Thymidine phosphorylase expression in metastatic sites is predictive for response in patients with colorectal cancer treated with continuous oral capecitabine and biweekly oxaliplatin

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The primary objective of this study was to determine the activity and safety profile of biweekly oxaliplatin combined with continuous oral capecitabine in the first-line treatment of metastatic colorectal cancer. A secondary endpoint was to investigate the correlation between thymidylate synthase and thymidine phosphorylase (TP) expression in metastatic tissues and tumor response. Forty-one patients received oral capecitabine 1331 mg/m^2 every day combined with intravenous oxaliplatin 85 mg/m^2 every 2 weeks. The overall response rate was 58.5% [95% confidence interval (CI): 43.3–73.6%], the median progression-free survival 9.4 months (95% CI: 7.7–11.2 months) and the median survival 22.3 months (95% CI: 16.1–27.5 months). There were no grade 4 toxicities, and grade 3 toxicity was also uncommon. High TP expression in metastatic tissue was significantly associated with response to treatment ($P=0.019$), and also with a trend towards a better median progression-free survival and overall survival compared with patients expressing low TP ($P=0.056$; $P=0.073$). This study suggests that biweekly oxaliplatin and continuous oral capecitabine is an active and well-tolerated chemotherapy regimen in the first-line treatment of metastatic colorectal cancer. Moreover, these findings add to a growing body of evidence that patients with high levels of intratumoral TP expression are the ideal candidates for capecitabine-based chemotherapy. ***Anti-Cancer Drugs*** **21:313–319 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Anti-Cancer Drugs 2010, **21**:313–319

Keywords: capecitabine, colorectal cancer, liver metastases, oxaliplatin, thymidine phosphorylase, thymidylate synthase

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Received 21 August 2009 **Revised form accepted** 2 November 2009

Introduction

The XELOX regimen, which is the combination of oxaliplatin (l-HOP) and capecitabine, is a highly effective first-line treatment for patients with metastatic colorectal cancer (CRC), with similar response rates, progression-free survival (PFS) and overall survival to 5-fluorouracil (5-FU)/leucovorin/l-HOP combinations [1,2]. The conventional schedule consists of intravenous (i.v.) l-HOP 130 mg/m^2 on day 1 followed by oral capecitabine 1000 mg/m^2 twice daily for 14 days every 21 days, and this 3-week regimen is also used as a platform for combination therapy with the new biologic agents cetuximab and bevacizumab [3,4]. Although toxicity from the conventional XELOX is generally acceptable and manageable, most trials reported the need for dose adjustment of both drugs in a substantial proportion of patients. Grade 3/4 diarrhea was observed in approximately 15% of patients, and grade 3/4 nausea and vomiting in approximately 10% of patients. Grade 3/4 hand-foot syndrome (HFS) developed in fewer than 5% of patients but grade 2 in 10–15% of cases, while severe neurotoxicity, related to the l-HOP use, was frequently observed in more than 15% of patients [2,5]. There are some clinical data suggesting that capecitabine administered daily without interruption might be useful in ensuring patient compliance and perhaps in reducing overall toxicity. In addition, capecitabine is a pro-drug of 5-FU that mimics an i.v. continuous administration of this antimetabolite, and there are theoretical reasons to administer S-phase specific agents such as 5-FU in continuous and protracted rather than intermittent schedules [6]. A few clinical studies have suggested that capecitabine administered at a continuous daily dose of 1331 mg/m^2 or at a fixed continuous daily dose is active and has a low toxicity profile, and can be used in conjunction with other chemotherapy drugs [7–9]. The primary objective of this study was to determine the activity and safety profile of l-HOP combined with continuous oral capecitabine in the first-line treatment of metastatic CRC.

