treated glioma patients, *MGMT* methylation has been associated with longer survival and seems to have a predictive value (17). Thus, it is conceivable that *MGMT* methylation might predict response to temozolomide and other alkylating agents also in other cancer types.

Alkylating agents failed to show interesting activity in unselected mCRC patients (18-20). On the other hand, interesting results have been obtained in selected patient populations (Table 1).

In a pilot study published in 2010, 86 patients with refractory metastatic tumors (arising from multiple different sites such as the ovary, colon-rectum, breast and other) had their tissue samples analyzed by multiple techniques (immunohistochemistry, fluorescent *in situ* hybridization or oligonucleotide microarray) in order to identify a potential therapeutic target: all the investigated factors (11 proteins and 51 mRNAs) covered a panel of targets for commercially available anticancer agents (*e.g.* EGFR, SPARC, c-KIT, hormone receptors, VEGF, MGMT and others). The authors compared the outcome (in terms of progression-free survival (PFS)) of a treatment regimen selected by molecular profiling with that achieved by the most recent regimen on which the patients had experienced disease progression (21). Intriguingly, a molecular target was detected in 84 patients, and 66 patients were treated according to molecular profiling. Temozolomide was the effective targeted treatment in 2 of the enrolled mCRC patients with *MGMT* promoter methylation, suggesting that it could be an interesting option in selected CRC populations.

A subsequent case report by Shacham-Shmueli *et al*. suggested that loss of the MGMT

expression might be a marker of temozolomide activity in CRC (22). A liver metastasis biopsy was obtained from two consecutive mCRC patients progressed to several lines of standard treatments: tissue analysis revealed a decreased expression of MGMT (evaluated by immunohistochemistry). Both patients were thus treated with single agent temozolomide and experienced objective response lasting for 5 to 6 months.

These findings were confirmed by Amatu and colleagues in a recently published phase II study with dacarbazine in mCRC patients whose tumor was refractory to standard treatments (23). Sixty-eight patients were enrolled in the study and received dacarbazine at the dose of 250 mg/sqm intravenously for 4 consecutive days (with cycles repeated every 21 days), and tumor tissue specimens were assessed for *MGMT* promoter hypermethylation. Overall response rate (RR) was 3%, and an additional 12% of the patients had stable disease (disease control rate (DCR): 15%). *MGMT* hypermethylation was detected in 40% of the 65 specimens analyzed. Of note, only patients with hypermethylated *MGMT* in the tumor achieved an objective response and *MGMT* status also predicted the benefit from treatment as measured by DCR (44% *vs*. 6% among methylated *vs*. non-methylated cases, p=0.0.12).

Two recent phase II trials evaluated temozolomide activity in refractory mCRC. In the study by Hochhauser *et al*. (24) the investigators applied an adaptive design to estimate the activity of temozolomide in patients with aerodigestive tract cancers (including esophageal, head and neck and non-small-cell lung cancers) and mCRC whose tumor and/or serum samples had *MGMT* promoter hypermethylation. Patients were treated with temozolomide 150 mg/sqm administered daily on a seven-day-on, seven-day-off schedule with 28 days-cycles. Among 740 patients screened, 137 (19%) had confirmed tissue and/or serum *MGMT* promoter methylation, including 25% (57 out of 229) of all mCRC cases: concordance of *MGMT* status evaluation between tumor tissue and cell-free tumor DNA in serum was 81%, even though this percentage fell to 32% among the 113 patients with *MGMT* promoter methylation in a tissue sample (suggesting that a significant proportion of *MGMT* methylated cases was not detected by the serum assay or that tumor heterogeneity may influence the results of different methods). A total of 86 patients with mCRC, esophageal cancer, head and neck cancer or lung cancer who were positive for *MGMT* promoter hypermethylation were treated: in the intention-to-treat population, 6% of the patients had partial response and 45% reported disease stabilization. Among the 37 mCRC patients treated with temozolomide, 1 patient (3%) experienced partial response and 15 (41%) had stable disease. Therefore, despite some signals of drug activity, the authors conclude that temozolomide is not promising for aerodigestive tract cancers and mCRC patients with confirmed *MGMT* status because of low RR: it is arguable that additional site-specific factors should be identified and considered (beyond *MGMT* status) to further select patients for alkylating agents. The authors also point out that serum assay alone may underreport gene promoter methylation, as only 32% of the subject with *MGMT* hypermethylation in tissue samples resulted hypermethylated also at the serum assay, and conclude that tissue assay remains the gold standard for methylation detection.