0959-4973 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e328334d88a

Another point to consider in the modern concept of metastatic CRC treatment is that the predictive testing of tumor biopsy samples might identify tumors likely to be responsive or resistant to antineoplastic agents. Thymidylate synthase (TS), a rate-limiting enzyme in the DNA synthetic pathway, is an important biomarker to predict response to 5-FU-based chemotherapy. In metastatic disease, higher response rates were observed in tumors with low versus high TS staining [10,11]. Thymidine phosphorylase (TP) is a fundamental enzyme for the activation of capecitabine and its intermediate metabolite, 5′-deoxy-5-fluorouridine, to 5-FU. The enzyme is known to be the same protein as platelet-derived endothelial cell growth factor, and its activity is higher in various tumor tissues than in normal tissues adjacent to the tumors [12,13]. Despite the fact that tumors with high concentrations of TP should respond better to capecitabine, the relationship between the tumoral TP expression and the efficacy of this oral fluoropyrimidine has not yet been clarified [14]. A secondary objective of this study was to investigate the correlation between TS and TP expression in metastatic tissues and tumor response.

Patients and methods

Eligibility criteria

The study enrolled patients with histologically proven metastatic adenocarcinoma of the colon or rectum, who had not received chemotherapy for metastatic disease earlier. Patients who had been treated with adjuvant 5-FU or capecitabine-based chemotherapy were eligible provided that they had remained disease-free for at least 6 months after the completion of adjuvant therapy. The other eligibility criteria included age more than 18 years; Eastern Cooperative Oncology Group performance status of 0–2; bidimensionally measurable disease; a life expectancy of at least 3 months; adequate hematological parameters (an absolute neutrophil count of $\geq 1.5 \times 10^9$/l and a platelet count of $\geq 100 \times 10^9$/l); creatinine and total bilirubin levels $< 1.25 \times$ the upper normal limit; aspartate and alanine aminotransferase of less than $3.0 \times$ the upper normal limit; and absence of a second primary tumor other than non-melanoma skin cancer or in-situ cervical carcinoma. Patients with operable metastatic disease were excluded from the study, as were those with severe cardiac dysfunction, chronic diarrhea or uncontrolled sites of infection. This study was approved by the local scientific ethics committee, and all of the patients gave their written informed consent.

Patient evaluation

The pretreatment evaluation, performed within the 2 weeks before study entry, included a detailed history and physical examination, a complete blood cell count with differential and platelet counts, whole-blood chemistry, the determination of serum carcinoembryonic antigen levels, and computed tomography scans and/or magnetic resonance imaging of the chest and abdomen. During treatment, a complete blood cell count with differential and platelet counts was performed every 2 weeks. In addition, the patients were clinically assessed every 2 weeks, and routine biochemical tests were performed. Treatment response by means of computed tomography scan and/or magnetic resonance imaging was evaluated after every four 2-week cycles or sooner if clinically indicated. Tumor response was assessed using the RECIST criteria.

Treatment delivery

Treatment consisted of 1331 mg/m^2 of oral capecitabine every day in two divided doses combined with a 2-hour infusion of 85 mg/m^2 of l-HOP every 2 weeks, and was continued until progression of disease, unmanageable toxic effects or withdrawal of consent. In case of l-HOP interruption, patients could continue with oral capecitabine as monotherapy.

Toxicity

Toxicity was assessed using the common toxicity criteria of the National Cancer Institute, version 2.0. Treatment was delayed if, on the planned day of treatment, the neutrophil count was less than 1500/mm^3, the platelet count was less than 100 000/mm^3 or the patient had persistent diarrhea or stomatitis greater than grade 1. Any patient who required more than 2 weeks for recovery from adverse reactions was excluded from the study. In the event of grade 4 hematologic or any other severe ($\geq$ grade 3) organ toxicity in individual patients, chemotherapeutic drugs doses were reduced by 25% for subsequent courses. The l-HOP was also reduced by 25% in case of persistent ($\geq$ 14 days) paresthesia or temporary (7–14 days) painful paresthesias or functional impairment. In case of persistent (> 14 days) painful paresthesia or functional impairment, l-HOP was omitted from the subsequent cycles until recovery.