The second trial enrolled mCRC patients only, and evaluated temozolomide at a dose of 150 mg/sqm/day for 5 consecutive days in 4 weeks cycles, administered after the failure of all approved treatments (25). Thirty-two patients with *MGMT* promoter methylation were treated and the schedule demonstrated a favorable safety profile. The objective RR was 12%, reaching the pre-specified level for promising activity. The median duration of response was 7 months (range, 3.7.-9.2. months). Six patients (19%) had stable disease and DCR was 31%. Tissue blocks were available for 31 patients for a biological ancillary study. *KRAS*, *NRAS* and *BRAF* mutations were highly represented (overall incidence was 71%) and patients with *KRAS*, *NRAS* and *BRAF* wild-type mCRC showed significantly higher response when compared with those with any *RAS* or *BRAF* mutation (44% *vs*. 0%, p=0.0.04). These data are in line for those reported among patients with glioblastoma, confirming that the MAP kinase (MAPK) signaling may represent a resistance mechanism to temozolomide (26). The reasons beneath such different results between the two discussed trials are unclear: it is arguable that differences in patient populations (due to different selection criteria) and in treatment exposure may have played a role.

The same authors recently presented the preliminary results obtained by a dose-dense schedule of temozolomide, that may result in enhanced activity and may restore treatment sensitivity in *RAS* mutant tumors (27). Enrolled patients are treated with temozolomide at the daily dose of 75 mg/sqm for 21 consecutive days in 4 weeks cycles. The primary end-point is RR, with a target accrual of 32 patients. Preliminary data about the first 21 patients enrolled showed an interesting RR of 24%: even more intriguingly, all patients with tumor response harbored a *KRAS* or *BRAF* mutation. Reasons explaining this discrepancy are still under investigation: it could be of particular interest to understand if treatment schedule is relevant in this context. DCR was 30%, further confirming a promising activity of temozolomide in *MGMT* hypermethylated heavily pretreated mCRC patients.

3.2. Open questions about *MGMT* methylation in mCRC: ready for prime time?

Despite the increasing needs for *MGMT* methylation testing in clinical practice, there is no

consensus about the best laboratory technique for its assessment even in glioma (28). Quillien *et al*. compared five different methods (methylation-specific polymerase chain reaction (PCR), methylight, pyrosequencing, methylation-sensitive high-resolution melting and immunohistochemistry) to analyze *MGMT* status in a series of 100 patients with glioblastoma who had received radiotherapy plus concomitant adjuvant chemotherapy with temozolomide (29). The authors found that the most accurate prediction of survival was obtained with pyrosequencing.

The abovementioned studies conducted in CRC patients used different techniques to assess *MGMT* hypermethylation. In the case reports by Shacham-Shmueli *et al*. (22) *MGMT* status was evaluated by immunohystochemistry, while in the studies by Amatu (23), Hochhauser (24) and Pietrantonio (25-27) *MGMT* status was assessed by methylation-specific PCR. More in detail, Amatu and colleagues (23) defined loss of expression of MGMT as a promoter hypermethylation of 25% or more, while in the other papers this cut-off is not specified, so that the accurate definition of *MGMT* hypermethylation remains an open issue.