To prevent nausea and vomiting, i.v. hydroxytryptamine-3 antagonists plus dexamethasone 8 mg i.v. were administered before the infusion. Oral loperamide of 2 mg every 2 h and oral rehydration were prescribed in the case of delayed diarrhea. No cytokine prophylactic treatment was recommended. The other concomitant medications were primarily used to palliate pain.

Thymidylate synthase and thymidine phosphorylase expression

Patients underwent tumor biopsies as part of this study. TS and TP expression was assessed on 2-μm sections of paraffin-embedded tissue samples from CRC metastases, and was evaluated with the avidin–biotin complex immunohistochemical technique using a rabbit polyclonal antibody to recombinant TS and TP (Santa Cruz Biotechnology, Santa Cruz, California, USA) [15]. Slides were examined under a light microscope and scored

independently by two pathologists (S.L. and C.B.) blinded to both the clinical and pathologic data. TS and TP expression was quantitated using a visual grading system based on the intensity of staining and classified into three groups from 0 (undetectable staining) to 3 (very high intensity of staining). Intensity levels from 0 to 1 were considered low expression and levels 2 and 3 were considered high expression. In the cases of disagreement, a final score was determined by consensus after re-examination.

Statistical considerations

The null hypothesis was that the overall response rate was $\leq 30\%$, and the alternative hypothesis was that the overall response rate was $\geq 50\%$. On the basis of Simon's two-stage design, with 0.05 level of significance and 80% power, at least six responses should be observed in the first stage by the recruitment of 19 patients. In the second stage, a total of 39 patients should be recruited.

Response duration was measured from the first documentation of a response to disease progression. PFS was the interval between the start of treatment and the date on which disease progression was first documented. Survival was measured from the date of the start of treatment to the date of death. The association between TS and TP expression and tumor response was assessed by the χ^2-test, and the association between TS and TP expression and PFS and survival was analyzed by log-rank test.

Results

Patient characteristics

Forty-one patients were enrolled from March 2005 to December 2007. The baseline characteristics of the patients are presented in Table 1. The median age was 69 years (range: 49–81). In all, 92.6% of patients had a performance status of 0 or 1, 68.2% had liver metastases and 34.1% had multiple metastatic sites. Sixteen patients (39.0%) had received prior 5-FU-based adjuvant chemotherapy (three patients had received prior l-HOP), while no patient had received prior capecitabine.

Treatment efficacy

All of the patients received at least one treatment cycle and were evaluable for response and toxicity. In an intent-to-treat analysis, documented complete responses were observed in two patients (4.9%) and partial responses in 22 (53.6%), for an overall response rate of 58.5% [95% confidence interval (CI): 43.3–73.6%]. Fifteen patients (36.6%) had stable disease, and two (4.9%) had progressive disease. The median response duration was 8.5 months (95% CI: 6.4–10.6 months). Surgical removal of residual metastases could be performed in eight patients (19.5%): six with liver involvement and two with lung involvement.

Table 1 Patient characteristics

Characteristics	No. of patients ($n=41$)
Age, years	
Median	69
Range	49–81
Sex	
Male	22
Female	19
ECOG PS	
0	27
1	11
2	3
Primary tumor	
Colon	32
Rectum	9
Metastatic sites	
Liver	28
Lung	7
Lymph nodes	5
Local abdominal mass	10
Peritoneum	6
Bone	1
Resection	
Yes	35
No	6
No. of metastatic sites	
1	27
≥ 2	14
Earlier adjuvant chemotherapy	16
Earlier adjuvant radiotherapy	5

ECOG PS, Eastern Cooperative Oncology Group performance status.

The median PFS of the patients was 9.4 months (95% CI: 7.7–11.2 months). At a median follow-up of 20.4 months, a total of 27 patients were deceased: the median survival was 22.3 months (95% CI: 16.1–27.5 months).