Due to the difficulties in the definition of a clear cut-off value for the identification of *MGMT* methylated CRC cases, alternative strategies for patient selection are warranted. A germline single nucleotide polymorphism (SNP) in the *MGMT* promoter region has been described (c-56C>T; rs16906252) (30,31). Interestingly, this SNP is strongly correlated with *MGMT* methylation and loss of MGMT expression in tumors. Indeed, *MGMT* methylation was found in 24% of C/C patients and 84% of C/T or T/T patients (multivariate odds ratio: 18.0.; 95% CI: 6.2.-52.1.). The T allele has a 12% prevalence among Caucasians (source: NCBI-SNP website at: http://www.ncbi.nlm.nih.gov/projects/SNP/), thus the c-56C>T SNP identifies a relatively common variant. Although the molecular mechanism is not clear, it has been hypothesized that the T allele reduces MGMT expression, thereby favoring gene methylation and silencing. Our group hypothesized that this genetic variant may be useful to predict *MGMT* methylation levels in normal patients and, consequently, temozolomide sensitivity in CRC patients. At our Institution we prospectively analyzed c-56C>T SNP in 88 heavely pretreated mCRC patients (results were available for 74 of them) (unpublished data). In 12 patients we found a C/T genotype and 6 of them were treated with metronomic temozolomide at the daily dose of 50 mg/sqm. All patients experienced progressive disease at first radiological evaluation (DCR: 0%) and the study was prematurely interrupted. On the basis of our experience the c-56C>T SNP evaluation does not seem promising as selection tool in mCRC and can not substitute the *MGMT* status evaluation on tumor tissue. Moreover, a metronomic schedule of temozolomide could be less effective than a higher dose schedule.

4. BEYOND MGMT IN MCRC: TOO MANY QUESTIONS AND STILL NO ANSWERS

As reported for MGMT in the previous chapter, DNA methylation is involved in the maintenance of DNA stability and the regulation of gene expression. Global DNA hypomethylation is more often detected in CpG dinucleotides found in satellite DNA sequences or long interspersed nuclear element (LINE) repeats and is responsible for the impairment of chromosomal stability, mainly by inducing the expression of normally silenced genetic elements and facilitating chromosomal damage (32,33).

In many human genes the promoter region is rich in CpG sequences, and at this level DNA methyltransferases (DNMTs) are responsible for DNA methylation: usually CpG regions are found methylated in silenced genes, as methylation prevents the interaction of DNA with trascription factors. Hypermethylation is often found in the promoter regions of oncosuppressor genes in CRC, resulting in a loss of expression. The CIMP phenotype is interpreted as the identification of promoter hypermethylation of tumor suppressor genes in tumorigenesis (6).

Epigenetic mechanisms can trigger resistance to some cytotoxic agents usually used in mCRC, such as 5-fluorouracil, irinotecan and oxaliplatin (34), so there is growing interest in developing new epigenetic drugs. Different classes of inhibitors of DNA methylation are actually under investigation: as epigenetic mechanisms are involved in treatment resistance, combining hypomethylating agents with conventional chemotherapy seems to offer the best results when trying to exploit DNA methylation as a therapeutic target. Most of the evidences however still come from the preclinical phase (34).

More recently, data of a potential synergistic effect of hypomethylating agents and even targeted drugs are emerging: in a phase I/II study among 20 *KRAS* wild-type mCRC patients the combination of decitabine (a hypomethylating agent) and panitumumab (a monoclonal antibody against the epidermal growth factor receptor) demonstrated a 10% RR and a DCR of 60% (35). Patients responsive to experimental treatment were pretreated with cetuximab: it is then difficult to understand if they benefit from the combination of decitabine and panitumumab or from a rechallenge with an effective anti-EGFR antibody after previous response (36).

Histone post-translational modifications usually occur in N-terminal tails of histones, and are responsible for genetic regulation in an epigenetic manner (34). Histone post-translational modifications include phopshorylation, methylation, acetylation and ubiquitination, orchestrating gene expression by modifying chromatin configuration. The two most important histone modifications are acetylation of histone tails (regulated by histone acetyltransferases (HATs)

and histone deacetylases (HDACs)) and methylation (mediated by histone methyltransferases (HMTs) and histone demethylases (HDMs)) (37). Histone acetylation is always associated with gene activation due to a reduced histone-DNA binding: some tumor suppressors seem to be hypo-acetylated, and thus silenced, in CRC (32).

Given the importance of histone post-traslational modifications in tumorigenesis, HDACs are intriguing targets in pharmacoepigenetics. Among the most studied compounds, vorinostat (a HDACs inhibitor) seems to down-regulate the expression of thymidylate synthase, with a consequent sinergistic anti-tumor activity with 5-fluorouracil. Unfortunately, the combination of vorinostat with 5-fluorouracil has been tested in clinical trials with disappointing results. In the trial reported by Fakih *et al*. (38), patients with refractory mCRC were randomized to receive vorinostat at two different dose levels (800 or 1400 mg daily for 3 days, repeated every 2 weeks) in association with 5-fluorouracil. The low-dose vorinostat arm accrued 43 patients reporting 1 partial response: the median PFS and OS in this arm were 2.4. and 6.5. months, respectively. On the other hand, the high-dose vorinostat arm did not even reach the pre-specified level of efficacy for completing accrual.