Treatment toxicity

A total of 457 cycles of l-HOP combined with continuous oral capecitabine were administered (median: 12, range: 4–18). Thirteen patients (most of them had undergone surgical resection of residual metastases) interrupted l-HOP after 12 cycles of treatment and continued with 1331 mg/m^2 of capecitabine every day until progression of disease. The incidence of toxicity is summarized in Table 2. The most common toxicities were diarrhea, nausea and vomiting, and neutropenia. However, there were no grade 4 toxicities, and grade 3 toxicity was also uncommon. Of the 41 patients, four (9.7%) experienced at least one episode of grade 3 diarrhea, one (2.2.%) experienced one episode of grade 3 nausea and vomiting, two (4.8%) at least one episode of grade 3 neutropenia, one (2.2%) had grade 3 thrombocytopenia and one (2.2%) grade 3 neurotoxicity. Among the other adverse effects, stomatitis was rare, HFS reached grade 2 in 9.7% of the patients, and grade 3 was observed in only one patient after 9 months of treatment. Increased bilirubin levels (grade 2–3) were observed in two patients (4.8%); this was not associated with concurrent severely increased transaminase levels, and retreatment after recovery was possible in both cases. A total of 62 cycles (13.5%) were delayed because of toxicity, and a dose reduction was required in five patients.

Table 2 Number of patients with the worst occurrence of adverse events

	Grade 1	Grade 2	Grade 3
Hematological toxicity			
Neutropenia	12	7	2
Anemia	9	2	0
Thrombocytopenia	14	4	1
Nonhematological toxicity			
Nausea/vomiting	6	4	1
Diarrhea	10	5	4
Mucositis	4	3	0
Hand-foot syndrome	15	4	1
Fatigue	9	6	0
Abdominal pain	8	5	0
Hyperbilirubinemia	12	1	1
Peripheral neuropathy	18	22	1

Thymidylate synthase and thymidine phosphorylase expression

Tissue from different metastatic lesions was available from 35 (liver, 25; lymph nodes, two; local recurrence, three; peritoneum, two; lung, three) of the 41 patients with metastases. Reasons for non- availability were refusal to undergo agobiopsy (two patients) and technical difficulties (four patients). The 14 patients with metastasis at multiple sites had TS and TP assessed only on liver (nine cases), local recurrence (two cases), lung (one case), peritoneum (one case) and lymph nodes (one case) samples, respectively.

Nineteen patients (54.3%) showed high levels of TS and 21 (60.0%) high levels of TP. Among the patients with low levels of TS expression, seven exhibited negative staining for TS and nine had a score of 1. Among the patients with low TP expression, six exhibited negative staining for TP, and eight had a score of 1. Representative metastatic samples of high and low TP levels are shown in Fig. 1a and b, respectively.

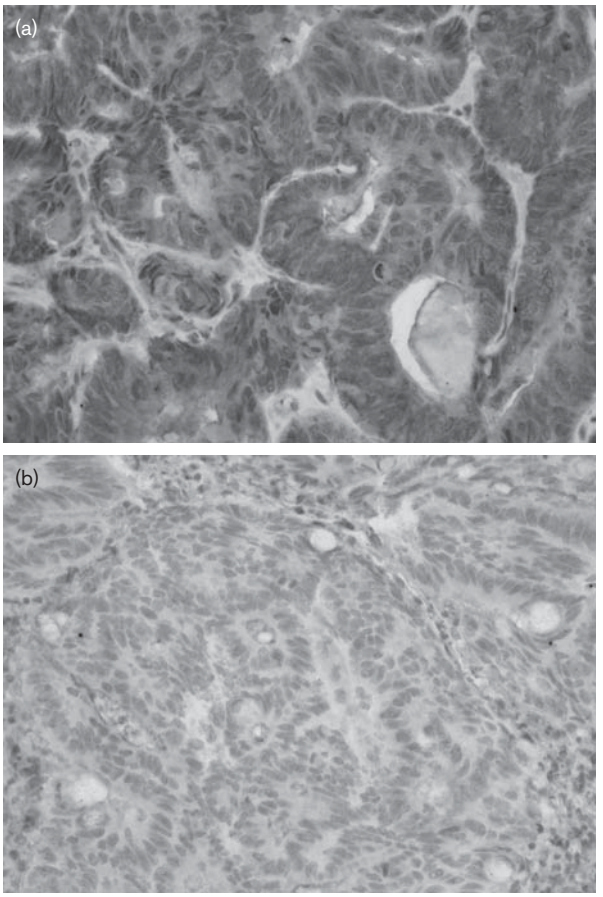
High TP expression on metastatic tissue was associated with 80.9% response (17 of 21) to continuous oral capecitabine and 1-HOP compared with 35.7% response (five of 14) in patients expressing low TP ($P = 0.019$). TS expression was associated with 75% response (12 of 16) in patients expressing low TS levels compared with 52.6% response (10 of 19) in patients expressing high TS ($P = 0.31$). The median PFS among patients expressing high TP was 10.4 months (95% CI: 8.3–12.6 months) compared with 8.8 months (95% CI: 7.4–10.3 months) among patients expressing low TP ($P = 0.056$) (Fig. 2). The median survival in patients with high TP was 25.6 months (95% CI: 18.2–32.3 months) compared with 18.2 months (95% CI: 13.4–26.7 months) in patients with low TP ($P = 0.073$) (Fig. 3). The median PFS and median survival were similar in patients with high TS compared with patients expressing low TS.

Discussion

The results of this study, which is the first clinical trial using this dose and schedule of capecitabine and l-HOP for CRC, indicate the feasibility and efficacy of continuous oral capecitabine combined with biweekly l-HOP in non-pretreated metastatic patients with CRC. Albeit with the limitations of a phase II study, the 58.5% response rate, the 9.4 month PFS and the 22.3 month median survival compare well with those usually reported with conventional XELOX and other l-HOP-based combinations [16,17]. The patients' characteristics do not suggest a better-than-average group performance with regard to patient selection, as is always the hazard of phase II trials. In addition, the daily capecitabine dose of 1331 mg/m^2 we used led to a planned dose intensity of 9.3 g/m^2/week, which is equal to that of the conventional XELOX with capecitabine administered at the dose of 2000 mg/m^2 for 14 days every 3 weeks.

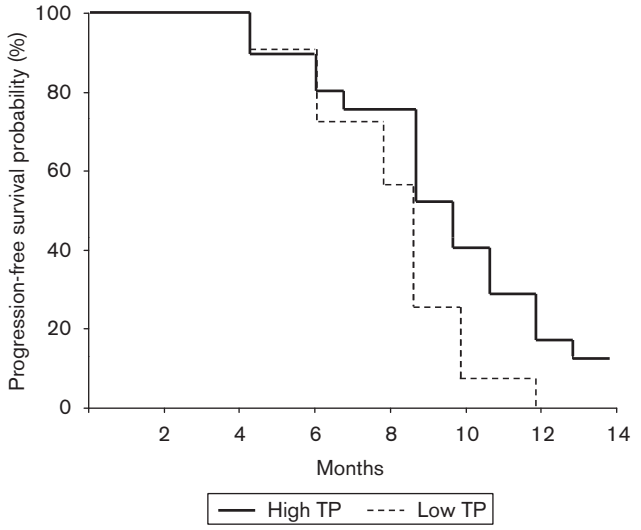
It must be underlined that a dose-reduced capecitabine is frequent in the standard XELOX regimen, mainly owing to a lower starting dose or to dose-limiting toxicities, and this may have a negative impact on dose intensity and