Among more promising fields of interest for the clinical development of the synergism observed in the preclinical phase between HDAC inhibitors and fluoropyrimidine,the treatment of locally advance rectal cancer offers the opportunity to combine such agents with radiotherapy. As HDAC inhibitors have shown activity in combination with radiotherapy (39), some authors are exploring the role of valproic acid plus capecitabine as companion to short-course radiotherapy in the treatment of low-moderate risk rectal cancer: valproic acid may in fact enhance the activity of capecitabine by up-regulating thymidine phosphorylase (the enzyme responsible for converting the oral prodrug to 5-fluorouracil) and by down-regulating thymidylate synthase (40).

As seen for hypomethylating agents, also the HDACs inhibitors are currently under investigation in combination with biologic agents, but results are still immature. As an example, valproic acid has been tested in combination with bevacizumab in a recently reported phase I study among 55 patients with advanced solid malignancies (CRC and non-colorectal tumors) (41). Disease stabilization lasting more than 6 months was reported in 7% of the patients, including 2 patients with colorectal cancer who had progressed previously on bevacizumab.

5. EPIGENETIC MECHANISMS AND TREATMENT OF ADVANCED NON-COLORECTAL GI MALIGNANCIES: STILL FAR FROM THE CLINICS

Over the years, advances in our understanding of the molecular biology of GI tumors have led to an increased interest for epigenetic processes also in non-colorectal tumors. Epigenetic changes, including aberrant DNA methylation and histone modifications, contribute significantly to the initiation and progression of gastric tumor (42-44). As regards *MGMT*, hypermethylation is frequently detected in gastric cancer and preclinical data suggest a potential prognostic value of this epigenetic event (45-47).

Moving from these data, novel therapeutic approaches are emerging. A phase I study of vorinostat combined with capecitabine and cisplatin as first-line chemotherapy was conducted in 30 patients with metastatic gastric cancer (48). Median PFS and OS were 7.1. months and 18.0. months, respectively. Dose limiting toxicities were represented by thrombocytopenia and non-hematologic events such as fatigue, stomatitis and anorexia and a phase II trial is currently ongoing with the established dose of vorinostat 400 mg once daily to better evaluate the activity of the combination. Another HDAC inhibitor currently under phase II investigation is panobinostat, which demonstrated the ability to overcome resistance to anthracyclines in preclinical model of gastric cancer (49).

The aberrant hypermethylation of cancer-related genes, such as *MGMT*, is a frequent event also in esophageal cancer (50): preliminary *in vitro* data suggest that *MGMT* methylation may be useful in selecting patients for temozolomide treatment even in this difficult disease (51). Hochhauser *et al*. reported promising results in the cohort of 32 patients with advanced esophageal cancer and *MGMT* promoter methylation in their recent already discussed study (24): 3 (9%) patients achieved a partial response and 17 (53%) reported a disease stabilization, for an overall DCR of 62% (which is the highest among the different patient subgroups enrolled in the trial according to disease location).

In pancreatic cancer the research on epigenetic mechanisms is paving the way for the rational development of novel epigenetic drugs (52,53): however, available results are still disappointing. A phase II study with panobinostat and bortezomib in patients progressing on gemcitabine-based therapy was suspended after the enrollment of 7 patients because of a complete lack of treatment responses and early treatment-related toxicity (54).