Fig. 1



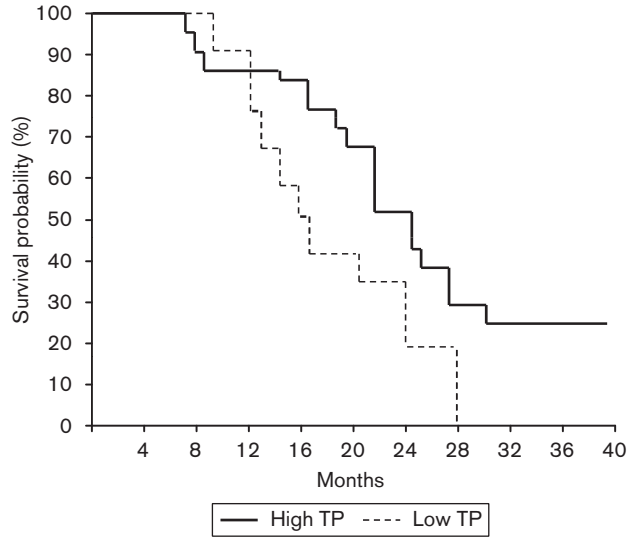
Immunohistochemical staining of colorectal liver metastasis using thymidine phosphorylase monoclonal antibody showing high (a) and low (b) intensity tumor cell staining for thymidine phosphorylase (magnification ×200).

Fig. 2



Estimated probability of progression-free survival based on thymidine phosphorylase (TP) expression in metastatic tissue in 35 patients with colorectal cancer treated with oxaliplatin and continuous oral capecitabine.

Fig. 3



Estimated survival probability based on thymidine phosphorylase (TP) expression in metastatic tissue in 35 patients with colorectal cancer treated with oxaliplatin and continuous oral capecitabine.

treatment efficacy [18]. In this setting, the chemotherapy regimen applied in this study was well tolerated, with a low percentage of delays and dose reductions. The proportion of patients suffering from grade 3 diarrhea and grade 3 nausea and vomiting appeared lower than that usually reported with the standard XELOX schedule. Moreover, grade 3 HFS was observed in only one patient. A lower incidence of grade 3 and 4 diarrhea and grade 3 and 4 HFS has been previously reported with the use of continuous oral capecitabine, and in one of our recent studies we found a low incidence of HFS in patients more than 75 years with metastatic colorectal and gastric cancer treated with fixed and continuous-dose capecitabine [8,19]. The use of 85 mg/m^2 of 1-HOP every 14 days instead of 130 mg/m^2 every 21 days may have also contributed to a low incidence of some toxicities. It was recently reported that l-HOP 85 mg/m^2 combined with capecitabine provided the same level of clinical efficacy in patients with advanced CRC, with reduced nausea and vomiting and reduced peripheral neuropathy compared with the same two-drug combination using a higher dose of l-HOP [20].

The conventional XELOX schedule, with a 1-week rest of capecitabine, remains the standard platform to consider for further clinical studies in CRC and other malignancies. However, our results suggest that continuous oral capecitabine combined with biweekly l-HOP is a valid alternative that may be proposed to patients that develop persistent gastroenteric toxicities during conventional XELOX, or to older patients that might prefer a lower daily number of pills. Nevertheless, the convenience of the modified protocol would have to be proven objectively, and be shown to have significant benefit before this regimen is preferred to the established XELOX. In the current antiangiogenics era, our research group is also investigating the activity and tolerability of continuous oral capecitabine and 1-HOP regimen combined with bevacizumab (data not shown).