Epigenetics is actually making its entry also in the field of hepatobiliary malignancies (55). Several epigenetic events are described in cholangiocarcinoma (56) and HDACs or HMTs inhibitors have been shown to inhibit the growth of cholangiocarcinoma cells *in vitro* (57,58). As regards hepatocellular carcinoma, multiple epigenetic aberrations (*e.g.* gene hypermethylation, Polycomb group protein deregulation, aberrant microRNA expression) have been identified in preclinical studies (59,60). A phase I/II study demonstrated potential anti-tumor activity of

belinostat with a favorable safety profile (61): this agent achieved partial response and stable disease rates of 2.4.% and 45.2.%, respectively, with median PFS and OS of 2.6. and 6.6. months, respectively. These limited data from a phase I/II study do not allow for a strict definition of the efficacy of belinostat in HCC, even though disease stabilization may be considered an interesting result in hepatocellular carcinoma due to the limited number of effective treatment options: future trials will clarify the role of this (and other) agents, and will probably help identify predictive biomarkers for a better patient selection.

6. DISCUSSION

As described in previous chapters, epigenetics offers a complementary view to cancer biology beyond the conventional framework of somatic gene mutations. This may translate into the identification of new molecular markers with prognostic or, more intriguingly, treatment predictive value. Among the so far explored parameters, *MGMT* promoter methylation is certainly the most extensively studied in GI tumors (mainly in mCRC). Preclinical and clinical evidences are now available that mCRC patients with tumors characterized by *MGMT* promoter methylation may benefit from treatment with alkylating agents such as dacarbazine and temozolomide: objective responses have been reported in heavily pretreated patients, and in some cases responses lasted for more than 6 months (Table 1).

However, there are still open questions before *MGMT* status may enter routine clinical testing and alkylating agents become part of the therapeutic armamentarium in mCRC. First of all, literature data were reported with different drugs and different schedules. Treatment schedules may have an impact on the safety profile (and ultimately on treatment compliance and exposure), and this may play a crucial role among pretreated patients who are at increased risk of toxicity with conventional cytotoxics. Moreover, temozolomide schedules may differ in the capacity to effectively exert antitumor activity in *RAS* mutant mCRC, as suggested by preliminary clinical data (27).

As discussed, *MGMT* methylation is an early event in CRC pathogenesis: it is arguable that anticipating treatment with alkylating agents in previous lines may therefore translate into higher RRs. Moreover, there is now renewed interest in the combination of temozolomide with other active agents in GI cancers, such as fluoropyrimidines and irinotecan (62,63): data from non-GI tumors show that there may be an important synergistic activity between these agents, and that time of administration may play a role in increasing the chances of response (62). Future trials will thus explore the role of temozolomide either alone or in combination with chemotherapy administered earlier in the course of mCRC.

Last, there is no consensus about the optimal methodology to be used in designing and conducting *MGMT*-based trials: *i*) the most reliable method of assessment has not been defined, at least in GI tumors (29); *ii*) it is not clear whether results between primary tumor and related metastases are superimposable (as some initial data seem to confirm (23) and as it could be anticipated from the evidence that such an alteration occurs early in colorectal carcinogenesis (10,11)); *iii*) the most suitable cut-off value for the identification of *MGMT* methylated cases is unclear, as underlined by the different strategies used in the reported mCRC studies (23-25,27). All these issues should be addressed before we design an ambitious plan of clinical trials with alkylating agents in different treatment lines for mCRC.

The road toward the INTRODUCTION of epidrugs (or epigenetic biomarkers) in the treatment of non-colorectal GI tumors appears still long and winding when compared with the advances already achieved in CRC. However, preclinical data suggest that similar epigenetic alterations occur also in other GI malignancies and *in vitro* experiences have identified multiple interesting compounds or therapeutic combinations, which are currently under evaluation in phase I/II clinical trials.

To conclude, pharmacoepigenetics may contribute to shed new light on old drugs, as well as identify new agents with innovative mechanisms of action: in both cases, however, only rigorously designed translational trials in selected patient populations will move forward the current therapeutic results in advanced GI tumors.

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Abbreviations: mCRC, metastatic colorectal cancer; DTIC, dacarbazine; TMZ, temozolomide; d, day; q, repeated every (days); No., number (of patients); *MGMT*+, *MGMT* methylated; PCR, (methylation-specific) polymerase chain reaction; RR, response rate; DCR, disease control rate; mPFS, median progression-free survival (months); mOS, median overall survival (months); NR, not reported. *preliminary results

Key Words: Alkylating Agents, Braf, Cancer Epigenetics, Colorectal Cancer, Gastrointestinal Tumors, Methylation, MGMT, RAS, Review

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