In this study, a high intratumoral TP expression in CRC metastases predicted a significantly higher response rate (80.9% vs. 35.7%) ($P = 0.019$), and was also associated with a trend towards a longer duration of time to progression and overall survival compared with patients with low TP (Figs 2 and 3). Therefore, although a large trial is needed to confirm the results, these findings suggest the intriguing probability that patients with high levels of intratumoral TP expression are the ideal candidates for a capecitabine-based chemotherapy. However, the measurement of tumor biomarkers was not the primary aim of this study, and the immunohistochemistry (IHC) measurement of predictors of response to chemotherapy was restricted to only two enzymes: TS and TP. The level of intratumoral TS remains the most important predictor of response to fluoropyrimidine-based chemotherapy, while TP has a main role in the metabolic activation of capecitabine. Other factors, such as the intratumoral expression of dihydropyrimidine dehydrogenase (DPD), are considered important predictors for response to 5FU-or capecitabine-based chemotherapy [21–25]. A recent study suggested that low TS, DPD, and TP expression were prognostic of better outcome in patients with stage III CRC treated with 5-FU [26].

A partially unexpected result of our study was the lack of a statistically significant relationship between the levels of TS expression on metastatic tissue and clinical response. These findings may simply reflect the small sample size available for these analyses, which were not the primary end-point, and, therefore, have to be considered only exploratory. Nevertheless, as TS expression is associated with response to 5-FU, and capecitabine is a prodrug of 5-FU, low intratumoral TS expression should be associated with a high response rate with either 5-FU or capecitabine treatment. A recent study reported that lower expression of TS mRNA in metastatic tumor was associated with a lower chance of early PD with XELOX therapy in patients with metastatic CRC, while high excision repair cross-complementation group-1 mRNA levels were associated with shortened time to treatment failure [27]. Meropol *et al.*, in line with our results, reported that TP expression, but not TS and DPD, was significantly associated with time to progression and overall survival in patients with metastatic CRC treated with capecitabine and irinotecan [28]. The results in the aforementioned study were less consistent with IHC when the potentially predictive value of TP gene expression was explored by reverse transcription polymerase chain reaction. In addition, although TP levels in the primary tumor significantly predicted response rate, a statistically significant difference was not found when considering TP expression in metastatic sites, likely owing to the decreased sample size of metastatic versus primary tumors. In this setting, our research group evaluated the TS and TP expression only in metastatic tissue, as the IHC measurement of biochemical markers on primary tumors often failed to predict the response to chemotherapy for advanced disease in earlier clinical studies [29]. Nevertheless, a difference in the biology of primary and metastatic colorectal tumor has been reported [30,31]. However, a comparison between clinical studies analysing the predictive role for response of intratumoral biomarkers is difficult, mainly because of the inadequate number of patients and the use of different chemotherapy regimens, and divergent results on their prognostic and/or predictive value are often reported [32]. Data from prospective studies large enough to evaluate multiple predictive markers are needed. Another point to consider is that different techniques are often used to evaluate the expression of biomarkers: IHC is inexpensive and widely available, but its evaluation is semi-quantitative and therefore difficult to standardize, while reverse transcription polymerase chain reaction allows mRNA quantitation, but does not yet have widespread use in clinical practice because of its difficult application [33,34].

In conclusion, this study suggests that continuous oral capecitabine and biweekly l-HOP is an active and well-tolerated chemotherapy regimen in first-line treatment of metastatic CRC. Moreover, these findings add to a growing body of evidence that the analysis of TP expression might help to rationally select a capecitabine versus a 5-FU-based treatment.

Acknowledgements

The authors thank Dr ssa S. Rossi for statistical assistance. The authors also thank the following members of the Multidisciplinary Oncology Group in Gastrointestinal Tumors for their participation in the study: Dr A. Bernini; Dr ssa S. Civitelli; Dr Calomino, Clinical Surgery; Professor A. De Martino; Professor F. Tani; Professor G. Marzocca; Professor M. Lorenzi, General Surgery; Professor F. Roviello; Professor D. Marrelli, Oncologic Surgery, University of Siena; Dr ssa C. Intrivici, University of Palermo; Dr ssa L. Paolelli, Hospital of Gallipoli; and Dr G. Giusti, USL 10, Pisa, Italy.

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